5-Hydroxytryptamine and Motor-Sensory Dysfunction: Do they Discriminate Functional Subtypes of Constipation?

A thesis submitted to the University of Manchester for the degree of Doctor of Medicine in the Faculty of Medical and Human Sciences 2011

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Abstract

University of Manchester


Recent studies suggest that patients identified by the Rome III criteria for functional constipation (FC) and irritable bowel syndrome with constipation (IBS-C) are not distinct groups. Previous studies have shown that patients with IBS-C exhibit no or limited 5-HT response to meal ingestion, with plasma concentrations remaining similar to those under fasting conditions. The aim of this study was to determine whether patients with FC show a similar 5-HT response to meal ingestion as patients with IBS-C, and to investigate any relationship to gastrointestinal transit and visceral sensitivity.

23 female IBS-C patients, 11 female FC patients and 23 healthy female volunteers (HV) were recruited. Platelet depleted plasma 5-HT concentrations were measured under fasting (2hrs) and fed (4hrs) conditions. Within 2 weeks, oro-caecal (hydrogen breath test) and colonic (radio-opaque markers followed by X-ray) transit, along with rectal sensitivity (barostat) were determined. The main findings of the study are:

1. The FC patients had no 5-HT response to meal ingestion, as previously seen in patients with IBS-C, compared with healthy volunteers.

2. Patients with FC had abdominal and bowel movement associated symptoms as well as delayed colonic transit (whole gut transit), similar to that seen in IBS-C compared with healthy volunteers.

3. The mean pain threshold in patients with FC was similar to that seen in healthy volunteers, with more patients with hyposensitivity or insensitivity in this group compared with IBS-C and no patients with hypersensitivity.

4. Patients with FC had a shift towards higher fasting and postprandial PDP 5-HT levels, unlike patients with IBS-C, compared to healthy volunteers.

This study show that based on symptoms, IBS-C and FC patients have more similarities than differences. However, although patients with FC had a similar 5-HT response to a test meal, they had different fasting 5-HT levels and some different physiological findings on assessment of visceral sensitivity with barostat.
Statement of Originality

I confirm that the project described in this thesis was performed by me except as stated below:

An initial protocol for the study was in existence before my appointment as a Clinical Research Fellow at the University of Manchester. I also received helping hand from my colleagues; Ms Maggie Hastings and Dr Basma Issa in conducting some of the physiological tests in a few subjects. Some of the data from these physiological tests may also be used in doctoral thesis of Dr Isaa as common subjects were jointly recruited in both studies.

Dr Phillip Monaghan and Dr Jo Adaway in the Department of Biochemistry performed the analysis on 5-HT and 5-HIAA, and platelet counts were done by technicians in the central laboratory at the University Hospital of South Manchester.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

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Dedications

I would like to dedicate my thesis work to my mother, whose sacrifices in my early years made it possible to achieve this level of education and to my wife, who had been an extraordinary support. Pooja has almost single-handedly raised our young children, while I was undertaking research in Manchester and then writing this thesis.
Acknowledgements

It is an honour to have worked with Prof Whorwell, my advisor and Prof Houghton, my supervisor. At the Neurogastroenterology Unit, I was given every opportunity and constant nurturing to learn and grow. Every interaction with Prof Whorwell was an opportunity to learn more from his vast clinical and life experience and gave completely new meaning to do research at this unit. His commitment to his patients was an inspiration and remains so. Prof Houghton had an eye for extraordinary detail, which always kept me on my toes and her constant encouragement, filled me with a desire to perform better. This thesis would not have been possible without her continual support, even when she moved to the Mayo Clinic, USA. I am also indebted to Prof Hamdy for agreeing to be my supervisor half way through my programme.

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A big thank you to Mrs. Julie Morris, who was always available to advise me regarding statistics and help with the SPSS package. I would also like to say thank you to Dr Helen Carruthers and Richard Coaton for preparing the illustrations for posters and presentations.

I am indebted to Pooja, my wife and our young kids; Naarain and Raaghav, for their ‘understanding’ and support as the majority of this work has come at the expense of their personal time.

Finally, yet importantly, I would like to thank the volunteers for this study, who agreed to undergo some cumbersome physiological assessments, without them, this research project and thesis would not have been possible.
Author

The author had his early medical training in India, before moving to the UK. He started his training in the NHS in 2003 and after completing his MRCP in 2004; he did his SpR training in Endocrinology and Diabetes for a couple of years, before changing to Gastroenterology. He has been in a specialist training programme in Gastroenterology in the West Midlands Deanery since 2008, where he is currently pursuing his training. He came to the University of Manchester to do research in functional gastrointestinal disorders in May 2008 until September 2010.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine</td>
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<tr>
<td>5-HIAA</td>
<td>5-Hydroxyindoleacetic acid</td>
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<td>5-HTOL</td>
<td>5-Hydroxtryptophol</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>Serotonin transporter linked polymorphic regions</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate gyrus</td>
</tr>
<tr>
<td>Acetyl-coA</td>
<td>Acetyl-coenzyme A</td>
</tr>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BOP</td>
<td>Basal operating pressure</td>
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<tr>
<td>BSS</td>
<td>Bristol stool scale</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CRF/CRH</td>
<td>Corticotrophin releasing factor</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter protein</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DZ</td>
<td>Dizygotic twins</td>
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<td>Abbr.</td>
<td>Term</td>
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</tr>
<tr>
<td>EC</td>
<td>Enterochromaffin</td>
</tr>
<tr>
<td>ENS</td>
<td>Enteric nervous system</td>
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<tr>
<td>EP</td>
<td>Evoked potential</td>
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<tr>
<td>EPS</td>
<td>Epigastric pain syndrome</td>
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<tr>
<td>EPSP</td>
<td>Excitatory post-synaptic potential</td>
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<tr>
<td>FC</td>
<td>Functional constipation</td>
</tr>
<tr>
<td>FD</td>
<td>Functional dyspepsia</td>
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<tr>
<td>FGIDs</td>
<td>Functional gastrointestinal disorders</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>HAD</td>
<td>Hospital anxiety and depression</td>
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<tr>
<td>HAPC</td>
<td>High amplitude propagated complexes</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IBS-C</td>
<td>Irritable bowel syndrome with constipation</td>
</tr>
<tr>
<td>IBS-D</td>
<td>Irritable bowel syndrome with diarrhoea</td>
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<tr>
<td>IPAN</td>
<td>Intrinsic primary neurones</td>
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<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
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<tr>
<td>MOA</td>
<td>Monoamine oxidase</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>MZ</td>
<td>Monozygotic twins</td>
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<tr>
<td>NTC</td>
<td>Normal transit constipation</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>OCT</td>
<td>Organic cation transporter</td>
</tr>
<tr>
<td>PDP</td>
<td>Platelet depleted plasma</td>
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<tr>
<td>PDS</td>
<td>Postprandial distress syndrome</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>SERT</td>
<td>Serotonin transporter protein</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>STC</td>
<td>Slow transit constipation</td>
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<tr>
<td>TPH</td>
<td>Tryptophan hydroxylase</td>
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<td>TRPV 1</td>
<td>Transient receptor potential vanilloid type 1</td>
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Chapter 1

Literature Review
1.1. 5-Hydroxytryptamine (5-HT)

1.1.1 History

The first mention of a substance resembling 5-hydroxytryptamine (5-HT, Serotonin) in medical literature can be found as early as 1884, when Stevens and Lee reported a vasoconstrictor matter in defibrinated blood, or blood serum, but not in whole blood (Pineyro and Blier 1999). This was later confirmed by Brodie in 1900 (Brodie 1900; Pineyro and Blier 1999).

In 1937, an Italian researcher, Vittorio Erspamer was studying enterochromaffin cells in molluscs and vertebrates for his PhD, when he stumbled upon a new cytoplasmic substance on histochemical staining of the enterochromaffin cells in the gastrointestinal mucosa. Based on its location and a probable ‘amine’ histochemical property, he suggested this cytoplasmic content be called ‘enteramind’ (entramine) (Renda 2000; Negri 2006). At about the same time Irvine Page, during his research into malignant hypertension became aware of the presence of an unknown substance, released on the breakdown of platelets (Green 2006). However, it was his student Maurice Rapport in 1949, who first independently isolated and described its chemical structure and named it ‘serotonin’, based on its presence in serum and its vasoconstrictor properties (Green 2006). It was later discovered that the ‘serotonin’ in platelets was the same substance as the ‘enteramine’, earlier identified by Erspamer. The role of 5-HT as a neurotransmitter was first proposed in 1957 after its discovery in the mammalian brain (Brodie and Shore 1957). Since then, 5-HT has been extensively researched for its pivotal role in brain functions and gastrointestinal physiology.

1.1.2 Location

80% of the 5-HT in the human body is present in the gastrointestinal tract (GI) tract; the rest of it is equally distributed in the brain and circulation (Spiller 2001). In the GI tract, 95% of 5-HT is stored in the secretory granules of the enterochromaffin (EC) cells, with small amounts in the cytoplasm (Nilsson, Dahlstrom et al. 1987) and 5% in the serotonergic
neurones of the myenteric plexus (Gershon and Tack 2007). The 5-HT synthesised in the GI mucosa spills over into the systemic circulation. It does not however, cross the blood brain barrier, hence its neurotransmitter functions in the brain remain separate from its effect on the GI tract.

In the blood, platelets uptake and store the major proportion of circulating 5-HT spilled over from the GI tract. They do not however, synthesise or metabolise 5-HT and release it in the process of thrombus formation (Hervig, Volundardottir et al. 1990). Serotonin producing cells can also be found in carcinoid tumours.

The distribution of EC cells in the GI tract varies significantly, for example the numbers, in the duodenum and rectum being much higher than in the stomach (Spiller 2001; van Lelyveld, Ter Linde et al. 2007). The EC cells in GI mucosa are mainly located at the base of the crypts with the secretory granules inside the EC cells being oriented closer towards the basement membrane (Spiller 2001), leading to release of 5-HT into the lamina propria on appropriate stimuli. In the GI tract, 5-HT is also present in the serotonergic neurones of the sub mucosa and myenteric plexus (Furness and Costa 1982), where it functions as a neurotransmitter. It can also be found in both animal and human mast cells, where it has been shown to act as a mediator in anaphylactic reaction in a murine model. In man, there is also evidence to suggest that human mast cells are not only able to store 5-HT, but may also have the capacity to synthesise it (Kushnir-Sukhov, Brown et al. 2007) and may play a part in mast cell mediated allergic reaction.

