Establishing Volumetric Biomarkers In MRI of the Digestive Tract.

Susan Elizabeth Pritchard MSc

Published works by staff candidate submitted to the University of Nottingham

for the degree of Doctor of Philosophy

December 2015

Acknowledgements

I would like to take this opportunity to acknowledge the help and support of my colleagues at the University of Nottingham. In particular Dr Luca Marciani and Prof Penny Gowland who recruited me back in 2008, after my prolonged career break, who have always encouraged and supported me as I have returned to research, culminating in this submission. Also Prof Robin Spiller both for inspiring my interest in the workings of the gut and for framing the questions that MRI had to answer.

The Sir Peter Mansfield Imaging Centre has been a tremendous environment in which to work due to the inclusivity and friendliness of my colleagues coupled with their scientific drive and excellence. I am truly grateful for the support that I have received, both professional and personal, from all the staff and students contained within. There are too many to mention individually but I am indebted to you all.

To Laurence and Ellie who take great pride in their Mum's achievements, whilst gently mocking the geek within, to John who has loved and supported me for 40 years and to my late Mother and Father who would have been overjoyed to see me attain a PhD towards the end of my working life.

Thank you.

Contents

1. Abstract

This extended abstract describes the background to the 14 research papers that the author, as staff candidate, is submitting for the award of PhD by published works. The core part of this work refers to the development of volumetric biomarkers within the human digestive tract using magnetic resonance imaging (MRI) and their application to answer novel biomedical research questions. In particular the author's work has focussed on applying these techniques within the human colon and the first two papers (which detail this work) were led and written by the author. This work was pioneering in its field, the first time that physiologically undisturbed colon volumes were measured in healthy human subjects and in patients suffering from irritable bowel syndrome (IBS) and provided novel insights into the post-prandial symptoms experienced. Subsequently the effect of an experimental stress on this post prandial response was evaluated in healthy subjects, also the first time such an effect had been measured. The third paper, also written by the author, describes her work on the first clinical application of similar volumetric techniques to assess the human nasal airways and their response to pharmacological intervention, in this case the efficacy of a nasal decongestant.

This document seeks to set the gastro-intestinal papers within their scientific and physiological background and to show their original contribution to the current understanding of the physiological processes within the human gastro-intestinal tract.

Between mouth and anus, a complex myriad of mechanical, chemical and biological procedures interact to liquefy and transport food; to break it down into increasingly simpler chemical forms; absorb nutrients and then eject what is no longer required. MRI provides a unique window into the functions and form of this environment at the macroscopic level; a non-invasive tool for detecting and measuring the structure and physical movements of the abdominal organs and their contents, monitoring fluid transport and providing insights into the biological processing therein. This can provide quantitative biomarkers to rigorously assess the normal undisturbed physiology in health and disease and the effect of pharmacological interventions. It is a hitherto relatively unexplored area and it is the development and application of such measurements that form the bulk of the author's research contained within the presented publications.

2. A brief background to MRI as applied to the gastrointestinal tract

The first MRI image of the human abdomen was obtained in 1977 [1, 2] and since then rapid advances in both MRI theory and technology have led to an imaging modality uniquely able to image the distribution of water and fat in the human body without the radiological risks of high resolution computed tomography (CT). MRI not only provides information on the density of water and fat but on the environment, both physical and chemical, within which the hydrogen nuclei contained in water and fat molecules reside. It is these subtle differences in environment that enables detailed images of form and function within the human body, particularly those organs of predominantly high water content. Thus MRI has, historically, been primarily directed at the human brain where unparalleled detail of the anatomy and function have been obtained [3]. Early imaging techniques were highly susceptible to disturbance from physiological movement, however the development of more rapid imaging techniques has enabled visualisation and measurement of mobile structures such as heart, blood vessels and viscera within the timescale of individual breaths and even heartbeats.

2.1 MRI – a brief semi-classical explanation

The physics of MRI, as applied to in vivo imaging, is described very clearly elsewhere [4] [5] This section seeks to provide a quick background to the

terms and technologies applicable to abdominal imaging, in particular those used in the published works submitted by the author.

2.1.1 NMR

Nuclear magnetic resonance in its most basic form relies on the magnetic properties of atomic nuclei. In most nuclei the magnetic properties of the constituent baryons cancel out giving a net magnetic 'spin' of zero. However, when this is not the case then these 'un-paired spins' (present in such naturally abundant nuclei as hydrogen, fluorine, sodium and phosphorus) give rise to a net magnetic moment. The application of an external magnetic field will then result in these spins aligning parallel and anti-parallel to the applied field. However the number of spins aligning parallel is slightly greater than those aligning anti-parallel (as it requires more energy to be anti-parallel). This difference in numbers is extremely small and at temperatures concurrent with the human body (and even with an applied field of 1.0 Tesla -approximately 40,000 times the earth's magnetic field) is only 7 nuclei in one million. This results in a very small net longitudinal magnetisation. However, application of a time varying electromagnetic field (in this case within the radio frequency part of the electromagnetic spectrum) can cause these aligned spins to precess around the direction of the applied field (creating a transverse magnetisation). Absorption of this RF energy is a resonant phenomenon (due to quantum mechanical considerations energy can only be absorbed within very precise frequencies). Thus each of these elements has its own resonant frequency dependent on the nuclear properties of the nuclei and the applied field. For isolated hydrogen nuclei (protons) this resonant frequency is 42MHz at 1.0 Tesla but this frequency can be altered slightly by the structure of the chemical

bonds surrounding the proton (known as a 'chemical shift' in frequency and this is the basis for magnetic resonance spectroscopy). For example the protons in a water molecule resonate at a slightly (3ppm) higher frequency than those in a tri-glyceride [6] molecule. A brief pulse of RF energy at the resonant frequency will thus be absorbed and subsequently result in the emission of a small RF field and it is this field that becomes the detected MR signal.

This radiative energy will only last for a short time due to loss of magnetisation from any movement and reorientation of the resonating protons including that caused from physical processes such as collisions. This reduction in energy (and hence the intensity of signal) has an exponential form and is known as free inductive decay (FID). This can be parameterised by the time constants T_1 and T_2, where T_1 is an expression of how quickly the longitudinal magnetisation of the nuclei is reduced (it is the time taken for the signal to reduce to the fraction 'e' of its original value (where e= 2.72)) and T_2 relates to the decay of the transverse magnetisation. T_1 is often referred to as the spin-lattice time constant (T_1 is more affected by any chemical bonds surrounding the resonating proton) and T_2 as the spin-spin time constant (and is more affected by the degree of magnetic coupling with adjacent protons). T_1 and T_2 vary with the applied magnetic field ie the values at 1.5T will be different to values at 3T but in biological materials, T_1 and T_2 are higher in liquids and shorter near large complex molecules[7]. Typical values for human tissues are shown in table (1) [7], more recent in vivo measurements at 1.5 and 3 T can be found elsewhere [8]

	T_1 (ms)	T_2 (ms)
Water	2000-3000	2000-3000
Liver	586	46
Fat	343	58
Muscle	856	29

Table 1: Typical T_1 and T_2 values at 1.5T [7],

It is possible, by the addition of paramagnetic materials, to manipulate T_1 and/or T_2, in order to provide additional contrast to that obtained from unadulterated biological tissues. This has led to the development of contrast agents containing gadolinium that can be swallowed or injected into the blood stream to enhance normal or pathological structures.

2.1.2 MRI

The 3D image produced in MRI relies on subtle shifts in frequency and phase of the resonant absorption/ emission caused by variations in the applied magnetic field. Addition of a spatially varying applied magnetic field (or magnetic field gradient) localises the emitting nuclei due to the fact that the frequency of their RF emission changes according to the exact value of the total applied field (frequency encoding). In a similar manner the phase of the emitted RF field will also rely on the location within this spatially changing field (phase encoding). Precisely timed excitation pulses and gradient switches in three dimensions (pulse sequences) can construct echo trains of RF pulses as the precessing magnetic dipoles come in and out of phase. These ‘spin-echo’ or ‘gradient-echo’ trains can then be used to derive spatial and intensity maps (i.e.

the image). Hence the physical principles described result in a map of the location of resonating protons within water and fat, with the subtle differences in signal intensity introduced by density, T_1, and T_2 changes all delineating the tissues within individual organs of the abdominal cavity and any contents therein.

Since the original 3D imaging of the human abdomen was demonstrated in 1988 [9] there has been an explosion of technical innovations in magnet technology. Scanners capable of imaging the human body with static fields of 1.5T, 3T are now available within the clinical environment (and up to 7T within the research environment). However with higher fields come increasing problems with variations of magnetic susceptibility and motion artefacts and abdominal imaging (with its large tissue and gas contrasts) at the 7T field is in its infancy. Advances in gradient coil design and modes of operation enable fast application of the 2D and 3D image acquisition and this has been coupled with enormous leaps in RF signal processing and computational capability. Data acquisition times have been reduced by orders of magnitude so that it is now possible to acquire high resolution 3D images within seconds and visualise them almost instantly.

The majority of the data in the submitted published works were acquired using the 1.5T Philips Achieva^{TM} scanner based in the Sir Peter Mansfield Imaging Centre at the University of Nottingham. Nasal data were acquired on the 3T scanner and the hyperoxia study (paper 14) used the 7T scanner, also at the Sir Peter Mansfield Imaging Centre.

2.2 Pulse sequences relevant to submitted papers

Imaging the abdomen has different technical challenges to those in brain imaging. The required volumes are larger and there is much more variation in MRI parameters between large blood vessels (e.g. aorta, vena cava, hepatic and portal veins), tissues, free water and gas within the stomach and bowel than within grey and white matter. Imaging of nasal morphometry has similar challenges with the proximity of bone and air-filled spaces. Motional blurring and artefacts from respiration and heart motion and peristalsis are much greater within the abdomen leading to image degradation. Within the often adipose abdomen fat/ water chemical shifts can also have an effect. Due to all of these technical challenges it has proved necessary for the development of bespoke imaging techniques for the abdomen with varying sequences of resonating RF, applied field gradients and enhanced signal detection and computation. These differing pulse sequences are optimised to the acquisition of the precise data that is required e.g. high water content or fat imaging, capture of motion or flow etc. The main sequences generally used for these applications at Nottingham and elsewhere (and contained within the published works) are summarised below.

Many of the features of interest within the digestive system are related to fluid secretion, transport and absorption (see section 3) and as such MRI pulse sequences that are sensitive to freely mobile water are useful for the visualisation and measurement of stomach and small bowel contents. Water

has both a long T_1 and T_2 whereas liver and fat have a much shorter T_2 than T_1 (Table (1). Therefore a pulse sequence which relies on the transverse T_2 relaxation of water (a T_2 weighted sequence which generally has a long time between the echoes) will result in an image where the water has a high signal (as there has been less signal decay) and the signal from the surrounding visceral fat and tissue will be much lower (as shown in Fig 1a and b)

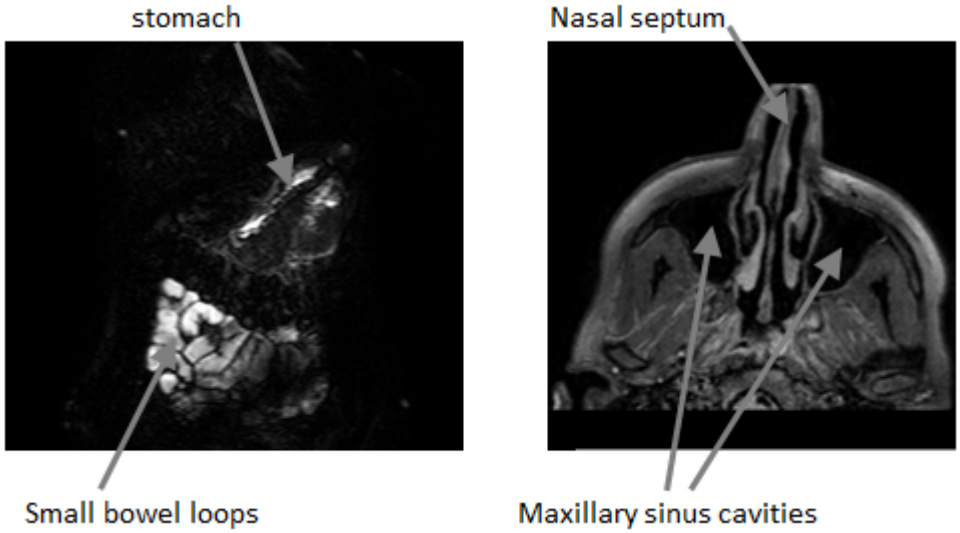

Fig 1: T_2 weighted images of a) small bowel [10]

b) nasal structure (paper 3)

A fast spin echo RARE (rapid acquisition with relaxation enhancement) heavily T_2 weighted image was used to image and measure fluid volumes in the stomach (paper 5), pancreatic and bile ducts (paper 13), small bowel (papers 5 8 9 11 12) and colon (paper 1). The rapid acquisition and the sensitivity of fast spin echo techniques was also utilised for the imaging and volume measurement of inflamed, water loaded nasal membranes (paper 3).

Other pulse sequences rely on the ratio of T_1 and T_2 to provide contrast. Gradient echo sequences (where the re-phasing of the precessing dipoles is achieved by manipulation of magnetic gradients) are more sensitive to small changes in $T_1$and T_2 and a balanced gradient echo sequence has been used to image stomach contents whether food (paper 4 6 7 9 10), foams and gels (paper 4) and gall bladder volumes (paper 13). A similar pulse sequence was used to image ascending colon contents (paper 11) as in Fig 2

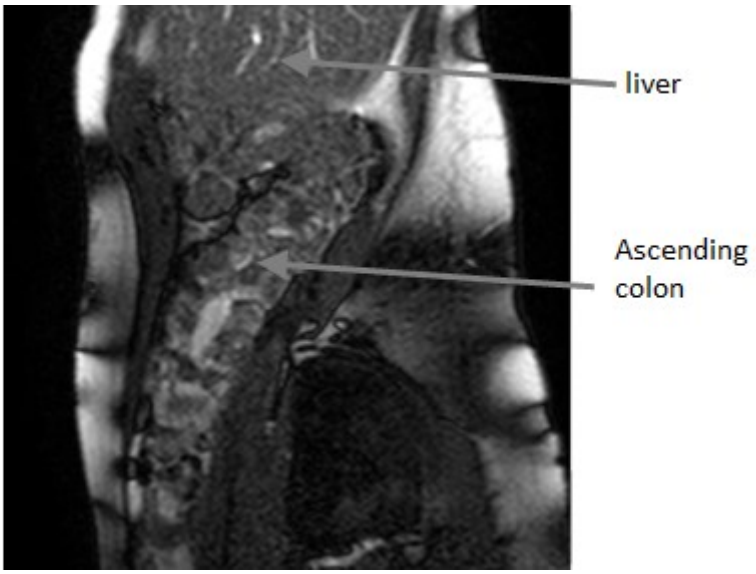

Fig 2: High resolution balanced gradient echo image of ascending colon (paper 11).

Another way of creating contrast using gradient echo techniques within the abdomen is aided by the chemical shift in resonant frequency between fat and water. Two images are created, the first has an echo time of 2.3ms, designed such that the echoes from fat and water are out of phase (at 1.5T) and the second has twice this echo time so that the echoes from fat and water are in phase. The out of phase image creates a black border at the fat/tissue interface and this often helps to highlight anatomical details within the abdomen (as

seen in fig 3). This sequence was used to provide anatomical landmarks for successful segmentation of the small bowel (papers 5 8 9 11 12) and also for the identification and measurement of the colon (papers 1 2 8)

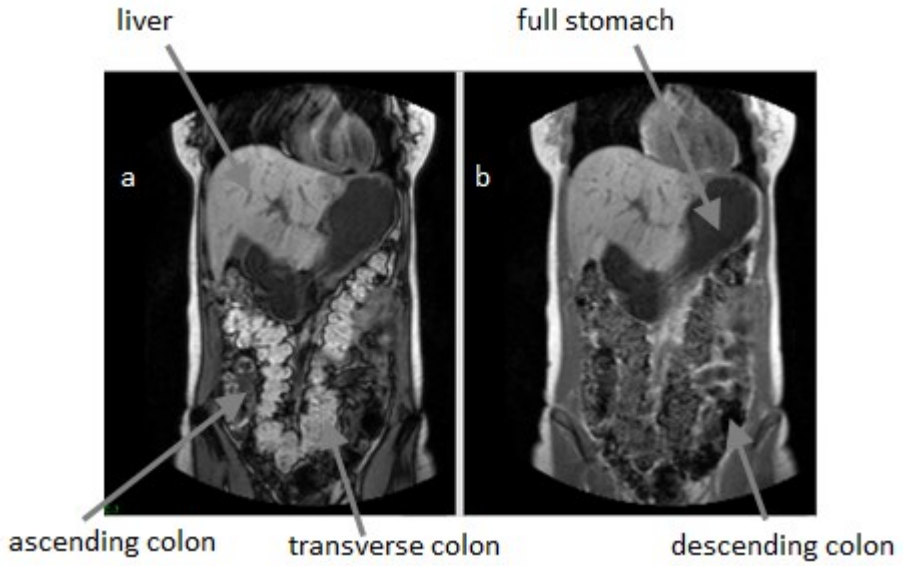

Fig 3: Dual echo images of human abdomen

a) fat / water out of phase b) fat / water in phase

(data acquired by author[11])

The pulse sequences used for brain imaging at 7T are very different to those used in GI imaging. There are fewer susceptibility differences, indeed the BOLD technique relies on the small differences between oxygenated and deoxygenated blood to provide information on blood flow within the brain. In this case the emphasis is to achieve a good signal to noise ratio, with high resolution and rapid imaging and a gradient echo planar imaging (EPI) sequence was used (paper 14). Cerebral blood flow was assessed using an arterial spin labelling technique FAIR where RF excitation occurs upstream of the blood flow and signal readout is targeted on regions downstream [12].

3. Context of submitted papers- MRI and the physiology of digestion

The process of digestion has previously been a difficult field to explore. Despite the fact that the alimentary canal is technically outside of the body (it is lined by epithelium) access to its realms historically relied upon invasive procedures, both surgical and endoscopic, that disrupted the very processes to be studied.