1.1.3 Synthesis

5-hydroxytryptamine is synthesised exclusively from tryptophan. Tryptophan is an essential amino acid, which can only be derived from dietary sources and is a precursor for the majority of proteins in the body, with only about 1% of total dietary tryptophan being involved in the synthesis of 5-HT (Bender 1983). Common foods rich in tryptophan are eggs, milk, meat, bananas, fish, and walnuts etc. There are studies to suggest that with
modification of diet, it is possible to be able to change body (brain) serotonin levels for clinical benefits (Bruce, Steiger et al. 2009). Besides serotonin and melatonin synthesis, tryptophan is also metabolised through the kynurenine pathway, leading to the synthesis of nicotinic acid and Acetyl-coenzyme A (Acetyl-coA) [Figure 1.1].

Tryptophan undergoes hydroxylation with the help of the enzyme tryptophan hydroxylase (tryptophan 5-monoxygenase) (TPH) followed by decarboxylation to produce 5-HT, with TPH being the rate-limiting step in 5-HT synthesis (Gershon and Tack 2007). In the human body, TPH is present in only selected cells capable of synthesising 5-HT, such as EC cells, pinealocytes, mast cells and serotonergic neurones in brain and enteric nervous system. Until recently, it was believed that TPH had only one isoform, but in 2003, another isoform of TPH was reported, which was found exclusively in the brain (Walther, Peter et al. 2003). TPH-1, the originally known TPH, is present in the peripheral organs as well as being expressed in the brain, whereas TPH-2, the more recently identified entity, is present primarily in the brain and to a very small extent in the serotonergic neurones in the myenteric plexus of the GI tract (Neal, Parry et al. 2009). In an experiment, TPH-1 homozygous null mouse had 94% reduction in blood 5-HT levels and 99% reduction in GI 5-HT levels with no significant effect on brain 5-HT levels (Liu, Yang et al. 2008). This study also demonstrated that although TPH-2 is present in the enteric nervous system (ENS), but it only contributes 1% of the total 5-HT in the GI tract. With the exception of the area postrema in the medulla, the synthesis of 5-HT in the tissue is site specific, with 5-HT synthesised in peripheral organs not crossing the blood brain barrier, (Roth, Walton et al. 1970). Besides changes in the dietary intake of tryptophan, any change in the rate of metabolism of tryptophan in conditions such as liver failure, increases the amount of 5-HT synthesis (Herneth, Steindl et al. 1998). Once synthesised, 5-HT is stored in the cell cytoplasm in membrane bound small vesicles, ready to be released on appropriate stimuli.
Figure 1.1 Tryptophan metabolic pathways. TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; MAO, Monoamine oxidase; 3-HAO, 3-hydroxyanthranilic acid oxidase; ACMSD, aminocarboxymuconate-semialdehyde decarboxylase; QPRT, quinolinic acid phosphoribosyl transferase; TPH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; ALDH, aldehyde dehydrogenase; ALDR, aldehyde reductase (Keszthelyi, Troost et al. 2009).
1.1.4 Release

5-HT is stored in secretory granules, mainly towards the basement membrane of the mucosal EC cells, whose apices extend into the lumen of the GI tract, exposing them to its contents (Spiller 2001). The presence of food in the lumen of the upper GI tract stimulates the release of 5-HT along with other gut hormones, preparing it for the digestive process (Richter, Stockmann et al. 1986). In vitro studies suggest that physical stimuli, such as stroking the jejunal mucosa (Kellum, Albuquerque et al. 1999), enterotoxins such as cholera toxin or Escherichia coli toxin, as well as cytotoxic drugs, such as cisplatin, can stimulate 5-HT secretion. The receptor stimulation is mediated via β-adrenergic, purinergic A2A/B and muscarinic receptors; α-2 adrenergic, whilst histamine type 3 and purinergic receptors A1 receptors have inhibitory effect on 5-HT secretion (Spiller 2002). 5-HT modulates its own release through autoreceptors on the EC cells. The stimulation of 5-HT3 receptors augments 5-HT release, whereas 5-HT4 receptor activation inhibits release (Spiller 2001).

5-HT release takes place by calcium dependent exocytosis (Schwarzer, Racke et al. 1987; Ruddick, Evans et al. 2006). The influx of calcium is initiated by L-type calcium channel activation (Spiller 2001) or through a second messenger, such as cGMP / cAMP. This initiates fusion of the 5-HT storage vesicle to the cell membrane and release of 5-HT into the surrounding intercellular space (Spiller 2001; Ruddick, Evans et al. 2006).

1.1.5 Metabolism

The large quantities of 5-HT released into the surrounding tissue from the EC cells acts as a paracrine messenger, stimulating the nearby nerve endings and receptors (Wade and Westfall 1985) [See Figure 1.2]. However, such large amounts also pose certain physiological problems, and animal studies in mice suggest that excessive 5-HT release can cause anaphylactic shock (Gershon and Ross 1962). Moreover, the continuous presence of high concentrations of 5-HT can also cause over stimulation and desensitisation of receptors (Chen, Pan et al. 1998). Monoamine oxidase (MOA) and other enzymes required for the
metabolism of 5-HT are intracellular, however, at physiological pH, 5-HT is polarised and thus cannot diffuse into the cell and transport has to be by active process.

The serotonin transporter protein (SERT) actively transports 5-HT across the plasma membrane into the enterocytes, where it is metabolised (Wade, Chen et al. 1996; Chen, Pan et al. 1998). This terminates the action of 5-HT on the receptors, preventing over stimulation or desensitisation.

![Figure 1.2](image)

Figure 1.2 Electron micrograph of an EC cell from the mouse ileum. Note the clustering of dark serotonin-containing storage granules in the basolateral cytoplasm. Serotonin is released (5-HT, arrow) into the loose areolar connective tissue of the lamina propria where it can gain access to nerve fibers (nerve). After it has acted on 5-HT$_3$ (extrinsic nerves; myenteric IPANs) or 5-HT$_{1P}$ (submucosal IPANs) receptors expressed by these nerves, serotonin is inactivated by SERT-mediated uptake (dotted arrow) into enterocytes where it is catabolised. (Gershon and Tack 2007).
The large quantities of secreted 5-HT results in some diffusing into the GI lumen and the portal circulation (Gershon 2004). Hepatocytes then take up 5-HT for metabolism along with platelets with help of the SERT for storage in granules. A small amount entering the systemic circulation is metabolised in kidneys and lung epithelial cells (Read and Gwee 1994). Free 5-HT in the blood, represents only a very small amount of the total 5-HT in circulation with the majority stored in the platelets. Any 5-HT secreted by the serotonergic neurones at the synapses in the ENS is recaptured by the neurones with help of SERT (Wade, Chen et al. 1996; Ruddick, Evans et al. 2006).

Any alteration in SERT activity could have a potential significant effect on 5-HT levels within the mucosa, plasma, and at the serotonergic neurones (Chen, Pan et al. 1998). However, studies in SERT knockout mice would suggest the presence of alternative transport mechanisms for 5-HT. A dopamine transporter (DAT) is present within the central nervous system (CNS) and the ENS, which is a low affinity but high capacity back up transporter for serotonin (Chen, Li et al. 2001). Non-specific organic cation transporter-1 (OCT-1) is present in the ENS and enterocytes, where as OCT-2 is present in enterocytes alone (Chen, Li et al. 2001). These alternative transport mechanisms have a much lower affinity for 5-HT, but appear to be able to compensate for this by up regulation as suggested by increased OCT1 in the brain and intestines of SERT knockout mice (Chen, Li et al. 2001).

Once inside the enterocytes, 5-HT is metabolised by MAO to 5-hydroxyindoleacetaldehyde and then oxidised by the aldehyde dehydrogenase enzyme to 5-hydroxyindolacetic acid (5-HIAA) (Some and Helander 2002). 5-hydroxyindoleacetaldehyde can also be converted to 5-hydroxytryptophol (5-HTOL) by the enzyme aldehyde reductase but this constitutes only 1% of total 5-HT metabolism. Factors such as consumption of alcohol however, can change this equilibrium. After alcohol consumption, more 5-HT is metabolised through the 5-HTOL pathway as the aldehyde dehydrogenase enzyme is engaged in metabolising alcohol (Helander, Beck et al. 1995). The 5-HIAA and 5-HTOL produced in the enterocytes is secreted back into the circulation and eventually excreted in the urine. Other minor pathways for 5-HT metabolism includes intracellular conjugation with
sulphuric or glucuronic acid. In the pineal gland 5-HT undergoes a different metabolic pathway and is converted to N-acetylserotonin and then to melatonin (Ruddick, Evans et al. 2006).

1.1.6 Site of Action

5-HT has a wide range of physiological activities, even far from its main site of origin, the GI tract. In the GI tract not only does it play a role as a neurotransmitter with the ENS but also acts as a neurotransmitter integrating the ENS with CNS through descending tracts (Gershon 2005). It also acts as a mucosal signalling molecule, released from EC cells which acts as a signal transducer in response to luminal changes in pressure and nutrient composition modulating both peristalsis (Grider, Kuemmerle et al. 1996) and secretions (Holstein and Cederberg 1984). In the circulation, the majority of 5-HT is stored in the platelets and is released in the process of thrombosis, causing vasoconstriction and thus playing a role in maintaining the haemostasis (Hervig, Volundardottir et al. 1990). In the pineal gland, it is the substrate for melatonin synthesis, which functions as a neurohormone in the brain and plays a role in maintaining the circadian rhythm, sleep pattern, sexual maturation, reproductive behaviour, and thermoregulation (Ruddick, Evans et al. 2006). 5-HT present in the mast cells acts as a mediator in the anaphylactic reaction in mice and in man it may play a part in the mast cell mediated allergic reaction (Kushnir-Sukhov, Brown et al. 2007).