The advent of x-ray imaging techniques opened a window onto the undisturbed physiology, particularly when combined with the ingestion/ introduction of highly contrasting materials, such as barium. Although most barium studies are performed to highlight abnormalities within the digestive tract i.e. tumours, hernias, strictures etc. it is possible to highlight the passage of food through the alimentary canal. This could be done directly e.g. swallowing a barium laced meal, or by monitoring the passage of a standard meal by ingestion of radio-opaque markers prior to eating. These techniques are more suited to the examination of the upper GI tract where the only preparation required is a period of fasting and the transit times involved are in the region of hours rather than days (and hence require fewer exposures). However greater preparation is required for the examination of the lower digestive tract where the presence of faeces interferes with the visualisation of the bowel. Here fasting and purging

are required to cleanse the colon and frequently gaseous distension of the bowel is also required.

The radiological hazards of such examinations are acceptable within the clinical setting, where examination can provide substantial diagnostic benefit to the patient, but such exposures are unacceptable for the study of basic physiology where serial imaging of healthy volunteers would be required. Gamma scintigraphy techniques, where a radioactive substance is ingested and detected, are used in the clinical setting [6] to assess motor disorders of the upper GI tract and could potentially be used to provide information on the progress and absorption of radio-actively labelled food components e.g. the absorption and dissemination of sugars and/or fats. However the radiological safety requirements, when using these techniques on human subjects, have also restricted their use in basic physiological research.

More recent developments of ingestible wireless pills equipped with camera and chemical sensors have proved particularly useful in examination of the small bowel [13] where traditional endoscopic techniques cannot be used. They provide exquisite insight into the environment in close locality to the devices but lack the ability to see 'the whole picture'.

Since the advent of rapid imaging techniques [9, 14, 15] using MRI to visualise and monitor the digestive tract is a rapidly expanding field and comprehensive reviews of its use can be found elsewhere [16-18] This section briefly reviews the processes of digestion and the way that these have been

monitored using MRI in order to contextualise the submitted papers within the wider field. Attention is drawn to the work at Nottingham and, in particular, the work of the author, detailed in the submitted papers.

3.1 Upper GI tract

3.1.1 Mouth

Digestion begins with the biting and chewing of food. The act of chewing reduces the size of the food components, releases saliva and prepares a bolus for the act of swallowing. Swallowing requires the coordinated action of the tongue and the oro-pharyngeal structures to propel the food into the oesophageal tract, without the dangers of inhalation. Real-time 2D MRI has been used to assess chewing and swallowing in healthy volunteers, dysphagia patients [19] and acromegaly patients [20] where the larger tongue volumes of the patients (measured in this study) can lead to abnormalities in the swallowing process. In both cases visualisation of the food bolus was enhanced by use of an oral gadolinium contrast agent.

3.1.2 Gullet

Transit of the food bolus down the oesophagus can be hindered by the presence of physical obstruction (due to stricture or pathology) and also relies upon the presence of efficient, coordinated peristalsis and this can be affected by age and disease causing achalasia. Passage into the stomach is regulated by the gastro-oesophageal junction, which not only needs to relax to allow food into the stomach but also prevents reflux of highly acidic gastric contents into the relatively unprotected oesophageal epithelium. MRI has been used to research motility [21], gastro-oesophageal function [22] [23] and reflux [24]

but generally these are routinely clinically assessed using high resolution manometry [25, 26].

3.1.3 Stomach

The food then accumulates in the stomach where the lumen stretches to accommodate the volume of food and this stretching is an important part of both function and satiety responses. Failure of accommodation can lead to premature satiety, nausea and dyspepsia. The stretching of the stomach wall is also an important regulator of appetite and hunger, it is the detection of a full stomach that leads to cessation of eating. MRI can provide a radiologically safe method to measure these volume increases (and hence stretching) [27] [28] without the disturbance that balloon inflation methods introduce [29].

The necessary secretion of acid and digestive enzymes from the stomach wall is stimulated by the presence of food and these secretions can also be measured using MRI [30] [31, 32]. Substantial contractile activity is also triggered by food within the stomach, with primarily antegrade peristaltic waves churning and mixing the contents. Distension of the stomach walls will precipitate high motility whereas little or no distension will result in little motility. This ensures efficient and sufficient exposure of contents to the gastric acid and enzymes and promotes the mechanical breaking down of large food particles (antral grinding). Again rapid MRI techniques have been used to visualise and quantify this gastric motility[33] [34] and grinding [35] and establish the effect that factors as meal composition and viscosity [36] have on these functions.

Food resides in the stomach until it is of such a liquid form that it can exit the pylorus and enter the duodenum. The precise timing of this process (usually 1-4 hours) is influenced by many factors and there are many feedback mechanisms that control this process and at least five different sensory receptors have been identified in the mucosa, which respond to mechanical, thermal, osmotic and chemical stimuli. A liquid meal will tend to empty faster than a solid and the emptying rate of a solid meal will depend heavily on the efficiency of the antral grinding. The liquid phase will often empty before the solid phase as the combination of peristalsis and constriction of the pylorus sieves liquid from the more solid particles (antral sieving). A low nutrient meal will empty much faster than a meal high in fat which, when detected in the duodenum slows motility (and emptying) in order to match the available delivery of bile and pancreatic secretions. Any disruption to these mechanisms e.g. due to the nerve damage frequently seen in diabetes, can result in delayed gastric emptying (gastroparesis) with associated nausea, pain and vomiting.

MRI has been used in many studies to assess the gastric emptying process in health [37-40] and disease [41-48] and response to alteration in meal form [49] and pharmacological intervention [50, 51]. Several papers in the author's submitted published works are concerned with the measurement of gastric emptying using both a manual segmentation as shown in Fig 4 (papers 4, 6, 7, 9, 10) and a semi-automated method (paper 5).

Fig 4: Manual segmentation of stomach volume

(data acquired by author paper 9)

Measurement of the stomach volume as a function of time provides a quantitative estimate of gastric half-emptying time and this can be compared with the subjective responses of human volunteers in terms of satiety and hunger. The ability to discriminate between the volume of liquid or solid stomach contents and the distension due to swallowed air provides indicators as to the predominant mechanisms of satiety.

This is important when meal form has been altered to increase satiety by 'foaming' (paper 4) or 'souping' (paper 10) or by preventing separation/ layering of fat (paper 7). Satiety is also increased when a meal composition has been altered (paper 6), with a high fat meal promoting a greater feeling of fullness despite emptying faster than a high carbohydrate meal. Also consumption of a bread based meal increases the gastric emptying time compared to an equicaloric rice-based meal (paper 9) but did not appear to

affect satiety. There was a similar effect seen with bran additives which also delayed the gastric emptying time (paper 12). In addition, the measurement of the rapid emptying of water into the small bowel provides crucial information for the parameterisation of drug delivery and absorption (paper 5).

3.2 Small Bowel

3.2.1 Duodenum

As a meal enters the duodenum the gall bladder releases bile (and decreases in volume), and the pancreas directly secretes a fluid containing mixture of enzymes and bicarbonate. Both fluids are released from the common bile duct into the duodenum through the sphincter of Oddi. Bile contains water, bicarbonate and bile acids and these acids have detergent action which causes fat globules to break down (emulsify) into much smaller droplets. Emulsification greatly increases the surface area of fat, making it available for digestion by lipases (secreted from the pancreas). Pancreatic secretion is also alkaline to change the pH from the highly acidic environment of the stomach (pH 1.5-3.5) to the slightly more alkaline environment (pH 6-7.4) tolerated by the small bowel. The amount of bile released and pancreatic fluid is generally controlled by feedback from the receptor cells of the enteric nervous system and a highly fatty meal results in increased secretion [52]. Pharmacological intervention can change these secretory processes (and/or affect the function of the releasing sphincter) and paper 13 describes the author's quantitative assessment of the effect on the gall bladder, common bile duct and pancreatic duct of morphine-neostigmine and secretin in healthy volunteers. Both agents

were found to increase gall bladder volume but the morphine-neostigmine challenge caused greater distension of the pancreatic duct due to contraction of the Sphincter of Oddi. The effect of amount and form of fat droplets on bile release from the gall bladder was also investigated in paper 7 but no difference was found in the change in gall bladder volumes whether the fat emulsions were stabilized or whether there were fine or coarse fat droplets present.

3.2.2 Jejunum and Ileum

Upon leaving the duodenum the mixture of food, bile and pancreatic juice pass into the small bowel, and the rate of transport is now dominated by the peristaltic activity of the small bowel wall. Both antegrade and retrograde contractions occur, continuing the mixing process and creating small 'pools' in the convoluted intestinal loops. These 'pools' (or pockets) are thereby gradually moved through the small bowel allowing the absorption of simple sugars, amino acids and fatty acids, before emptying into the ascending colon through the ileocaecal valve. Mean transit times for non-nutrient fluids have been measured [53] at 84 minutes but there is a large natural variation in addition to those changes caused by meal composition. Large amounts of fat have been found to increase the small bowel transit time [54, 55] and the presence of disease or strictures (common in Crohns disease) can also retard the flow leaving large distended loops of fluid which can be prone to bacterial overgrowth.