The actions of 5-HT are mediated through several 5-HT receptors, located widely on EC cells, enterocytes, smooth muscles, immune cells, ENS, and the visceral connections of the autonomic nervous system and central nervous system (Gershon 2004). All 5-HT receptors mediate the action through the G protein coupled second messenger system, except the 5-HT₃ receptor, which is a ligand-gated Na⁺, K⁺, and Ca²⁺ ion channel (Barnes and Sharp 1999; Hannon and Hoyer 2008). [Figure 1.3]
1.1.7 5-HT Receptors Classification

5-HT receptors have been studied for a long time but their classification generally remained ad hoc until interest was laid by the sub-classification of the adrenoceptors to α- and β-types in 1948 (Hoyer, Clarke et al. 1994). The most important driver for classifying receptors was to understand their functions better and develop specific agonist and antagonist drugs. The first evidence to suggest the existence of different sub-types of 5-HT receptors was provided by the work of two British scientists, who suggested the presence of two types of ‘tryptamine receptors’; one which was inhibited by low concentrations of 5-HT receptors have been studied for a long time but their classification generally remained ad hoc until interest was laid by the sub-classification of the adrenoceptors to α- and β-types in 1948 (Hoyer, Clarke et al. 1994). The most important driver for classifying receptors was to understand their functions better and develop specific agonist and antagonist drugs. The first evidence to suggest the existence of different sub-types of 5-HT receptors was provided by the work of two British scientists, who suggested the presence of two types of ‘tryptamine receptors’; one which was inhibited by low concentrations of lysergic acid (Hoyer, Hannon et al. 2002).
diethylamide (LSD) and one which was not (Gaddum and Hameed 1954). Based on this work the first formal classification was proposed in 1957, sub-classifying into 'M' receptors which could be blocked by morphine, and 'D' receptors which could be blocked with dibenzyline (phenoxybenzamine) (Gaddum and Picarelli 1957). The current classification categorises 5-HT receptors into seven main types, named as 5HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆ (HT₆), 5-HT₇, some of which have been further sub-typed (Hoyer, Clarke et al. 1994; Barnes and Sharp 1999; Hannon and Hoyer 2008) [See Figure 1.4]

![5-HT Receptors Diagram]

**Figure 1.4 Schematic representations of 5-HT receptor sub-types**

The receptors, which have been cloned but not fully characterised functionally and pharmacologically as yet are denoted in lower case (Green 2006). However, with continuous research and new information available about 5-HT₅ and 5-HT₆ receptors (Nelson 2004; Woolley, Marsden et al. 2004), there are already arguments to update the classification, at
least for the status of the 5-HT$_5$ receptor (Woolley, Marsden et al. 2004). The receptors belonging to 5HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, and 5-HT$_7$ groups have identified GI functions (De Ponti 2004) with all receptors shown to exist in the ENS but with varied representation in other components of the GI tract (Sanger 2008).

1.1.7.1 5-HT$_1$ Receptors:

5-HT$_1$ and 5-HT$_2$ receptors were first localised in the rat brain cortex using radio ligand binding studies with $[^3]$H]LSD and $[^3]$H]Spiroperidol (Peroutka and Snyder 1979). All sub types of 5-HT$_1$ receptors are located in the human brain with the exception of 5-HT$_{1p}$. The receptor 5-HT$_{1A}$ is found in the ENS and enterocytes, whereas 5-HT$_{1B}$ and 5-HT$_{1D}$ are found in the ENS, smooth muscles and extrinsic nerves of the GI tract (Sanger 2008). Previous studies using sumatriptan, a 5-HT$_{1B}$ and 5-HT$_{1D}$ agonist, have demonstrated an effect on gastric motility and sensation (Houghton, Fowler et al. 1992), which perhaps reduce nausea and vomiting symptoms in patients with migraine through its action on 5-HT receptors in the CNS.

5-HT$_{1p}$ is an 'orphan receptor' and has not yet been cloned. It was designated as '1p' before the formal classification of 5-HT receptors, based on its high affinity for 5-HT (i) and its peripheral location (p) and has not yet been renamed (Gershon 1999). It is believed that 5-HT$_{1p}$ receptors are present on the mucosal processes of the sensory/intrinsic primary afferent neurones (IPAN). 5-HT released from the EC cells activates 5-HT$_{1p}$ receptors on the submucosal sensory neurones, leading to slow excitatory post synaptic potential (EPSP) and modulation of peristaltic and secretory responses (Gershon 2005) [see Figure 1.5).
Figure 1.5 5-HT secreted by EC cells activates intrinsic primary afferent neurones (IPANs) of the submucosal plexus. These cells, which are analogous in function to sensory neurones of dorsal root and cranial nerve ganglia, initiate peristaltic and secretory reflexes. The receptor activity that mediates stimulation of these cells by 5-HT is called 5-HT1P. (Gershon 2005).

1.1.7.2 5-HT2 Receptors

Three subtypes, 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ have been identified so far (Hoyer, Clarke et al. 1994). 5-HT$_{2A}$ is present on the intestinal smooth muscle cells and stimulation of these receptors with 5-HT induces contraction (Kuemmerle, Murthy et al. 1995). An in vitro study has suggested that 5-HT$_{2B}$ agonists lead to the neuronally mediated contractile response in the human colon (Borman, Tilford et al. 2002). Further evidence for this comes from studies using a 5-HT$_{2B}$ receptor antagonist in an animal model, which demonstrated that it reduces pain behaviour induced by acute colitis and maternal separation, suggesting its role in large
bowel sensitivity (Bulmer, O'Mahony et al. 2008). These animal studies indicate, 5-HT$_2$ receptors have a role in the large bowel physiology (Sanger, Bassil et al. 2008).

1.1.7.3 5-HT$_3$ Receptors

Unlike all other 5-HT receptors, 5-HT$_3$ are ligand-gated ion channels consisting of five subunits of an as yet unknown, composition (Niesler, Kapeller et al. 2008); namely 5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT$_{3C}$, 5-HT$_{3D}$, and 5-HT$_{3E}$. The 5-HT$_{3A}$, 5-HT$_{3B}$, and 5-HT$_{3C}$ are present in the CNS and periphery, whereas 5-HT$_{3D}$ is present in the kidney, liver and the GI tract, 5-HT$_{3E}$ is only present in the colon, and intestine (Niesler, Frank et al. 2003). 5-HT$_3$ receptors activate extrinsic sensory nerves, playing a role in vagal signalling to the CNS (Blackshaw and Grundy 1993), and it also activates mucosal afferents of the myenteric IPANs, through which it increases intestinal motility by initiation of giant migrating contraction through serotonergic transmission in the ENS (Gershon 2004).

1.1.7.4 5-HT$_4$ Receptors

Presence of 5-HT$_4$ receptors was first described in the CNS (Dumuis, Sebben et al. 1988) and later reported in guinea pig ileum (Craig, Eglen et al. 1990). The 5-HT$_4$ receptors act via the G$_s$ protein second messenger system coupled with cAMP. The excitatory or inhibitory response to the stimulation of 5-HT$_4$ receptors depends on its anatomical location and the species being studied. In rats, 5-HT$_4$ receptor stimulation in the oesophagus and ileum leads to relaxation (Reeves, Bunce et al. 1991; McLean, Coupar et al. 1995). In the guinea pig stomach, ileum and colon, 5-HT$_4$ receptor stimulation leads to contraction of smooth muscles (Taniyama, Makimoto et al. 2000). In the human GI tract, 5-HT$_4$ receptors are present in the ENS, EC cells, enterocytes, smooth muscles and the interstitial cells of Cajal (Sanger 2008). 5-HT released in the GI mucosa leads to stimulation of IPAN through 5-HT$_{1P}$ and this response is amplified by 5-HT$_4$ receptors (Gershon 1999). 5-HT$_4$ receptors are present on pre-synaptic cholinergic neurones and on smooth muscles cells. The stimulation
of receptors at the former site leads to an excitatory response, which leads to hyper motility and stimulation of the latter leads to relaxation of smooth muscle cells (Taniyama, Makimoto et al. 2000).

5-HT₄ receptors have also been shown to be present in the urinary bladder, adrenal glands and atrial myocytes of the pig and humans (Hegde and Eglen 1996). However, the 5-HT₄ receptors present in the myocardium are a different isoform from the GI and brain receptors and possibly play a role in atrial arrhythmias (Lezoualc'h, Steplewski et al. 2007).

1.1.7.5 5-HT₅ Receptors

The 5-HT₅ receptor family has two subtypes, 5-HT₅A and 5-HT₅B. 5-HT₅ receptors have been identified in the mouse, rat and humans (Nelson 2004). In humans, they are localised in the cerebral cortex, hippocampus and the cerebellum (Plassat, Boschert et al. 1992). Although, these 5-HT₅ receptors have been cloned and localised in the human tissue, and they have pharmacological characteristics similar to those of 5-HT₁ receptors, their exact functions in humans are still not known (Hoyer, Clarke et al. 1994).

1.1.7.6 5-HT₆ (HT₆) Receptors

5-HT₆ receptors were first cloned in 1993 by three separate groups (Plassat, Boschert et al. 1992; Monsma, Shen et al. 1993; Ruat, Traiffort et al. 1993) and since then have been localised in the brain and in the rat stomach (Ruat, Traiffort et al. 1993). The role in the human GI tract is still unknown, however, recently a lot of interest has been generated in the brain 5-HT₆ receptor pharmacology with its clinical implications for cognitive and memory functions (Mitchell and Neumaier 2005).
1.1.7.7 5-HT₇ Receptors

Three isoforms, 5-HT₇ₐ, 5-HT₇ᵇ and 5-HT₇ᶜ have been identified in the rat brain and peripheral tissues, which are coupled to Gₛ protein with the ability to stimulate cAMP production (Heidmann, Szot et al. 1998). Simultaneously, another study reported that humans did not have the 5-HT₇ᶜ isoform, instead another distinct isoform existed, which was identified as 5-HT₇ᵈ, which is absent in rats (Heidmann, Metcalf et al. 1997). In the human colon, 5-HT₇ mediates the relaxation of circular muscles, similar to 5-HT₄ receptors (Prins, Briejer et al. 1999). In vitro studies have also suggested a role for inhibiting 5-HT stimulated 5-HT peristalsis in guinea pig ileum (Tuladhar, Ge et al. 2003). This receptor subtype is also present in rat primary afferent nociceptor like neurones, terminating on the dorsal horn and may have a role in nociceptor activation by 5-HT (Meuser, Pietruck et al. 2002). Recently it has been suggested that 5-HT₇ receptors are expressed on enterocytes along with 5-HT₁ₐ and have a role in modulating SERT activity (Iceta, Mesonero et al. 2009).

1.1.8 Physiological Role of 5-HT in the GI tract

1.1.8.1 Secretion

The effect of 5-HT on intestinal secretion is mediated and regulated through several 5-HT receptor sub-types, which are present on enterocytes as well as in the ENS. In experimental studies on the human ileum and jejunal mucosa, evidence suggests that the 5-HT₄ receptors increase secretion, which is not blocked by 5-HT₃ receptors, antagonists or neuronal signal blockers such as 5-HT₁ antagonists or tetradoxin (Burleigh and Borman 1993; Budhoo, Harris et al. 1996). In contrast the secretory response to 5-HT in the human colonic mucosa is mediated through both the 5-HT₄ and 5-HT₂ₐ receptors (Borman and Burleigh 1996).

In studies using full thickness rat colon, the 5-HT₃ antagonist blocks the majority of the secretory response, which suggests a neuronally mediated response is more important in the presence of an intact myenteric plexus (Budhoo, Harris et al. 1996). However, in the same experiment, the secretory response was partially blocked by 5-HT₄ receptor antagonists,
emphasising the complex interplay of the different receptors in modulating intestinal secretions (Budhoo, Harris et al. 1996). Cholera toxin induces 5-HT release from the EC cells and induces a protective secretory response, which is perhaps mediated via 5-HT$_3$ receptors as this can be blocked with granisetron (Mourad, O'Donnell et al. 1995).

1.1.8.2 Motility and Gut Transit

The effect of 5-HT on intestinal motility is multifarious, mediated through 5-HT receptors, which are present on the nerve endings, interneurones and muscle cells. The 5-HT$_3$ receptors are present on the nerve endings of motor neurones, whereas 5-HT$_4$ receptors are present, both on motor neurones and on interneurones with their effect being mediated via acetylcholine (Ach) and substance P (Briejer and Schuurkes 1996). 5-HT$_{2B}$ receptors mediate contraction in human ileal smooth muscles (Borman and Burleigh 1995), whereas 5-HT$_{2A}$ and 5-HT$_4$ receptors mediate, jejunal smooth muscle contraction and relaxation, respectively (Kuemmerle, Murthy et al. 1995). In colonic circular muscles, relaxation is mediated via 5-HT$_4$ (Tam, Hillier et al. 1995) and 5-HT$_7$ receptors (Prins, Briejer et al. 1999). However, the physiological significance of these in vitro studies, examining the direct effects of 5-HT on smooth muscle cells, is unknown, as little or no 5-HT comes in direct contact with smooth muscle cells and effects on smooth muscles are mediated via serotonergic neurones in the myenteric plexus (Spiller 2001).

The effect on peristalsis and the whole gut transit time is even more intricate. 5-HT affects both secretion and motility, which in turn has an effect on distal propulsion of gut contents (Spiller 2001). One in vitro study demonstrated the initiation of the peristaltic reflex by mucosal application of the 5-HT$_4$ receptor agonist HTF 919, in human and guinea pig intestine (Grider, Foxx-Orenstein et al. 1998). Intra aortic infusion of 5-HT accelerates ileocolonic junction transit in rats (Oosterbosch, von der Ohe et al. 1993). Patients with carcinoid syndrome have excessive 5-HT secretion, leading to increased secretions and motility/transit and hence diarrhoea (von der Ohe, Camilleri et al. 1993; Gershon and Tack
Intravenous administration of Citalopram, a selective serotonin reuptake inhibitor (SSRI) in humans, increases colonic phasic contractions and the frequency of high amplitude propagated complexes (HAPC), perhaps by increasing mucosal serotonin content by inhibiting reuptake (Tack, Broekaert et al. 2006). Stimulation of IPANs by 5-HT is mediated via 5-HT₁P receptors and this is enhanced further by 5-HT₄ receptors, which are present on the presynaptic neurones [See Figure 1.6]. IPANs have calcitonin gene related peptide (CGRP) neurones, which cause ascending contraction via excitatory neurotransmitters and relaxation inhibitions via inhibitory neurotransmitters, thus triggering the peristaltic reflex (Kim 2009). A highly selective 5-HT₄ receptor agonist prucalopride, reduces colonic transit time in healthy volunteers (Poen, Felt-Bersma et al. 1999).

Figure 1.6 5-HT₄ receptors are presynaptic and are found at the terminals of submucosal IPANs, at synapses within the myenteric plexus, and at the neuromuscular junction. 5-HT₄ stimulation enhances the secretion of ACh and CGRP from stimulated nerve endings. The effect of activating 5-HT₄ receptors is to strengthen neurotransmission in prokinetic pathways (Gershon 2005).
1.1.8.3 Sensation

In order for the ENS to regulate bowel functions, it needs to be aware of its intra luminal milieu, that is pressure, pH, nutrient concentration etc. (Gershon 2005). As mentioned above, the EC cells in the GI mucosa are in a unique position with their apices directly in contact with the luminal content. Indeed, they have been shown to respond to changes in pressure and/or content by releasing 5-HT into the mucosa (Bulbring and Crema 1959; Kim, Cooke et al. 2001), which initiates physiological responses in the gut. [See Figure 1.6]. This effect is further amplified by 5-HT through 5-HT4 receptors, which are present on the terminals of submucosal IPANs (Grider 2003).

5-HT also plays a role in conveying gut sensation to the CNS via 5-HT3 receptors, which are present on the extrinsic sensory neurones terminal in the gut (Gershon 2005) [See Figure1.7]. However, some of the extrinsic nerves may be stimulated indirectly by 5-HT through 5-HT2A receptor mediated smooth muscle contraction, which in turn stimulates 5-HT insensitive mechanoreceptors in the bowel wall (Gershon 1999). An animal study assessing the role of 5-HT3 receptor antagonist on visceral pain induced by colorectal distension, suggests that 5-HT3 receptors are involved in visceral nociceptive stimuli, perhaps through primary afferent or spinal neurones (Kozlowski, Green et al. 2000). 5-HT3 receptor antagonists modify visceral sensation in animal and human models by mechanisms, which are not yet very clear, nevertheless, this effect is used to therapeutic advantage in treatment of visceral hypersensitivity in irritable bowel syndrome(Crowell 2004). Similarly, capability of these antagonists to inhibit vagal afferent neuron receptors, makes them most successful treatment for chemotherapy induced nausea and vomiting (Hesketh 2008).
Figure 1.7 5-HT3 receptors are postsynaptic and are a ligand-gated ion channel. They are found on the terminals in the bowel of extrinsic sensory neurones and transmit noxious signals to the CNS. 5-HT3 receptors are also located on neurones in the myenteric plexus, where they mediate fast excitatory neurotransmission from serotonergic interneurones and on the mucosal terminals of myenteric IPANs. Antagonism of 5-HT3 receptors interferes with visceral hypersensitivity, but because of the location of 5-HT3 receptors in the ENS and on myenteric IPANs, 5-HT3 antagonists may also be constipating (Gershon 2005).

1.1.8.4 Summary

5-HT plays a fundamental role in the gut physiology and any alteration in its synthesis, uptake and metabolism could have a profound effect on the functions of the gut. In addition, 5-HT is a biochemical intermediary, connecting various gut sensations in the ENS with the CNS, through its receptors and serotonergic neurones. 5-HT is also a key neurotransmitter in the brain and plays a considerable role in a wide range of functions such as mood, appetite, sleep, memory and learning, homeostasis, and sexual behaviour (Crowell
There is an increased prevalence of psychiatric disorders in clinic patients with functional gastrointestinal disorders (FGIDs) (Levy, Olden et al. 2006) with the possibility of an alteration in gut-brain-gut communications (Crowell 2004). Abnormalities in serotonin availability/metabolism have long been associated with behavioural and psychiatric disorders (Nordquist and Oreland 2010), and development of visceral hypersensitivity (Farmer and Aziz 2009). Both these factors have an impact on brain-gut dysfunction, which is proposed as one of the crucial links in understanding multifactorial pathophysiology of FGIDs (Grundy, Al-Chaer et al. 2006). Moreover, alteration in 5-HT signalling is also linked to other proposed pathophysiological mechanisms for FGIDs such as visceral hypersensitivity (Houghton, Atkinson et al. 2003), dysmotility (Houghton, Atkinson et al. 2007), post-infectious (Lee, Kim et al. 2008) and inflammation (Spiller 2008; Faure, Patey et al. 2010).

1.2 Functional Gastrointestinal Disorders

1.2.1 Introduction

A disease or a disorder can have either an organic or a functional origin. The diagnosis of an organic disease is generally based upon the presence of anatomical, metabolic or physiological abnormalities, which can be measured or assessed and hence, treatments devised to rectify these abnormalities. The absence of these abnormalities defines a functional disorder. However, with advancement of science and availability of more complex tools for assessment, there are studies to indicate that so called functional disorders might be associated with significant physiological abnormalities, which were not measurable in the past. The principle of classification and diagnosis of a disease state based on symptoms only is well established and accepted in the field of psychiatry. In other disciplines such as gastroenterology, this approach has led to difficulty in accepting the very existence of symptom based or functional disorders (Drossman 2006). Moreover, in clinical practice, dealing with disorders in which symptoms are difficult to explain using conventionally established concepts and in which there are complex management issues, makes the whole
experience unnerving for some physicians (Drossman 2001). Functional gastrointestinal disorders (FGIDs) are one such group of disorders, which are commonly seen in clinical practice.

1.2.2 Epidemiology

FGIDs are extremely common; although the true prevalence of these disorders in a population is difficult to gauge as the methods of assessment usually rely on self-reported symptom based questionnaires, which are open to interpretation by the responders. Verification of such studies and the assessment of any changes in prevalence over time are further complicated by changing definitions and the classification system over the years. Moreover, change in symptom profile over a period in the same group of subjects is also common. In a prospective questionnaire study, 9% of new subjects developed symptoms consistent with FGIDs, whereas 38% no longer met criteria after 12-20 month follow up, who initially qualified for the diagnosis of irritable bowel syndrome. However, the over all prevalence of FGIDs remained stable suggesting high turnover rate with onset and disappearance of symptoms of FGIDs (Talley, Weaver et al. 1992). Similar findings have been confirmed in other studies (Halder, Locke et al. 2007) (Olafsdottir, Gudjonsson et al. 2010).

In a US household survey, using a questionnaire based on the Rome multinational diagnostic criteria (Rome-1, 1990), of over five thousand responders, 69% of responders had symptoms suggestive of at least one FGID over the last three months (Drossman, Li et al. 1993). Likewise, a similar survey using Rome II criteria, reported prevalence of 62% of FGIDs in the Canadian population (Thompson, Irvine et al. 2002). A longer 12 years prospective study from the US reported the prevalence of FGIDs at 42.3% (Halder, Locke et al. 2007). However, this study only screened for the most common FGIDs; IBS, FD (functional dyspepsia), FC (functional constipation), and functional abdominal pain, which might partly explain the major difference in the prevalence reported in the study.
In the UK, FGIDs constitute 50% of all gastrointestinal consultations in general practice, with the majority being diagnosed and managed by the GP and up to one third being referred to a specialist service (Thompson, Heaton et al. 2000). The total direct and indirect annual cost of FGIDs have been estimated to be 41 billion US dollars for the eight major industrialised nations (Fullerton 1998).

1.2.3 Classification

FGIDs are a group of heterogeneous disorders, in which symptoms are believed to originate from the gastrointestinal tract. Description of similar symptom patterns can be dated back to 1818. However, the first attempt to describe and classify such disorders was made with the publication of the first symptom based criteria for the diagnosis of IBS in 1978 (Manning, Thompson et al. 1978) and the first outline for the classification of FGIDs was published in the book “The Irritable Gut” in 1979 (Thompson 1979; Thompson 2006). The process of Rome classification began with the first publication of Rome guidelines for IBS in 1989 followed by more comprehensive classification of FGIDs (Thompson 2006). This allowed for the first time more accurate comparison between research studies. Since 1990, the criteria have been updated a number of times (see Table 1.1).