MRI is particularly useful in the study of this region of the digestive tract as intubation is not only difficult but disturbs these processes (in particular it

increases motility). The liquid nature of the intestinal chyme enables rapid and accurate imaging using T_2 weighted sequences that provide a large contrast with surrounding viscera and fat. Motility studies using a combination of oral contrast agents and unabsorbable hydrogels are reported in health [56, 57], post-surgery [58] and after pharmacological intervention [59, 60]. More recently semi-automated methods of assessing small bowel wall motility in health and Crohns disease have been reported [61-66]. The direct observation of fluid flow in the small bowel has also been described using an MRI tagging technique [62, 67].

The lumen of the small bowel can both secrete and absorb water and pioneering work at Nottingham has established a validated MRI measurement of the volume of free water within the small bowel [10]. This volume measurement has been used to evaluate the effect of bran additives [68], and the author subsequently used this measurement method applied to a bread based meal vs a rice based meal (paper 9) where the bread meal was found to reduce the small bowel water compared to the equicaloric rice based meal. In addition the effect of the form of food on small bowel water has been investigated. A solid/liquid meal was found to increase the small bowel water more rapidly than a 'souped' meal (paper 10) - as the liquid phase emptied from the stomach almost immediately- although during the later phase of digestion the total water content was greatest for the 'souped' meal. Small bowel water content is also significantly reduced in patients with disordered bowel function such as IBS-D (paper 12), but significantly increased in coeliac disease and scleroderma [69] and constipation (private communication

from C Lam -awaiting publication). The effect of dietary components such as fermentable oligo –di –mono saccharides (FODMAPS) on both healthy volunteers and subjects with bloating is beginning to be explored [11, 70, 71].

In addition the rate and extent of drug dissolution and absorption may be highly influenced by the precise water distribution within the stomach and small bowel and quantification of this distribution was evaluated in 12 healthy volunteers, both in the fasted state and post-ingestion of a 240ml water dose (paper 5). In this case the water formed distinct small pockets within the small bowel of around 5ml.

Evaluation of pharmacological interventions at Nottingham include:- a study on the effects of ondansetron on small bowel water in healthy volunteers [72] and IBS-D patients [73]; the effect of oral polyethylene glycol to speed transit in healthy volunteers (paper 8) and in constipated patients [74] - which caused a fourfold increase in small bowel water from the fasted state ; the use of loperamide and simethicone to slow transit in an experimental model of diarrhoea (paper 11) where, despite accelerating the gastric emptying of a mannitol drink the interventions both reduced the amount of small bowel water and increased the oro-caecal transit time compared to a placebo.

3.3 Large Bowel

The large bowel is defined as the section of GI tract from ileocaecal valve to rectum. It consists of 4 regions, the ascending, transverse, descending and the sigmoid colon. It is vitally important for the absorption of nutrients and the

recovery of water and electrolytes as well as the transmogrification of watery intestinal chyme to the solid faecal matter passed to the rectum. [75]

3.3.1 Ascending colon

Upon exiting the small bowel the intestinal chyme enters the ascending colon via the ileocaecal valve. This valve has two functions. Firstly it regulates flow into the colon thereby slowing transit and aiding absorption in the terminal ileum as well as preventing overload of the large bowel (with subsequent rapid transit, poor colonic absorption and diarrhoea). Secondly it acts as a non-return valve to prevent backflow of the bacterially loaded colonic contents into the relatively sterile small bowel. Failure to regulate backflow is thought to lead to overgrowth of bacteria in the small bowel (SIBO) which is associated with rapid transit and malabsorption as well as clinical symptoms such as pain, bloating and diarrhoea [76]. The main function of the ascending colon is to facilitate the efficient fermentation and degradation of so-far unabsorbed complex sugars and fats. The chyme is thoroughly mixed with resident bacteria, which are present in enormous populations (the ‘microbiome’ [77]). This is accomplished by antegrade and retrograde peristaltic contractions that create flow within the large volume of the ascending colon.

Basic physiological data on colon morphology has to date mainly been obtained from resected cadaver specimens, inter-operative measurements or from radiological assessments of the emptied and distended colon. Nottingham has led the way in the measurement of the undisturbed colon using MRI using

a manual 3D segmentation method. An example of the reconstructed volume of the colon is shown in fig 5.

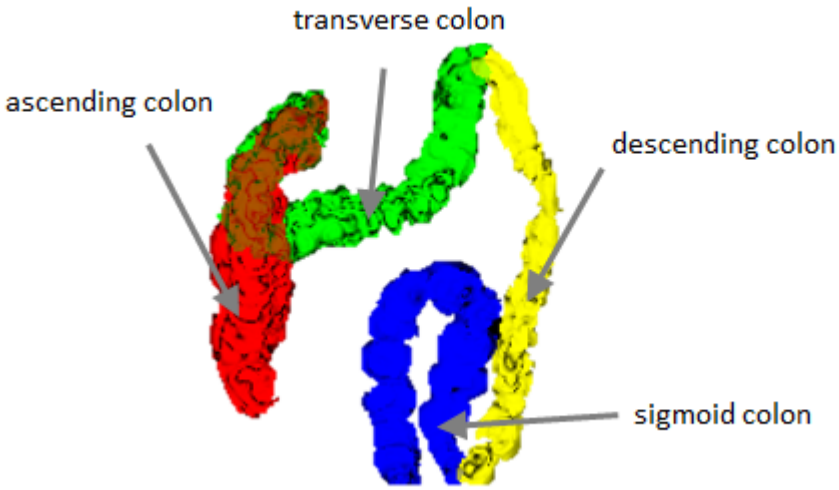

Fig 5: Volumetric reconstruction of segmented colon

(data acquired by author [11])

This has been important in establishing the range of normal fasting values in vivo both in health (75 subjects) and in IBS-D (25 subjects) (paper 1) and coeliac disease and scleroderma [69]. Surprisingly there are no significant differences in these fasted values in healthy subjects compared to those suffering from IBS-D although the post-prandial behaviour of both small bowel water [78-80] and the ascending colon volume (paper 1) has been shown to differ in a manner consistent both with the accelerated transit seen in IBS-D and in the manifest clinical symptoms. These symptoms are often worsened by stress [81] and both psychological and pharmacological treatments of the underlying stress have been reported to be effective in lessening symptoms [82, 83]. Therefore the effect on the small bowel and colon of an ethically acceptable experimental stress (ice cold hand immersion) and a pharmacological stress (corticotrophin releasing hormone-CRH) was evaluated at Nottingham (paper 2) using 36 healthy volunteers. This showed that both

stressors constricted the small bowel and CRH both increased the volume of the ascending colon and reduced the normal post-prandial expansion and was associated with the sensation of distension and bloating. Subsequent to this work (which utilised a laborious manual segmentation of the colon from surrounding viscera) further development has been reported on semi-automated techniques to measure these volumes [84] (although a good degree of manual segmentation is still required). The colon volumes reported in this study (on 4 subjects) were similar to those previously published by the author.

Fermentation within the ascending colon also produces gas, and this can be easily visualised using MRI. Abnormal handling of the gas produced has been implicated in the symptoms [85] such as bloating [86] of both IBS [87] and constipation [88, 89]. Dietary modification to reduce fermentable sugars have been reported to improve symptoms in IBS patients [90] and measurement of colon volumes and gas loading at Nottingham have shown consistent differential responses to FODMAPs (fermentable-oligo-, di-, mono-saccharides and polyols) compared to simple sugars in healthy volunteers [71] with the largest increase in diameters seen in the transverse colon. Recent work by the author and colleagues at Nottingham with patients reporting bloating [11] has suggested that it is the sensitivity of the colon to stretching which differs in patients rather than the amount of gas produced.

3.3.2 Transverse colon

The main function of the transverse colon is the absorption of short chain fatty acids produced by the fermentation in the ascending colon, (although further

fermentation- including proteins-does take place in this region) and the reabsorption of water and electrolytes from the chyme. It is more mobile than the ascending and descending colon and also exhibits a greater range in form and volume, both in the fasted and fed state (paper 1). The gastro colic reflex has been found to have less effect here than in the ascending colon (paper 1) [91]. Propulsive and mixing activity also continues throughout this region and lack of coordinated motility can lead to slow transit constipation. In contrast, excessive or uncoordinated (or retrograde) contractions can lead to the pain associated with IBS [92], with either diarrhoea or constipation.

3.3.3 Descending and Sigmoid Colon

Water and electrolyte absorption continue as the mixture of living and dead bacteria and undigested food residue gradually becomes more viscous and solid faeces form. Approximately 90% of the water is normally reabsorbed within the colon leaving around 100-150ml /day within the faeces. [93]. Faecal material and gas are stored in the mobile, expandable sigmoid colon until preparation for defecation occurs. Certainly large quantities of gas have been observed in this region in our MRI studies [11, 71].

3.4 Colo-rectal

3.4.1 Rectum

Around 1 hour prior to defecation antegrade contractile activity increases throughout the whole colon [94] and faeces are propelled through the sigmoid

colon and into the rectum where distension of the rectal walls produces the desire to defecate and the relaxation of the internal anal sphincter. IBS sufferers have been shown to have greater sensitivity to this distension [95, 96], experiencing both higher pain and urgency levels.