Table 1.1 History of the Rome Diagnostic Criteria (Thompson 2006).

<table>
<thead>
<tr>
<th>Year</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>The Manning Criteria for IBS</td>
</tr>
<tr>
<td>1984</td>
<td>The Kruis Criteria for IBS</td>
</tr>
<tr>
<td>1989</td>
<td>The Rome Guidelines for IBS (Rome-2)</td>
</tr>
<tr>
<td>1990</td>
<td>The Rome Classification System for FGIDs (Rome-1)</td>
</tr>
<tr>
<td>1999</td>
<td>The Rome II Criteria for IBS and the FGIDs (1999)</td>
</tr>
<tr>
<td>2006</td>
<td>The Rome III Criteria</td>
</tr>
</tbody>
</table>
The latest, the Rome III criteria, published in 2006 (Drossman 2006) are now widely approved by researchers, the drug manufacturing industry and the regulatory agencies, as the best way to categorise and standardise patient selection for research studies and clinical drug trials.

In Rome III classification, the FGIDs are broadly classified into six different categories based on the likely area of the origin of symptoms in the GI tract; functional oesophageal disorders, functional gastroduodenal disorders, functional bowel disorders, functional abdominal pain syndrome, functional gallbladder and sphincter of Oddi disorders, and functional anorectal disorders, with further sub-classification in most of the categories (Drossman 2006) (see Table 1.2).

**Table 1.2 Rome III Functional Gastrointestinal Disorders (Adults)**

**A. Functional oesophageal disorders**
- A1. Functional heartburn
- A2. Functional chest pain of presumed oesophageal origin
- A3. Functional dysphagia
- A4. Globus

**B. Functional gastroduodenal disorders**
- B1. Functional dyspepsia
  - B1a. Postprandial distress syndrome
  - B1b. Epigastric pain syndrome
- B2. Belching disorders
  - B2a. Aerophagia
  - B2b. Unspecified excessive belching
- B3. Nausea and vomiting disorders
  - B3a. Chronic idiopathic nausea
  - B3b. Functional vomiting
  - B3c. Cyclic vomiting syndrome
- B4. Rumination syndrome in adults

**C. Functional bowel disorders**
- C1. Irritable bowel syndrome
- C2. Functional bloating
- C3. Functional constipation
1.2.4 Functional Bowel Disorders

Among all FGIDs, functional bowel disorders constitute the most common group with prevalence rate of 41.6% out of all FGIDs (Thompson, Irvine et al. 2002). Both IBS and FC are part of functional bowel disorders, with IBS being further classified into four subtypes based on the predominant stool pattern; IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), mixed IBS (IBS-M), and unsubtyped IBS.

1.2.4.1 Irritable Bowel Syndrome

1.2.4.1.1 Definition

“IBS is a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of disordered defecation” (Longstreth, Thompson et al. 2006) (see Table 1.3 for detailed criteria).
1.2.4.1.2 Diagnostic Criteria and Sub-typing

In the literature IBS has also been described as “irritable colon syndrome” or “spastic colon” (Thompson 2006). In 1978, Manning first described a symptoms based criteria to help in making a positive diagnosis of IBS and discriminating it from organic diseases, however, initial criteria only described IBS associated with loose stools (Manning, Thompson et al. 1978).

From then on, after several modifications, the diagnostic criteria became more specific and IBS was classified into subtypes based on the stool consistency, and frequency (Rome II). Over the time, the consensus has moved to classify bowel habits based on the stool consistency (Rome III) alone to overcome discrepancies due to patient’s understanding of the terms diarrhoea and constipation. For example, patients with increased frequency of stool despite normal or even harder stool consistency often report their bowel habit as diarrhoea (‘pseudo-diarrhoea’), perhaps these patients may have increased frequency due to the sense of incomplete evacuation and possibly passing very small amounts of stool every time they open their bowels, and may in fact be suffering with constipation. The consistency of stool has been shown to correlate with the intestinal transit time more accurately and it can easily exclude ‘pseudo-diarrhoea’ (O’Donnell, Virjee et al. 1990).

Similarly, associated symptoms with bowel habits such as urgency, straining, and incomplete evacuation, are also poor discriminating factors as they have been shown to be associated with all types of stool forms in patients with IBS (Heaton, Ghosh et al. 1991). Besides bowel symptoms, other extra colonic symptoms such as constant tiredness, backache, dyspareunia, frequency, and, urgency etc. are also common in patients with IBS (Whorwell, McCallum et al. 1986).
Table 1.3 Rome III Diagnostic Criteria for Irritable Bowel Syndrome (Longstreth, Thompson et al. 2006)

<table>
<thead>
<tr>
<th>Recurrent Abdominal Pain or Discomfort at least 3 days per month in last 3 months associated with 2 or more of the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Improvement with defecation</td>
</tr>
<tr>
<td>2. Onset associated with a change in frequency of stool</td>
</tr>
<tr>
<td>3. Onset associated with a change in form (appearance) of stool</td>
</tr>
</tbody>
</table>

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

Despite classifying patients into individual subtypes (see Table 1.4), the variability and interchangibility in these groups has also been reported. Studies have shown that over 1 year, 75% of patients will change subtypes from either IBS-C/IBS-D to IBS-M, with a further 29% changing from IBS-C to IBS-D (described as IBS-A, “alternator”) and vice versa (Drossman, Morris et al. 2005). This creates a difficulty in maintaining the consistency in patient selection for research; depending upon the timing of selection, the same patient may be categorised into different subtypes of IBS. Nonetheless, even with its shortcomings, the Rome III criteria remain widely accepted standard for the research studies and drug trials.

Table 1.4 Sub-typing IBS by Predominant Stool Pattern (Longstreth, Thompson et al. 2006)

| 1. IBS with constipation (IBS-C) – hard or lumpy stool >25% and loose (mushy) or watery stools <25% of bowel movements. |
| 2. IBS with diarrhoea (IBS-D) – loose (mushy) or watery stools > 25% and hard or lumpy stools <25% of bowel movements. |
| 3. Mixed IBS (IBS-M) – hard or lumpy stools > 25% and loose (mushy) or watery stools > 25% of bowel movements |
| 4. Unsubtyped IBS- insufficient abnormality of stool consistency to meet criteria for IBS-C, D or M. |
1.2.4.1.3 Epidemiology

IBS is one of the most common functional bowel disorders with the prevalence rate of around 10-20%, which affects women slightly more than men (1.4:1) (Jones and Lydeard 1992; Hungin, Whorwell et al. 2003). However, few studies have reported prevalence of IBS in females is twice of that in males (Saito, Schoenfeld et al. 2002). This gender difference cannot be explained by higher health seeking behaviour in females as higher prevalence have been reported in population based studies as well (Halder, Locke et al. 2007). The prevalence is approximately the same across all age groups (Saito, Schoenfeld et al. 2002). In addition, the prevalence of the various subgroups is almost equally divided between IBS-D (35.6%), IBS-C (33.8%) and IBS-M (30.6%).

Only one fourth of patients who have IBS consult healthcare professionals for their IBS symptoms. However, those who consult, use health care services more often, even for non-gastrointestinal symptoms and costs 50% more to the health care services as compared with patients without IBS (Longstreth, Wilson et al. 2003). IBS sufferers also have significantly increased rate of cholecystectomy, appendisectomy and hysterectomy as compared with controls (Longstreth and Yao 2004).

Studies published to date suggest no effect of IBS on the long-term survival (Owens, Nelson et al. 1995) but it is related to increased morbidity. IBS patients experience poor health related quality of life, similar to that associated with other chronic illnesses like diabetes and end stage renal failure (Gralnek, Hays et al. 2000), and there are also reports of increased suicidal ideation (Miller, Hopkins et al. 2004).

1.2.4.2 Functional Constipation

1.2.4.2.1 Definition

“Functional constipation is a functional bowel disorder that presents as persistently difficult, infrequent, or seemingly incomplete defaecation, which does not meet the criteria for IBS” (Longstreth, Thompson et al. 2006)
1.2.4.2 Diagnostic Criteria

Abdominal pain, which is an essential criteria for diagnosis of IBS, has also been reported in patients with severe constipation (Preston and Lennard-Jones 1986). Apart from the abdominal pain or discomfort, other symptoms related to the consistency and frequency of bowel motions are similar in these two subgroups of functional bowel disorders to the extent that the patient who falls short of the criteria for IBS, by default will be classified as FC (see Table 1.5).

Table 1.5 Rome III Diagnostic Criteria for Functional Constipation (Longstreth, Thompson et al. 2006)

<table>
<thead>
<tr>
<th>Diagnostic Criteria for Functional Constipation (modified from Rome III constipation module questionnaire)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Must include two or more of the following</strong></td>
</tr>
<tr>
<td>a. Straining during at least 25% of defecation</td>
</tr>
<tr>
<td>b. Lumpy hard stool at least 25% of defecation</td>
</tr>
<tr>
<td>c. Sensation of incomplete evacuation at least 25% of defecation</td>
</tr>
<tr>
<td>d. Sensation of anorectal obstruction / blockage at least 25% of defecation</td>
</tr>
<tr>
<td>e. Manual manoeuvres to facilitate at least 25% of defecation</td>
</tr>
<tr>
<td>f. &lt;3 defecations per week</td>
</tr>
<tr>
<td><strong>2. Loose stools are rarely present without use of laxatives</strong></td>
</tr>
<tr>
<td><strong>3. Insufficient criteria for IBS (diagnostic criteria for IBS not met)</strong></td>
</tr>
<tr>
<td><em>Criteria fulfilled for at least 3 months with symptom onset at least 6 months prior to diagnosis</em></td>
</tr>
</tbody>
</table>

IBS-C and FC share several clinical characteristics; in fact, subjects who have symptoms similar to IBS-C but fall short of meeting the required criteria are classed as FC. This makes its clinical characterisation as a separate clinical entity slightly debatable. Recent studies have also highlighted interchangebility of the diagnosis in a significant proportion of patients based on symptoms over a 12-month follow up (Wong, Palsson et al. 2010) Another study
reported that a significant proportion of patients with constipation failed to meet the criteria for FC despite being clinically similar (Palsson, Turner et al. 2009). However, to date the Rome III classification remains a valid tool to identify subjects with FC for the research studies.

1.2.4.2.3 Epidemiology

Functional constipation is as prevalent as most of the other major FGIDs, with prevalence rates up to 20%. In fact, some studies suggest it is slightly more widespread than the IBS, making it probably the most common FGID (Thompson, Irvine et al. 2002; Halder, Locke et al. 2007). A large epidemiological study from Spain reported a significant difference in self reported constipation (29.5%) and FC identified by the Rome II criteria (14.%), such that only 47% of subjects, who considered themselves as constipated met the Rome II diagnostic criteria (Garrigues, Galvez et al. 2004). Defining constipation has been challenging in the clinical practice too. Out of 676 patients diagnosed with constipation by clinicians, only 20.2% met the Rome III criteria for FC (Palsson OS 2009). The clinical importance of understanding constipation is highlighted by a study from the US in which it was estimated that the cost of annual health care delivery was approximately 50% ($7522 Vs $5049 per patient) more expensive for constipation than the treatment of IBS (Nyrop, Palsson et al. 2007).

1.2.5 Pathophysiology

FGIDs are a group of heterogeneous disorders affecting different parts of the GI tract, which perhaps share some common pathophysiology, as well as subtle changes manifesting differently in their clinical presentation. Any attempt to explain the pathophysiology of FGIDs based on biomedical theories, such as altered visceral sensitivity (Ritchie 1973; Camilleri, McKinzie et al. 2008), dysmotility (Kellow, Miller et al. 1987; Quartero, de Wit et al. 1998) or neurohormonal changes (Romijn, Corssmit et al. 2008) or classifying them as purely behavioural (functional or psychological) disorders alone have not been successful. In a
biomedical model for FGIDs, measurable abnormalities such as altered visceral sensitivity and dysmotility are only found in a sub-set of patients with FGIDs and the distribution of these findings are neither uniform nor consistent. Besides, this model does not consider the effect of psychosocial aspects on disease development and clinical presentation, despite evidence of a strong association with FGIDs (Whitehead, Palsson et al. 2007). Hence, three decades ago a common unifying bio-psycho-social model was first proposed to explain the pathophysiology of these complex disorders (Engel 1977). Over the years, the argument for the bio-psycho-social model has developed further and has gained favour perhaps as the unifying factor for different pathophysiological mechanisms and resultant clinical manifestation of FGIDs.

Based on this model, the development of disease is the result of a complex interplay between exposure to the environmental, social and genetic factors, which has an effect on the psychosocial development of a person and their predisposition to an illness. The illness on the other hand produces symptoms, which affects a person physically as well as the individual’s psychosocial state, and the interplay between these factors directly relates to the clinical presentation and outcome of the disease state. In the case of FGIDs, this might increase susceptibility to intestinal dysmotility and visceral hypersensitivity through interaction in gut-brain axis (Jones, Dilley et al. 2006) and eventually symptom expression and clinical outcome (Drossman 1996; Drossman 2006)[see Figure 1.8].

![Figure 1.8 Schematic representation of bio-psycho-social model (Drossman; 1998)]
In recent years, an increased interest in FGIDs has led to a sudden increase in research in this area, particularly exploring potential 'organic' pathophysiological causes of FGIDs. For example studies have explored changes in measurable physiological markers, such as visceral sensitivity (Bouin, Lupien et al. 2004), dysmotility (Keller and Layer 2009) or changes in blood flow in different areas of the brain (Mertz, Morgan et al. 2000) in FGIDs. More recently, evidence has also emerged to suggest a proportion of patients with FGIDs have an infective aetiology (Saps, Pensabene et al. 2008) and low, but demonstrable, levels of inflammatory changes in the GI tract (Spiller, Jenkins et al. 2000), further smudging the boundary line between functional and organic disease. Despite the emergence of these new evidences, the bio-psycho-social model remains a comprehensive model to understand the role of different pathophysiological factors in the development of FGIDs (Drossman 2006). These pathophysiological factors will be discussed below, particularly in relationship with functional bowel disorders.

### 1.2.6 Altered Visceral Sensitivity

Altered visceral sensitivity has long been considered as a key factor in the pathophysiology of FGIDs, especially in IBS and FD (Mertz, Naliboff et al. 1995; Bouin, Plourde et al. 2002). A reduction in the pain threshold (visceral hypersensitivity) to colonic balloon distension in IBS (irritable colon syndrome) was first reported in 1973 (Ritchie 1973). Since then it has been confirmed by many studies, although there is a wide variability in its reported prevalence in IBS ranging from 20% (Camilleri, McKinzie et al. 2008) to 94% (Mertz, Naliboff et al. 1995). One study, however has suggested that depending on the threshold used to identify visceral hypersensitivity, it may be a useful discriminating factor in identifying IBS patients’ from healthy controls with 95.5% sensitivity and 71.8% specificity (Bouin, Plourde et al. 2002). Apart from the different distension methods (e.g. ascending methods, staircase, tracking etc.) and thresholds used to determine the altered visceral sensitivity, prevalence also depends on the subtypes of patients studied. Visceral hypersensitivity is more consistently reported in patients with IBS-D and post infectious IBS than in patients with IBS-C (Prior,
Maxton et al. 1990; Gwee, Leong et al. 1999; Agrawal, Houghton et al. 2008; Camilleri, McKinzie et al. 2008). Moreover, visceral hypersensitivity has been shown to correlate with the severity of the two most common IBS related symptoms, which are pain and bloating (Posserud, Syrous et al. 2007). Another study from our centre has shown that visceral hypersensitivity is related to bloating but not distension, suggesting visceral hypersensitivity may play a role in the perception of distension by patients with IBS (Agrawal, Houghton et al. 2008).

Evidence of altered visceral sensation in patients with IBS-C is less congruous. A previous study from our group has shown that a similar percentage of IBS-C patients are either viscerally hypersensitive (28%) or hyposensitive (28%) to rectal balloon distension (Agrawal, Houghton et al. 2008). Visceral hyposensitivity however, tends to be more common in patients with FC although the distribution varies significantly from 16 % (Gladman, Scott et al. 2003) to 68% (Shouler and Keighley 1986). A study in chronic constipation (FC) has reported a correlation between rectal hyposensitivity and slow gastrointestinal transit time (De Medici, Badiali et al. 1989). However, visceral hyposensitivity has also been identified in the absence of delayed transit (Wald, Hinds et al. 1989). The methods of evaluation of rectal hyposensitivity and patient selection are not consistent in these studies and the prevalence of rectal hyposensitivity in patients with FC in the community is unknown, as the majority of studies have evaluated patients’ in tertiary referral centres.

In FD, 67% of patients have been shown to have decreased pain threshold to gastric balloon distension compared with dyspepsia due to an organic cause (20%) (Mertz, Fullerton et al. 1998). Another study showed only 29% of patients with functional dyspepsia exhibit visceral hypersensitivity but identified an association between postprandial pain and weight loss (>5%) (Tack, Caenepeel et al. 2001). Others have shown decreased pain thresholds but no correlation to symptoms (Rhee, Kim et al. 2000). Hyper vigilance and somatisation have been linked with increased visceral sensitivity in patients with IBS. However, recent studies using electrical rectal stimulation while simultaneously recording cerebral evoked potential (EP) provide objective data of visceral hypersensitivity in patients with IBS (Sinhmahapatra,
Saha et al. 2001). In this experiment, increased afferent recruitment and heightened conduction were demonstrated, along with normal somatosensory EP, suggesting altered afferent sensory mechanisms rather than generalised anxiety or arousal as a cause of decreased threshold (visceral hypersensitivity) for rectal stimulation. Other studies support this data, demonstrating an increased pain threshold for electrocutaneous stimulation in patients with IBS (Cook, van Eeden et al. 1987).

There is limited data exploring the role of endogenous 5-HT in altered visceral sensitivity in FGIDs. One study reported a significant increase in postprandial levels of plasma 5-HT, correlating with dyspepsia scores in patients with untreated coeliac disease (Coleman, Foley et al. 2006). They also showed a significant increase in the number of EC cells in biopsies from the duodenum of these patients, compared with controls. The significant reduction in the concentration of mucosal 5-HIAA and 5-HIAA/5-HT ratio, along with increased mucosal and plasma 5-HT, suggest perhaps that there is a problem with reuptake or metabolism of 5-HT in these patients. It is possible that increased postprandial dyspeptic symptoms reported by patients with coeliac disease are mediated by the effect of 5-HT on visceral sensitivity. A similar study in patients with IBS-D reported a positive correlation between increased postprandial plasma 5-HT with increased postprandial symptoms (Houghton, Atkinson et al. 2003). Another study demonstrated that patients with IBS-D exhibiting visceral hypersensitivity (reduced maximally tolerable pressure) had a significantly higher number of EC cells in rectal mucosa compared to those without visceral hypersensitivity (Park, Rhee et al. 2006). Although the average numbers of EC cells in the terminal ileum, ascending colon and rectum was not significantly different in patients with IBS-D compared with healthy controls.

On the other hand, in an experimental study in patients with IBS, where a citalopram (SSRI) challenge was used to artificially increase levels of serotonin in the plasma, no significant effect on visceral perception was seen despite the rise in plasma 5-HT levels (Kilkens, Honig et al. 2005). Similarly, a study using paroxetine (SSRI), which demonstrated an improvement in gastric accommodation in healthy volunteers, did not demonstrate any
change in the thresholds for perception or discomfort on gastric balloon distension (Tack, Broekaert et al. 2003). These results were consistent with a previous study, which demonstrated no effect on the visceral sensory threshold in healthy volunteers after 6 weeks of oral sertraline, another common SSRI (Ladabaum and Glidden 2002).

There are very few studies exploring the relationship between 5-HT levels and FD. In a polish study, fasting serum 5-HT levels were measured in patients with FD. The patients with epigastric pain syndrome (EPS) demonstrated higher levels of 5-HT, while lower levels were measured in patients with postprandial distress syndrome (PDS) when compared with healthy controls (Harasiuk, Klupinska et al. 2007). Another study published by the same research group has shown an increase in the number of the EC cells in patients with EPS and a decreased number in patients with PDS compared with controls (Kulig, Klupinrska et al. 2009). A pilot study from our centre has shown increased post-prandial plasma 5-HT levels in FD correlating with dyspeptic symptoms (Lea, Houghton et al. 2002). There is no data on the relationship between 5-HT and visceral hyposensitivity, which has been reported in patients with IBS-C as well as functional constipation.