3.4.2 Defecation

Defecation in healthy subjects is normally a voluntary act involving relaxation of the puborectalis muscle and the external anal sphincter, coupled with an increase in inter-abdominal pressure. Voluntary and involuntary contraction of the rectum then expels the faeces. In cases where defecation is obstructed, either by a functional inability to relax the puborectalis or by physical obstruction due to abnormal anatomy, the inability to empty the rectum leads to constipation and delayed gut transit. Previously investigated by contrast barium and x-ray defecography using MRI is becoming increasingly available, with the advent of scanners where the sitting position is possible within the magnetic field (so-called upright scanners). The rectum is filled with a viscous MRI contrast material and dynamic imaging performed as the act of defecation is attempted. The high resolution moving images of the musculature of the rectum and anus, combined with the surrounding anatomy can provide a great deal of useful information regarding mode of action and efficiency of defecation [97, 98].

3.5 Whole gut transit

The motility of the digestive system varies greatly between individuals, with the time taken to transit from mouth to anus ranging from hours to days[99, 100]. This transit time has been found to be significantly altered in irritable bowel syndrome and constipation [79, 101]. Work at Nottingham has established a novel MRI method for measurement of whole gut transit time using MRI marker capsules (and compared this technique with that using radio-opaque markers [102]).This has then been used to evaluate the stimulation of colonic motility using a polyethylene glycol bowel preparation in healthy volunteers (paper 8) and in constipated subjects [74].

4. Gastrointestinal MRI: going forward

The increasing availability of MRI, its acceptability as a non-invasive alternative to CT and the technical advances in spatial and temporal resolution will open up new applications within the digestive tract as increased detail is obtained. Abdominal imaging is moving from 1.5T to 3T both in clinical and research applications and the advent of abdominal imaging at 7T holds the potential of imaging the finer details within the stomach and bowel lumen, as well as more detailed resolution of associated hepatic and pancreatic structures. 7T imaging also holds the potential for more rapid magnetic resonance spectroscopy of gut contents, increasing the understanding of nutrient transport and absorption within these regions.

Improved motion compensation and post-processing will lead to longer image acquisition times- not constrained by the duration of a breath hold- both improving the signal to noise ratio in images and enabling real-time cine MRI capture of peristalsis in small bowel and particularly in the colon where contractual activity is much less frequent. MRI measurement of flow patterns within these structures could also be useful in the assessment of motility disorders and their response to pharmaceutical intervention, and indeed in the mechanics of drug absorption within the gut.

MRI has contributed greatly to the understanding of gut physiology and function since those simple images were obtained 37 years ago. Nottingham is at the forefront of gastro-intestinal MRI and well placed to drive the techniques and expertise further over the decades to come.

5. List of peer reviewed publications from UoN- including author contribution

1. SE Pritchard, L Marciani, KC Garsed, CL Hoad, W Thongborisute, E Roberts, PA Gowland and RC Spiller. Fasting and post-prandial volumes of the undisturbed colon: normal values and changes in diarrhoea-predominant Irritable Bowel Syndrome measured using serial MRI. *Neurogastroenterol Motil* 26, 124-130, 2014.

This paper was the first to measure colon volumes in the unprepared and undisturbed human colon enabling the evaluation of the normal physiological response to feeding.
Author contribution- Assisting MRI Data acquisition (10%), measurement of colon volumes (100%), analysis (60%) and reporting of results (60%).

2. SE Pritchard, KC Garsed, CL Hoad, M Lingaya, R Banwait, W Thongborisute, E Roberts, C Costigan, L Marciani, PA Gowland and RC Spiller. Effect of experimental stress on the small bowel and colon in healthy humans. *Neurogastroenterol Motil* 27,542-549 2015

This paper used the techniques developed in paper 1 to assess the effect of stress on the large bowel
Author contribution- Assisting MRI Data acquisition (20%), measurement of colon volumes (100%), analysis (60%) and reporting of results (60%).

3. S Pritchard, M Glover, G Guthrie, J Brum, D Ramsey, G Kappler, P Thomas, S Stuart, D Hull, and P Gowland. Effectiveness of 0.05% oxymetazoline (Vicks Sinex Micromist (R)) nasal spray in the treatment of objective nasal congestion demonstrated to 12 h post-administration by magnetic resonance imaging. *Pulmonary Pharmacology and Therapeutics* 27, 121-126, 2014.

This paper was the first clinical trial using MRI to demonstrate the effect of a nasal decongestant in shrinking inflamed nasal membranes in subjects suffering from colds.
Author contribution- Protocol design following own pilot study (30%), subject recruitment (100%), data acquisition (80%), measurement of nasal morphometry (100%) and data analysis (80%) and reporting (50%).

4. K Murray, E Placidi, EAH Schuring, CL Hoad, W Koppenol, LN Arnaudov, WAM Blom, SE Pritchard, SD Stoyanov, PA Gowland, RC Spiller, HPF Peters and L Marciani. Aerated drinks increase gastric volume and reduce appetite as assessed by magnetic resonance imaging: a randomized balanced cross-over trial. *Am J Clin Nutr* 101,270-278 2015

This paper applied the gas/ liquid manual segmentation for the measurement of gastric emptying.

Author contribution- measurement of gastric emptying for inter-observer comparisons

5. DM Mudie, K Murray, CL Hoad, SE Pritchard, MC Garnett, GL Amidon, PA Gowland, RC Spiller, GE Amidon and L Marciani. Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Mol Pharmaceut* 11, 3039-3047, 2014.

Author contribution- measurement of gastric emptying using semi-automated method (100%).

6. L Marciani, EF Cox, SE Pritchard, G Major, CL Hoad, M Mellows, MO Hussein, C Costigan, M Fox, PA Gowland and RC Spiller. Additive effects of gastric volumes and macronutrient composition on the sensation of postprandial fullness in humans. *Eur J Clin Nutr* 6, 380-384, 2014

This paper used the manual segmentation method for gastric volumes (liquid and air) to compare the effect of a high fat and a high carbohydrate meal.
Author contribution- Assisted MRI data acquisition (20%), measurement of gastric emptying (100%), data analysis (20%) and reporting (20%).

7. MO Hussein, CL Hoad, J Wright, G Singh, MC Stephenson, EF Cox, E Placidi, SE Pritchard, C Costigan, H Ribeiro, E Ciampi, A Nandi, N Hedges, P Sanderson, HPF Peters, P Rayment, RC Spiller, PA Gowland and L Marciani. Fat emulsion intragastric stability and droplet size modulate gastrointestinal responses and subsequent food intake in young adults *Journal Nutrition* 145, 1170-1177

Author contribution- measurement of small bowel water for inter observer comparison

8. L Marciani, KC Garsed, CL Hoad, A Fields, I Fordham, SE Pritchard, E Placidi, K Murray, G Chaddock, C Costigan, C Lam, J Jalanka-Tuovinen, WM DeVos,PA Gowland and RC Spiller. Stimulation of colonic motility by oral PEG electrolyte bowel preparation assessed by MRI: comparison of split versus single dose. *Neurogastroenterol Motil* 26, 1426-1436, 2014.

This paper used the colon volume measurement technique developed in paper1 to stratify response to a laxative challenge in healthy volunteers.
Author contribution- measurement of colon volumes (100%)

9. L Marciani, SE Pritchard, C Hellier-Woods, C Costigan, CL Hoad, PA Gowland and RC Spiller. Delayed gastric emptying and reduced postprandial small bowel water content of equicaloric whole meal bread versus rice meals in healthy subjects: novel MRI insights. *Eur J Clin Nutr* 67, 754-758, 2013.

Author contribution- measurement of gastric emptying (100%), data analysis (10%) and reporting of results (10%).

10. L Marciani, N Hall, SE Pritchard, EF Cox, JJ Totman, M Lad, CL Hoad, TJ Foster, PA Gowland and RC Spiller. Preventing gastric sieving by blending a solid/water meal enhances satiation in healthy humans. *J Nutr* 142, 1253-1258, 2012.

Author contribution- measurement of small bowel water (100%), data analysis (30%) and reporting (20%)

11. E Placidi, L Marciani, CL Hoad, A Napolitano, KC Garsed, SE Pritchard, EF Cox, C Costigan, RC Spiller and PA Gowland. Effect of loperamide and loperamide plus simethicone on the distribution of gut water as assessed by MRI in a mannitol model of secretory diarrhea. *Aliment Pharmacol Ther* 36, 64-73, 2012.

Author contribution- measurement of gastric emptying and gas content (50%)

12. L Marciani, EF Cox, CL Hoad, S Pritchard, JJ Totman, S Foley, A Mistry, S Evans, PA Gowland and RC Spiller. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 138, 469-477, 2010.

Author contribution- Assisting in MRI data acquisition (20%) and measurement of small bowel water in study 2 (healthy volunteers) (100%)

13. AH Chowdhury, DJ Humes, S Pritchard, L Marciani, P Gowland, J Simpson and DN Lobo. The effects of morphine-neostigmine and secretin provocation on pancreaticobiliary morphology in healthy subjects: A randomised, double blind cross-over study using serial MRCP. *World J Surgery* 35, 2102-2109, 2011.

Author contribution- assisted experimental protocol design, MRI data acquisition (50%) and measurement of gall bladder volume, common bile duct and pancreatic duct diameter and length (100%).

14. ID Driver, EL Hall, SJ Wharton, SE Pritchard, ST Francis and PA Gowland. Calibrated BOLD using direct measurement of changes in venous oxygenation. *Neuroimage* 63: 1178-1187, 2012.

Author contribution- responsible for operation of Respiract ^{TM} system used for inducing stable hyperoxia and hypercapnia.

6. References

1. Mansfield P, Pykett IL, Morris PG, Coupland RE. Human whole body line-scan imaging by NMR. *The British journal of radiology* 1978; **51**: 921-922.
2. Damadian R, Goldsmith M, Minkoff L. Nmr in Cancer .16. Fonar Image of Live Human-Body. *Physiol Chem Phys M* 1977; **9**: 97-&.
3. Buxton RB. The physics of functional magnetic resonance imaging (fMRI). *Reports on Progress in Physics* 2013; **76**.
4. Stewart C Bushong GC. Magnetic Resonance Imaging:Physical and Biological Principles. 2014.
5. Schwizer W, Steingoetter A, Fox M. Magnetic resonance imaging for the assessment of gastrointestinal function. *Scandinavian Journal of Gastroenterology* 2006; **41**: 1245-1260.
6. Mariani G, Boni G, Barreca M, Bellini M, Fattori B, AlSharif A, Grosso M, Stasi C, Costa F, Anselmino M, Marchi S, Rubello D, Strauss HW. Radionuclide gastroesophageal motor studies. *J Nucl Med* 2004; **45**: 1004-1028.
7. Bazelaire CMJd, Duhamel GD, Rofsky NM, Alsop DC. MR Imaging Relaxation Times of Abdominal and Pelvic Tissues Measured in Vivo at 3.0 T: Preliminary Results. *Radiology* 2004; **230**: 652-659.
8. Chen Y, Lee GR, Aandal G, Badve C, Wright KL, Griswold MA, Seiberlich N, Gulani V. Rapid volumetric t1 mapping of the abdomen using three-dimensional through-time spiral GRAPPA. *Magnetic Resonance in Medicine* 2015: n/a-n/a.
9. Evans DF, Stehling MK, Lamont G, Coxon R, Howseman AM, Ordridge RJ, Hardcastle JD, Mansfield P. Investigation of gastrointestinal motility with echo-planar magnetic resonance imaging (EPI). *Gut* 1988; **29**: A1452.
10. Hoad CL, Marciani L, Foley S, Totman JJ, Wright J, Bush D, Cox EF, Campbell E, Spiller RC, Gowland PA. Non-invasive quantification of small bowel water content by MRI: a validation study. *Phys Med Biol* 2007; **52**: 6909-6922.
11. Major G, Pritchard S, Murray K, Hoad C, Marciani L, Gowland P, Spiller R. Mechanisms underlying FODMAP induced symptoms in patients with irritable bowel syndrome: a double-blind crossover trial using MRI. *DDF-15 1409* 2015; **London**.
12. Garcia DM, Duhamel G, Alsop DC. Efficiency of inversion pulses for background suppressed arterial spin labeling. *Magnetic Resonance in Medicine* 2005; **54**: 366-372.
13. Neumann H, Fry LC, Nagel A, Neurath MF. Wireless capsule endoscopy of the small intestine: a review with future directions. *Curr Opin Gastroen* 2014; **30**: 463-471.
14. Evans DF, Lamont G, Stehling MK, Blamire AM, Gibbs P, Coxon R, Hardcastle JD, Mansfield P. Prolonged monitoring of the upper gastrointestinal tract using echo-planar magnetic resonance imaging. *Gut* 1993; **34**: 848-852.
15. Stehling MK, Evans DF, Lamont G, Ordidge RJ, Howseman AM, Chapman B, Coxon R, Mansfield P, Hardcastle JD, Coupland RE. Gastrointestinal tract: dynamic MR studies with echo-planar imaging. *Radiology* 1989; **171**: 41-46.
16. McSweeney SE, O'Donoghue PM, Jhaveri K. Current and emerging techniques in gastrointestinal imaging. *J Postgrad Med* 2010; **56**: 52-59.
17. Maccioni F. Current status of gastrointestinal MRI. *Abdominal Imaging* 2002; **27**: 358-360.

18. Marciani L. Assessment of gastrointestinal motor functions by MRI: a comprehensive review. *Neurogastroenterology and Motility* 2011; **23**: 399-407.
19. Barkhausen J, Goyen M, von Winterfeld F, Lauenstein T, Arweiler-Harbeck D, Debatin JF. Visualization of swallowing using real-time TrueFISP MR fluoroscopy. *Eur Radiol* 2002; **12**: 129-133.
20. Ajaj W, Goyen M, Herrmann B, Massing S, Goehde S, Lauenstein T, Ruehm SG. Measuring tongue volumes and visualizing the chewing and swallowing process using real-time TrueFISP imaging - initial clinical experience in healthy volunteers and patients with acromegaly. *Eur Radiol* 2005; **15**: 913-918.
21. Panebianco V, Habib FI, Tomei E, Paolantonio P, Anzidei M, Laghi A, Catalano C, Passariello R. Initial experience with magnetic resonance fluoroscopy in the evaluation of oesophageal motility disorders. Comparison with manometry and barium fluoroscopy. *Eur Radiol* 2006; **16**: 1926-1933.
22. Kulinna-Cosentini C, Schima W, Cosentini EP. Dynamic MR imaging of the gastroesophageal junction in healthy volunteers during bolus passage. *Journal of Magnetic Resonance Imaging* 2007; **25**: 749-754.
23. Curcic J, Fox M, Kaufman E, Forras-Kaufman Z, Hebbard GS, Roy S, Pal A, Schwizer W, Fried M, Treier R, Boesiger P. Gastroesophageal junction: structure and function as assessed by MR imaging. *Radiology* 2010; **257**: 115-124.
24. Manabe T, Kawamitsu H, Higashino T, Shirasaka D, Aoyama N, Sugimura K. Observation of gastro-esophageal reflux by MRI: a feasibility study. *Abdominal Imaging* 2009; **34**: 419-423.
25. Fox M, Hebbard G, Janiak P, Brasseur JG, Ghosh S, Thumshirn M, Fried M, Schwizer W. High-resolution manometry predicts the success of oesophageal bolus transport and identifies clinically important abnormalities not detected by conventional manometry. *Neurogastroenterology and Motility* 2004; **16**: 533-542.
26. Fox MR, Bredenoord AJ. Oesophageal high-resolution manometry: moving from research into clinical practice. *Gut* 2008; **57**.
27. Boulby P, Gowland P, Adams V, Spiller RC. Use of echo planar imaging to demonstrate the effect of posture on the intragastric distribution and emptying of an oil/water meal. *Neurogastroenterology and Motility* 1997; **9**: 41-47.
28. Schwizer W, Steingotter A, Fox M, Zur T, Thumshirn M, Bosiger P, Fried M. Non-invasive measurement of gastric accommodation in humans. *Gut* 2002; **51**: I59-I62.
29. de Zwart IM, Haans JJL, Verbeek P, Eilers PHC, de Roos A, Masclee AAM. Gastric accommodation and motility are influenced by the barostat device: assessment with magnetic resonance imaging. *American Journal of Physiology* 2007; **292**: G208-G214.
30. Fidler J, Bharucha AE, Camilleri M, Camp J, Burton D, Grimm R, Riederer SJ, Robb RA, Zinsmeister AR. Application of magnetic resonance imaging to measure fasting and postprandial volumes in humans. *Neurogastroenterology and Motility* 2009; **21**: 42-51.
31. Treier R, Steingoetter A, Goetze O, Fox M, Fried M, Schwizer W, Boesiger P. Fast and optimized T1 mapping technique for the noninvasive quantification of gastric secretion. *Journal of Magnetic Resonance Imaging* 2008; **28**: 96-102.
32. Goetze O, Treier R, Fox M, Steingoetter A, Fried M, Boesiger P, Schwizer W. The effect of gastric secretion on gastric physiology and emptying in the fasted and fed state assessed by magnetic resonance imaging. *Neurogastroenterology & Motility* 2009; **21**: 725-e742.