The reason for the lack of consistency between studies, especially in relation to prevalence of visceral sensitivity in FGIDS, may be related to the heterogeneity of the samples used. The different definitions, descriptions and protocols used to measure and describe visceral hypo or hypersensitivity lead to differences in the correlation of symptoms to visceral sensitivity. For example, some studies have used balloon ‘volume’ and others ‘pressure’ to define threshold for pain, discomfort and the desire to defaecate. Some studies have even used just one component of abnormal sensations, such as higher threshold for urge to defecate as a marker for visceral hyposensitivity in patients with IBS-C (Steens, Van Der Schaar et al. 2002). Although the same group of patients also had higher pain perception scores on VAS scale at similar pressure levels, when compared with controls, which could be interpreted as a marker for visceral hypersensitivity. More recently, the use of the barostat, which uses a computerised incremental increase in pressure to define threshold, together
with the consistent use of the Rome diagnostic criteria to define study population has provided a more standardised and uniform approach to the assessment of visceral sensation.

**1.2.6.1 Assessment of Visceral Sensitivity**

Different methods have been used to assess visceral sensation for the research purpose, such as mechanical stimulation of viscera by using balloon distension or electrical stimulation. Balloon distension has been used in different parts of the body; the stomach (Bradette, Pare et al. 1991), oesophagus (Trimble, Farouk et al. 1995), colon (Ritchie 1973) and rectum (Bouin, Plourde et al. 2002). Rectal barostat became the preferred method of assessment in patients with IBS as the rectum is the most easily accessible part of the large bowel. The balloon is inflated to a steady volume (isovolumic) or pressure (isobaric). The volume methods are considered to be less reliable due to the non linear relationship between volume and balloon pressure and its diameter (Whitehead and Delvaux 1997). Whereas, the pressure studies are more reproducible between subjects and laboratories, as variance in shapes of bags used in barostat, and muscle compliance and contractility are compensated by the pressure scale (Whitehead and Delvaux 1997). In isobaric distension, ascending method of limits is one of the commonly used protocols, in which pressure is increased in a stepwise, phasic or in a continuous ramp method until subjects report pain. It is assumed that at a certain threshold pain, being a distinct sensation, would be perceived separately from the discomfort and other sensations. However, due to predictability of the distension in this method, it is considered to be prone to a response bias (Whitehead and Delvaux 1997). To use a completely random order of distension would to be unethical as subjects might receive stimulation much higher than their pain tolerability. To avoid this bias, a tracking technique has been suggested. The pressure sequences become random once the subject first reports pain sensation. The computer randomly selects the same or lower pressure for the next distension for every time the subject reports pain; otherwise, it goes to the next higher level up, to a predefined maximum pressure. After a certain number of set readings, the pain threshold is defined as the average of all the pressure sequences,
since pain was first reported. This method was proposed to identify hyper vigilance and visceral hypersensitivity in patients with IBS. This method ascending methods of limits, followed by tracking technique protocol has been used in several published studies (Agrawal, Houghton et al. 2008).

1.2.7 Dysmotility and Transit

Dysmotility, which is an abnormal contraction and relaxation of the GI tract and abnormal propagation of its contents, is considered to be another major factor in the pathophysiology of FGIDs. Relaxation of the upper part of the stomach (fundal relaxation/accommodation) is a normal physiological response to the ingestion of food, which is impaired in a significant proportion of patients with FD (Tack, Broeckaert et al. 1998; Tack, Piessevaux et al. 1998; Kim, Delgado-Aros et al. 2001; Bredenoord, Chial et al. 2003). Besides abnormal fundic relaxation, delayed gastric emptying is also present in up to 37% of patients with FD (Quartero, de Wit et al. 1998). Other studies have shown that patients with postprandial distress syndrome, a subset of FD, demonstrate increased early and mid postcibal gastric emptying when compared with healthy volunteers, which directly correlated with symptoms. The relationship between motor abnormalities and certain dyspeptic symptoms are present in only a minority of patients (Quartero, de Wit et al. 1998; Tack, Broeckaert et al. 1998) (Mimidis and Tack 2008), perhaps suggestive of multi factorial pathogenesis of FD.

Decreased duodenal propagation (Simren, Castedal et al. 2000) and an abnormal motility response to stimulants such as fat and cholecystokinin (Kellow and Phillips 1987) have been reported in both IBS-C and IBS-D patients. Studies have shown significantly faster small bowel transit in patients with IBS-D compared with IBS-C (Cann, Read et al. 1983; Lu, Chen et al. 1998). Increased high amplitude propagated colonic contractions and accelerated oro-caecal transit has also been demonstrated in patients with IBS-D (Chey, Jin et al. 2001), whereas a decrease in high amplitude propagated colonic contractions has been reported in both patients with IBS-C and FC (Bassotti, Chistolini et al. 2003). Delayed small bowel transit
in a small proportion (17%) of patients with IBS-C has been observed in some studies (Cann, Read et al. 1983; Agrawal, Houghton et al. 2009). Delayed whole gut / colonic transit has been reported in a higher proportion (47%) of patients with IBS-C (Agrawal, Houghton et al. 2009). Slow whole gut transit is also present in patients with FC. However, the prevalence of slow transit varies significantly from 13% to 27% in different studies (Cook, Talley et al. 2009). Furthermore, there is a proportion of patients with constipation, who have evacuation disorder confirmed on physiological assessments and also slow transit, making it difficult to identify the predominant pathology and primary versus secondary phenomenon (Cook, Talley et al. 2009). The discrepancies in these findings in FC are chiefly due to the definition of the disease itself. FC has been described as chronic constipation or idiopathic constipation and a percentage of these would have functional defaecation disorder, often described as evacuation disorder, pelvic outlet obstruction, obstructive defaecation or dyssynergic defaecation. This variance in terms used in the literature makes it difficult to assimilate and interpret the results.

Postprandial increased motor activity in the colon (Houghton, Atkinson et al. 2007) and ileum is the normal physiological response to meal ingestion (Kerlin, Zinsmeister et al. 1983). There is an increase in colonic propagating sequences on waking up and after meals (Dinning, Szczesniak et al. 2008). This response is exaggerated in patients with IBS-D (Chey, Jin et al. 2001). Results of a recent study in patients with FC (chronic constipation), who underwent 24-hour jejunal manometery demonstrated an abnormal pattern in all patients with slow transit and in the majority of patients with normal transit. However, motility patterns were normal in patients with predominant obstructive defaecation (Seidl, Gundling et al. 2009). Another study into FC with slow transit, demonstrated abnormal gall bladder emptying (Hemingway, Neilly et al. 1996; Gunay, Gurbuz et al. 2004), delayed gastric, and small bowel transit suggesting that these patients have a pan gastrointestinal motility disorder (van der Sijp, Kamm et al. 1993; Gunay, Gurbuz et al. 2004). Other studies supporting this hypothesis reported decreased proximal gastric compliance and postprandial fundus relaxation in these patients (Penning, Vu et al. 2001) and abnormal antroduodenal
contraction patterns (Glia and Lindberg 1998). However, whether these phenomena are primary or secondary to the constipation is yet not known.

Altered levels of 5-HT have been shown to be associated with GI dysmotility in a few studies. In patients with IBS-C, a low rise in postprandial 5-HT levels compared with healthy controls and post-infectious IBS patients has been shown to be associated with a trend towards increased colonic transit time (Dunlop, Coleman et al. 2005). A study from our centre has shown increased sigmoid colonic motility, both in the fasting and postprandial state, in direct proportion to 5-HT levels in IBS patients compared with controls (Houghton, Atkinson et al. 2007). Other conditions associated with increased 5-HT levels, such as carcinoid syndrome, have also been shown to have faster small bowel and colonic transit and increased colonic tone (von der Ohe, Camilleri et al. 1993). To date there are no published studies exploring an association between 5-HT and dysmotility in patients with functional constipation. Some indirect evidence supports the role of 5-HT in colonic motility in patients with FC. The use of the 5-HT4 agonist, prucalopride, has been reported to accelerate colonic transit in healthy volunteers (Bouras, Camilleri et al. 1999) and patients with FC (Bouras, Camilleri et al. 2001).

With the recent improvement in technology, new tools have become available to assess bowel motility and the intraluminal milieu of the GI tract. Wireless capsules can measure pH, temperature, pressure, and transit time throughout the GI tract (Rao, Kuo et al. 2009; Camilleri, Thorne et al. 2010). Using this technique, a recently published study reported that patients with IBS-C had a higher number of colonic contractions compared with controls irrespective of transit time. The numbers of contractions were higher in FC with moderately slow transit but not in patients with severe slow transit (Hasler, Saad et al. 2009).

The colonic motor activity pattern can now be studied better with spatiotemporal mapping of colonic propagating sequences, suggesting that in obstructive defaecation, a subset of functional constipation, there is a significant reduction in propagating sequence and increased retrograde sequences with a significantly reduced response to meal ingestion (Dinning, Szczesniak et al. 2008). Spiller and colleagues from Nottingham have come up
with a novel application of MRI (magnetic resonance imaging) to measure the small bowel water content as a means of assessing transit and other factors. A pilot study has demonstrated significantly faster small bowel transit in patients with IBS-D (Marciani, Cox et al. 2010).

In a recent pilot study using spatiotemporal mapping of colonic propagating sequences, researchers have demonstrated increased retrograde sequences (motility) with significantly reduced response to a meal ingestion in patients with obstructive defaecation (Dinning, Szczesniak et al. 2008). In future, with the availability of these more advanced tools to assess physiological changes in motility, together with the standardisation of patient selection, hopefully a clearer understanding will be gained into dysmotility in FGIDs.

1.2.7.1 Assessment of Transit

1.2.7.1.1 Small Bowel Transit

The scintigraphic method, using a radioisotope labelled meal, is considered to be the most reliable in assessing regional and total small bowel transit time. However, it requires multiple scans with an expensive gamma camera, available in select tertiary care centres only. Besides, this method has a very wide range of normal values, hence only very extremes of abnormal small bowel transit can be captured by this test and it has varying degree of inter and intra subject variability (Argenyi, Soffer et al. 1995). Alternative methods such as, breath tests are based on measuring gases, which are produced by the fermentation of organic compounds by colonic bacteria, which in turn are transported in to the circulation and excreted in exhaled breath. Measuring oro-caecal transit time, using the hydrogen breath tests are based on the principle of hydrogen gas production and excretion in humans, as first described in 1969 (Levitt 1969). Lactulose Hydrogen Breath Test (LHBT) is one of the methods, which has been used to measure small bowel transit time. There has been wide differences between results obtained from LHBT and scintigraphy method(Miller, Parkman et al. 1997) possibly due to the lactulose itself acting as a osmotic laxative in the GI tract.
However other studies using radio labelled lactulose as substrate suggested both tests give comparable results (Barrow, Steed et al. 1992). More recent tests have used solid meals containing fermentable carbohydrates, as they are more physiological and these tests have proven to be reproducible. A similar solid meal will be used in measuring oro-caecal transit in this study, which has been used in several studies in the past with consistent results (Houghton, Foster et al. 2000; Agrawal, Houghton et al. 2008).