33. Wright J, Evans D, Gowland P, Mansfield P. Validation of antroduodenal motility measurements made by echo- planar magnetic resonance imaging. *Neurogastroenterology and Motility* 1999; **11**: 19-25.
34. de Zwart IM, Mearadji B, Lamb HJ, Eilers PHC, Masclee AAM, de Roos A, Kunz P. Gastric motility: Comparison of assessment with real-time MR imaging or barostat measurement - Initial experience. *Radiology* 2002; **224**: 592-597.
35. Marciani L, Gowland PA, Fillery-Travis A, Manoj P, Wright J, Smith A, Young P, Moore R, Spiller RC. Assessment of antral grinding of a model solid meal with echo- planar imaging. *American Journal of Physiology* 2001; **280**: G844-G849.
36. Marciani L, Manoj P, Wright J, Young P, Moore RJ, Smith A, Fillery-Travis A, Gowland PA, Spiller RC. MRI assessment of the grinding forces in the antrum effects of solid food breakdown strength and meal viscosity on gastric emptying and satiety. *Gastroenterology* 2000; **118**: 844.
37. Fruehauf H, Goetze O, Steingoetter A, Kwiatek M, Boesiger P, Thumshirn M, Schwizer W, Fried M. Intersubject and intrasubject variability of gastric volumes in response to isocaloric liquid meals in functional dyspepsia and health. *Neurogastroenterology and Motility* 2007; **19**: 553-561.
38. Goetze O, Steingoetter A, Menne D, van der Voort IR, Kwiatek MA, Boesiger P, Weishaupt D, Thumshirn M, Fried M, Schwizer W. The effect of macronutrients on gastric volume responses and gastric emptying in humans: a magnetic resonance imaging study. *American Journal of Physiology* 2007; **292**: G11-G17.
39. Fruehauf H, Menne D, Kwiatek MA, Forras-Kaufman Z, Kaufman E, Goetze O, Fried M, Schwizer W, Fox M. Inter-observer reproducibility and analysis of gastric volume measurements and gastric emptying assessed with magnetic resonance imaging. *Neurogastroenterology and Motility* 2011; **23**: 854-861.
40. Kwiatek MA, Steingoetter A, Pal A, Menne D, Brasseur JG, Hebbard GS, Boesiger P, Thumshim M, Fried M, Schwizer W. Quantification of distal antral contractile motility in healthy human stomach with magnetic resonance imaging. *Journal of Magnetic Resonance Imaging* 2006; **24**: 1101-1109.
41. Fruehauf H, Steingoetter A, Fox MR, Kwiatek MA, Boesiger P, Schwizer W, Fried M, Thumshirn M, Goetze O. Characterization of gastric volume responses and liquid emptying in functional dyspepsia and health by MRI or barostat and simultaneous C-13-acetate breath test. *Neurogastroenterology and Motility* 2009; **21**: 697-E637.
42. Marciani L, Young P, Wright J, Moore RJ, Evans DF, Spiller RC, Gowland PA. Echo-planar imaging in GI clinical practice: assessment of gastric emptying and antral motility in four patients. *Journal of Magnetic Resonance Imaging* 2000; **12**: 343-346.
43. Lehmann R, Borovicka J, Kunz P, Crelier G, Boesiger P, Fried M, Schwizer W, Spinas GA. Evaluation of delayed gastric emptying in diabetic patients with autonomic neuropathy by a new magnetic resonance imaging technique and radio-opaque markers. *Diabetes Care* 1996; **19**: 1075-1082.
44. Borovicka J, Lehmann R, Kunz P, Fraser R, Kreiss C, Crelier G, Boesiger P, Spinas GA, Fried M, Schwizer W. Evaluation of gastric emptying and motility in diabetic gastroparesis with magnetic resonance imaging: effects of cisapride. *American Journal of Gastroenterology* 1999; **94**: 2866-2873.
45. Marciani L, Coleman NS, Dunlop SP, Singh G, Marsden CA, Holmes GK, Spiller RC, Gowland PA. Gallbladder contraction, gastric emptying and antral motility: Single visit assessment of upper GI function in untreated

Celiac disease using echo-planar MRI. *Journal Of Magnetic Resonance Imaging* 2005; **22**: 634-638.
46. Parker HL, Hoad CL, Hudders N, Costigan C, Marciani L, Cox E, Gowland PA, Fox MR. Validation of a novel, non-Invasive assessment of gastric function and gastric emptying (GE) after a large liquid nutrient meal by magnetic resonance imaging (MRI). *Gastroenterology* 2012; **142**: S610.
47. Parker HL, Tucker E, Hoad CL, Hudders N, Blackshaw PE, Perkins AC, Marciani L, Costigan C, Gowland PA, Fox MR. Effects of Age, Sex and Obesity on Satiation Assessed by Nutrient Drink Test and Gastric Emptying (GE) Assessed by Non-Invasive Gastric Scintigraphy (GS) and Magnetic Resonance Imaging (MRI): Analysis and Comparison of Methods. *Gastroenterology* 2012; **142**: S293-S293.
48. Tucker E, Parker HL, Hoad CL, Hudders N, Perkins AC, Blackshaw PE, Marciani L, Costigan C, Gowland PA, Fox MR. 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. *Gastroenterology* 2012; **142**: S194-S194.
49. Hoad CL, Rayment P, Spiller RC, Marciani L, Alonso BD, Traynor C, Mela DJ, Peters HPF, Gowland PA. In vivo imaging of intragastric gelation and its effect on satiety in humans. *J Nutr* 2004; **134**: 2293-2300.
50. Ajaj W, Lauenstein T, Papanikolaou N, Holtmann G, Goehde SC, Ruehm SG, Debatin JF. Real-time high-resolution MRI for the assessment of gastric motility: pre- and postpharmacological stimuli. *Journal of Magnetic Resonance Imaging* 2004; **19**: 453-458.
51. Lauenstein TC, Vogt FM, Herborn CU, DeGreiff A, Debatini JF, Holtmann G. Time-resolved three-dimensional MR imaging of gastric emptying modified by IV administration of erythromycin. *American Journal of Roentgenology* 2003; **180**: 1305-1310.
52. Marciani L, Cox EF, Hoad CL, Totman JJ, Costigan C, Singh G, Shepherd V, Chalkley L, Robinson M, Ison R, Gowland PA, Spiller RC. Effects of various food ingredients on gall bladder emptying. *European journal of clinical nutrition* 2013; **67**: 1182-1187.
53. Kim SK. SMALL INTESTINE TRANSIT TIME IN THE NORMAL SMALL BOWEL STUDY. *American Journal of Roentgenology* 1968; **104**: 522-524.
54. Spiller RC, Trotman IF, Adrian TE, Bloom SR, Misiewicz JJ, Silk DBA. Further Characterization of the Ileal Brake Reflex in Man - Effect of Ileal Infusion of Partial Digests of Fat, Protein, and Starch on Jejunal Motility and Release of Neurotensin, Enteroglucagon, and Peptide Yy. *Gut* 1988; **29**: 1042-1051.
55. Spiller RC, Trotman IF, Higgins BE, Ghatei MA, Grimble GK, Lee YC, Bloom SR, Misiewicz JJ, Silk DBA. The ileal brake: inhibition of jejunal motility after ileal fat perfusion in man. *Gut* 1984; **25**: 365-374.
56. Umschaden HW, Szolar D, Gasser J, Umschaden M, Haselbach H. Small-bowel disease: Comparison of MR enteroclysis images with conventional enteroclysis and surgical findings. *Radiology* 2000; **215**: 717-725.
57. Patak MA, Froehlich JM, von Weymarn C, Ritz MA, Zollikofer CL, Wentz KU. Non-invasive distension of the small bowel for magnetic resonance imaging. *Lancet* 2001; **358**: 987-988.
58. Patak MA, Froehlich JM, von Weymorn C, Breitenstein S, Zollikofer CL, Wentz KU. Non-invasive measurement of small-bowel motility by MRI after abdominal surgery. *Gut* 2007; **56**: 1023-1025.
59. Froehlich JM, Daenzer M, von Weymarn C, Erturk SM, Zollikofer CL, Patak MA. Aperistaltic effect of hyoscine N-butylbromide versus glucagon

on the small bowel assessed by magnetic resonance imaging. *Eur Radiol* 2009; **19**: 1387-1393.
60. Gutzeit A, Binkert CA, Koh D-M, Hergan K, von Weymarn C, Graf N, Patak MA, Roos JE, Horstmann M, Kos S, Hungerbuehler S, Froehlich JM. Evaluation of the anti-peristaltic effect of glucagon and hyoscine on the small bowel: comparison of intravenous and intramuscular drug administration. *Eur Radiol* 2012; **22**: 1186-1194.
61. Bickelhaupt S, Froehlich JM, Cattin R, Patuto N, Tutuian R, Wentz KU, Culmann JL, Raible S, Bouquet H, Bill U, Patak MA. Differentiation between active and chronic Crohn's disease using MRI small-bowel motility examinations - Initial experience. *Clinical Radiology* 2013; **68**: 1247-1253.
62. Bickelhaupt S, Froehlich JM, Cattin R, Raible S, Bouquet H, Bill U, Patak MA. Software-Assisted Small Bowel Motility Analysis Using Free-Breathing MRI: Feasibility Study. *Journal of Magnetic Resonance Imaging* 2014; **39**: 17-23.
63. Cullmann JL, Bickelhaupt S, Froehlich JM, Szucs-Farkas Z, Tutuian R, Patuto N, Dawson H, Patak MA. MR imaging in Crohn's disease: correlation of MR motility measurement with histopathology in the terminal ileum. *Neurogastroenterology and Motility* 2013; **25**: 749-E577.
64. Hamy V, Menys A, Helbren E, Odille F, Punwani S, Taylor S, Atkinson D. Respiratory motion correction in dynamic-MRI: application to small bowel motility quantification during free breathin. *Medical image computing and computer-assisted intervention : MICCAI International Conference on Medical Image Computing and Computer-Assisted Intervention* 2013; **16**: 132-140.
65. Menys A, Taylor SA, Emmanuel A, Ahmed A, Plumb AA, Odille F, Alam A, Halligan S, Atkinson D. Global small bowel motility: assessment with dynamic MR imaging. *Radiology* 2013; **269**: 443-450.
66. Menys A, Plumb A, Atkinson D, Taylor SA. The challenge of segmental small bowel motility quantitation using MR enterography. *The British journal of radiology* 2014; **87**: 20140330.
67. van der Paardt MP, Sprengers AMJ, Zijta FM, Lamerichs R, Nederveen AJ, Stoker J. Noninvasive Automated Motion Assessment of Intestinal Motility by Continuously Tagged MR Imaging. *Journal of Magnetic Resonance Imaging* 2014; **39**: 9-16.
68. Marciani L, Totman JJ, Hoad CL, Evans S, Mistry A, Gowland PA, Spiller RC. Bran-induced small bowel secretion: Assessment by novel non-invasive MRI technique. *Gastroenterology* 2006; **130**: A743-A743.
69. Lam C, Sanders D, Lanyon P, Garsed K, Foley S, Pritchard S, Marciani L, Hoad C, Costigan C, Gowland P, Spiller R. Contrasting changes in small bowel water content in patients with diarrhoea: coeliac disease and scleroderma versus IBS and healthy controls. *United European Gastroenterology Journal* 2013; **1**: A108.
70. Major G, Krishnasamy S, Mulvenna C, Pritchard S, Hoad C, Marciani L, Lomer M, Gowland P, Spiller R. A double-blind randomised controlled trial of the effect of dietary fermentable carbohydrates on the colon using MRI:supplementary oligofructose increases colonic volume but so does the low FODMAP diet. *Proc UEG week 2015* 2015; **Barcelona**.
71. Murray K, Wilkinson-Smith V, Hoad C, Costigan C, Cox E, Lam C, Marciani L, Gowland P, Spiller RC. Differential Effects of FODMAPs (Fermentable Oligo-, Di-, Mono-Saccharides and Polyols) on Small and Large Intestinal Contents in Healthy Subjects Shown by MRI. *American Journal of Gastroenterology* 2014; **109**: 110-119.
72. Marciani L, Foley S, Hoad CL, Campbell E, Armstrong A, Manby P, Gowland PA, Spiller RC. Effects of Ondansetron on small bowel water content: a magnetic resonance imaging study. *Gut* 2007; **56**: A333.

73. Marciani L, Wright J, Foley S, Hoad CL, Totman JJ, Bush D, Hartley C, Armstrong A, Manby P, Blackshaw E, Perkins AC, Gowland PA, Spiller RC. Effects of a 5-HT3 antagonist, ondansetron, on fasting and postprandial small bowel water content assessed by magnetic resonance imaging. *Alimentary Pharmacology and Therapeutics* 2010; **32**: 655-663.
74. Chaddock G, Lam C, Garsed K, Hoad C, Costigan C, Pritchard S, Marciani L, Spiller R, Gowland P. MRI colonic stress test for differentiating different subtypes of constipation. *Proceedings ISMRM Milan 2014 p 2115* 2014.
75. Hull UMSTL. *The ASCRS textbook of colon and rectal surgery 2nd Edition*. Second edition edn: Springer, 2011.
76. Cserni T, Paran S, Kanyari Z, O'Donnell AM, Kutasy B, Nemeth N, Puri P. New Insights into the Neuromuscular Anatomy of the Ileocecal Valve. *Anat Rec* 2009; **292**: 254-261.
77. Owyang C, Wu GD. The Gut Microbiome in Health and Disease. *Gastroenterology*; **146**: 1433-1436.
78. Marciani L, Cox EF, Hoad CL, Pritchard S, Totman JJ, Foley S, Mistry A, Evans S, Gowland PA, Spiller RC. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 2010; **138**: 469-477.
79. Marciani L, Foley S, Hoad C, Campbell E, Totman J, Cox E, Gowland P, Spiller R. Accelerated small bowel transit and contracted transverse colon in diarrhoea-predominant irritable bowel syndrome (IBS-D): Novel insights from magnetic resonance imaging (MRI). *Gastroenterology* 2007; **132**: A141-A141.
80. Marciani L, Foley S, Hoad CL, Campbell E, Totman JJ, Cox E, Gowland PA, Spiller RC. Abnormalities of small bowel and colonic water content in diarrohea-predominant irritable bowel syndrome: novel insights from magnetic resonance imaging. *Gut* 2007; **56**: A65-A65.
81. Spiller RC, Humes DJ, Campbell E, Hastings M, Neal KR, Dukes GE, Whorwell PJ. The Patient Health Questionnaire 12 Somatic Symptom scale as a predictor of symptom severity and consulting behaviour in patients with irritable bowel syndrome and symptomatic diverticular disease. *Alimentary Pharmacology and Therapeutics* 2010; **32**: 811-820.
82. Drossman DA, Creed FH, Olden KW, Svedlund J, Toner BB, Whitehead WE. Psychosocial aspects of the functional gastrointestinal disorders. *Gut* 1999; **45**: 25-30.
83. Sykes MA, Blanchard EB, Lackner J, Keefer L, Krasner S. Psychopathology in irritable bowel syndrome: Support for a psychophysiological model. *Journal of behavioral medicine* 2003; **26**: 361-372.
84. Sandberg TH, Nilsson M, Poulsen JL, Gram M, Frokjaer JB, Ostergaard LR, Drewes AM. A novel semi-automatic segmentation method for volumetric assessment of the colon based on magnetic resonance imaging. *Abdom Imaging* 2015.
85. Harder H, Serra J, Azpiroz F, Passos MC, Aguade S, Malagelada JR. Intestinal gas distribution determines abdominal symptoms. *Gut* 2003; **52**: 1708-1713.
86. Salvioli B, Serra J, Azpiroz F, Lorenzo C, Aguade S, Castell J, Malagelada JR. Origin of gas retention and symptoms in patients with bloating. *Gastroenterology* 2005; **128**: 574-579.
87. Serra J, Azpiroz F, Malagelada JR. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut* 2001; **48**: 14-19.
88. Houghton LA. Bloating in constipation: relevance of intraluminal gas handling. *Best Practice & Research in Clinical Gastroenterology* 2011; **25**: 141-150.

89. Serra J, Azpiroz F, Malagelada JR. Mechanisms of intestinal gas retention in humans: impaired propulsion versus obstructed evacuation. *American journal of physiology Gastrointestinal and liver physiology* 2001; **281**: G138-143.
90. Staudacher HM, Whelan K, Irving PM, Lomer MCE. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAPs) versus standard dietary advice in patients with irritable bowel syndrome. *Journal of Human Nutrition and Dietetics* 2011; **24**: 487-495.
91. Bassotti G, Iantorno G, Fiorella S, Bustos-Fernandez L, Bilder CR. Colonic motility in man: Features in normal subjects and in patients with chronic idiopathic constipation. *American Journal of Gastroenterology* 1999; **94**: 1760-1770.
92. Bassotti G, Crowiell MD, Cheskin LJ, Chami TN, Schuster MM, Whitehead WE. Physiological correlates of colonic motility in patients with irritable bowel syndrome. *Zeitschrift fur Gastroenterologie* 1998; **36**: 811-817.
93. Chang E, Leung P. Intestinal Water and Electrolyte Transport. In: Leung PS, ed. *The Gastrointestinal System*: Springer Netherlands, 2014: 107-134.
94. Bampton PA, Dinning PG, Kennedy ML, Lubowski DZ, deCarle D, Cook IJ. Spatial and temporal organization of pressure patterns throughout the unprepared colon during spontaneous defecation. *American Journal of Gastroenterology* 2000; **95**: 1027-1035.
95. Tornblom H, Van Oudenhove L, Tack J, Simren M. Rectal tone response after a high-caloric meal in patients with Irritable Bowel Syndrome (IBS) and healthry controls is influenced by rectal sensitivity. *Neurogastroenterology and Motility* 2012; **24**: 37-38.
96. Wanrooij SV, Wouters MM, Vanbrabant W, Lambaerts K, Boeckxstaens GE. Rectal Sensitivity Measurement: Rectal Mucosal Capsaicin Applications and Rectal Distensions. *Gastroenterology* 2012; **142**: S832-S832.
97. Cappabianca S, Reginelli A, Iacobellis F, Granata V, Urciuoli L, Alabiso ME, Di Grezia G, Marano I, Gatta G, Grassi R. Dynamic MRI defecography vs. entero-colpo-cystodefecography in the evaluation of midline pelvic floor hernias in female pelvic floor disorders. *Int J Colorectal Dis* 2011; **26**: 1191-1196.
98. Carmel M, Khatri G, Bailey A, Zimmern P. Impact of Mri Defecography on Clinical Evaluation and Surgical Management of Pelvic Organ Prolapse. *Neurourol Urodynam* 2015; **34**: S36-S36.
99. Degen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. *Gut* 1996; **39**: 299-305.
100. Madsen JL. Effects of gender, age, and body mass index on gastrointestinal transit times. *Digestive Diseases & Sciences* 1992; **37**: 1548-1553.
101. Serra J, Azpiroz F, Malagelada JR. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut* 2001; **48**: 14-19.
102. Lam C, Chaddock G, Hoad CL, Costigan C, Cox E, Marciani L, Gowland PA, Spiller RC. A new validated MRI method for measuring whole gut transit time. *Gastroentrology* 2013; **144**: S-920.