1.2.7.1.2 Whole Gut (Colonic) Transit

The scintigraphic method of assessing the colonic transit time is considered the gold standard and can provide assessment of both whole gut transit and colonic transit including accurate segmental transit time assessment (Lin, Prather et al. 2005). However, as mentioned above the scintigraphy method has practical difficulties associated with it.

Alternative method of assessment of colonic transit time using solid radio-opaque markers was first described by Metcalf (Metcalf, Phillips et al. 1987). Though, it is a much simpler method compared with scintigraphy method but there are certain practical difficulties in accurately assessing segmental transit, usually due to the inability to sometimes define the large bowel outline on simple x-ray (Pomerri, Frigo et al. 2007) and it has been suggested that addition of barium meal might enhance the accuracy in defining colonic segments. Accurate measurement of segmental colonic transit time is seldom of importance in clinical context, such as before planning surgery as an option for the treatment of constipation such as in cases of slow transit associated with particular colonic segment or segments (Lundin, Graf et al. 2007). Tests using radio-opaque markers with little modification in the protocol (Metcalf, Phillips et al. 1987) have shown to provide comparable results (Abrahamsson, Antov et al. 1988; Lundin, Graf et al. 2007).

A method using radio-opaque marker cannot assess colonic transit time alone and is a measure of whole gut transit time. However, measuring oro-caecal time along with whole gut transit assessment with solid radio-opaque marker will provide a practical and simple
alternative to scintigraphic method (Lin, Prather et al. 2005). This method of assessment of colonic transit time is commonly used in research studies and clinical trials (Houghton, Foster et al. 2000; Dunlop, Coleman et al. 2005; Houghton, Atkinson et al. 2006; Agrawal, Houghton et al. 2008)

1.2.8 Infection and inflammation

In recent years infection and inflammation have been proposed as potential mechanisms in the pathophysiology of FGIDs. There are a number of studies that have suggested an increased prevalence of IBS and FD after an episode of infective enteritis (Tack, Demedts et al. 2002; Mearin, Perez-Oliveras et al. 2005), although the association between infection and developing post-infectious IBS appears strongest (Marshall, Thabane et al. 2006; Saps, Pensabene et al. 2008). Not all patients following enteric infection go on to develop post-infectious IBS. Factors such as younger age, smoking habit and psychosocial factors appear to be the risk factors for developing post infectious IBS (Spiller and Garsed 2009). It is thought that a combination of mucosal injury, persistent inflammation following infection and changes in the enteric nervous system (Spiller, Jenkins et al. 2000), in association with the above predisposing factors (Gwee, Leong et al. 1999) leads to the development of post infectious IBS in a proportion of patients.

Several studies have shown an increased prevalence of small intestinal bacterial overgrowth (SIBO) in patients with IBS. Prevalence varies from a tendency to be slightly increased in patients compared with controls (32.9% vs. 17.9%) (Grover, Kanazawa et al. 2008) to as high as 78%, with the disappearance of symptoms in up to half of these patients following antibiotic therapy. This variation probably results in part from the different techniques employed to measure SIBO and also due to the different populations studied. For example, when jejunal aspirate was cultured, only 4% of IBS patients qualified for the diagnosis of SIBO (Posserud, Stotzer et al. 2007). Although this is considered to be the most objective test for the diagnosis of SIBO the cut off values used for diagnosis and the technique itself
are not widely accepted. It has been suggested that the presence of SIBO, may have an impact on immunological responses, inflammation and gas production leading to changes in visceral sensitivity, intestinal motility and brain gut axis in IBS patients (Lin 2004).

Significantly increased pro inflammatory cytokines such as interleukin-1 (IL-1), IL-10 and tissue necrosis factor (TNF) have been reported in patients with IBS-D and post infectious IBS (Eugene Campbell 2006). Similarly, a recently published study, which looked at inflammatory cells; lymphocytes, eosinophils and mastocytes in the duodenal mucosa, reported significantly increased eosinophil counts in patients with FD, increased intraepithelial lymphocytes in patients with IBS-C and increased mast cells in patients with IBS-D (Walker, Talley et al. 2009). Other studies have also shown increased mast cell numbers in rectal mucosa biopsies taken from both patients with post-infectious and non-infectious IBS-D, but not from patients with IBS-C (Lee, Kim et al. 2008). An increase in pro inflammatory cells in duodenal mucosal biopsies have been confirmed in patients with post infectious FD (Kindt, Tertychnyy et al. 2009). A distinct study reported intraganglionic inflammation and the presence of neuronal degeneration in the majority of full thickness jejunal biopsies from patients with IBS. Tissue obtained from autopsies of subjects without any known bowel disorder was used for comparison (Tornblom, Lindberg et al. 2002). Similar findings were reported in another study reporting degenerative/inflammatory neuropathy or leomyopathy in all (19/20) but one patient with IBS (Knowles, Veress et al. 2008), however, no controls were used in this study and data from the first study were also included in the overall results. The clinical significance of these findings is as yet not known.

Inflammation, and perhaps infection, modulates 5-HT in the GI tract mucosa by various mechanisms. In various studies in animal models, trinitrobenzene sulfonic acid (TNBS) induced colitis has been associated with increased mucosal 5-HT content and linked to increased EC cells, mast cells, and decreased SERT in the mucosa, the latter being the most consistent finding (Linden, Chen et al. 2003; O'Hara, Ho et al. 2004; Linden, Foley et al. 2005). In humans with Campylobacter enteritis, increased numbers of EC cells were reported in the rectal mucosa along with increased pro inflammatory cells and intra epithelial
lymphocytes (Spiller, Jenkins et al. 2000). These changes persisted even after 12 months in one third of these patients and were also associated with increased gut permeability. Similar findings of EC cells hyperplasia along with decreased SERT have been reported in mice infected with T Spiralis (Wheatcroft, Wakelin et al. 2005).

The reports from studies assessing similar aspects in patients with IBS in comparison with inflammatory bowel disease have produced more conflicting results. Earlier studies have shown increased numbers of EC cells in the rectal mucosa of patients with IBS, and decreased in patients with ulcerative colitis (UC), when compared with healthy controls (Ahonen, Kyosola et al. 1976). Another more extensive study assessing different aspects of 5-HT metabolism showed no difference in EC cells in the rectal mucosa of patients with IBS-C, IBS-D, and UC compared with controls (Coates, Mahoney et al. 2004). However, the numbers of EC cells in patients with UC were significantly reduced in the sub-group with more severe inflammation, compared with non-severe inflammation. Decreased 5-HT content and SERT was also demonstrated in patients with IBS and UC when compared with the control group (Coates, Mahoney et al. 2004). Another recent study has also reported no difference in the number of EC cells in the mucosa of the colon, ileum or duodenum in patients with IBS-D or IBS-C compared with controls. The mucosal 5-HT content was decreased only in patients with IBS-C, but the number of mast cells was significantly higher in the terminal ileum of patients with both IBS-C and IBS-D (Wang, Dong et al. 2007). A study in paediatric patient with IBS and FD reported a slight but non-significant increase in inflammatory cells in all IBS patients along with increased mucosal 5-HT content and decreased SERT activity (Faure, Patey et al. 2010). No difference was reported in the 5-HT mechanism in patients with FD. A reduced number of EC cells in the colonic mucosa has also been reported in patients with FC (El-Salhy, Norrgard et al. 1999).

Research findings in inflammatory bowel disease are equally conflicting with both increased (El-Salhy, Danielsson et al. 1997) and decreased (Ahonen, Kyosola et al. 1976) numbers of EC cells being reported in different studies. In a novel experiment with TPH-1−/− deficient mice, a study demonstrated eloquently, that 5-HT has a central role in the development of
pathogenesis in experimental colitis (Ghia, Li et al. 2009). EC cells are the main source of 5-HT in the GI tract and SERT is an essential component in terminating 5-HT action on 5-HT receptors. Any changes in these as a direct or indirect result of infection or inflammation, would play a significant part in the modulation of secretion, motility and sensation of the GI tract by various receptor pathways as described above.

1.2.9 Gut Brain Axis

The gut-brain axis is important in the control of GI functions and may have a role to play in the pathogenesis of FGIDs. The GI tract relays sensory information from mechanical, chemical and nutrient stimuli through the vagal and spinal afferent nerves to the central nervous system. The brain in turn modulates information accumulated both from the GI tract and from other sensory and emotional inputs such as anxiety, fear, and anger and relays back signals to regulate GI activity via the autonomic nervous system. This interaction is closely controlled by general and organ specific hormones, secreted from the GI tract and from the brain as messengers (Romijn, Corssmit et al. 2008). The hollow viscera of the GI tract have sensory innervations at the mucosal and myenteric levels forming plexuses (the enteric nervous system or mini brain), which regulates the motility, secretion, mucosal transport and blood flow of the GI tract. However, this also receives input from the brain as a part of the gut-brain axis.

Experimental studies with functional brain imaging provide further evidence of deferential activity in the brain of patients with FGIDs. The areas of the brain that are stimulated on somatic painful stimulus are the contra lateral cingular cortex, thalamus and insula cortex (Casey, Minoshima et al. 1994). While non-painful visceral stimuli activate the central sulcus, insular cortex and frontal operculum, painful visceral stimuli activate additional areas with more intense activities in the anterior insular cortex and anterior cingulate cortex (ACC) (Aziz, Andersson et al. 1997). In patients with IBS, a trend towards an increased activity in the ACC and the insular cortex was seen on painful rectal balloon distension compared with
controls. In fact this increased activity was also shown to be related to the intensity of pain reported by the patients (Mertz, Morgan et al. 2000). Studies using positron emission tomography (PET) however, have shown different results in IBS patients with no significant increase in blood flow in the area of ACC (a normal expected response to painful visceral stimuli in healthy volunteers), instead an increased activity in the prefrontal cortex was noted (Silverman, Munakata et al. 1997). Despite the inconsistent results, there is certainly some evidence of aberrant processing of noxious visceral stimuli in the brain of patients with IBS compared with controls.

Central sensitisation is a process in which sensitisation of one area of the GI tract leads to sensitisation elsewhere. For example, acid infusion into the distal oesophagus of patients with non-cardiac chest pain has been shown to have the effect of lowering the threshold for pain on electrical stimulation of the proximal oesophagus, which lasted for longer than in healthy controls (Sarkar, Aziz et al. 2000).

Peripheral sensitisation on the other hand, is a process by which local inflammation and the release of inflammatory mediators results in normally non-noxious stimuli being relayed to the brain as noxious, leading to the development of visceral hypersensitivity (Knowles and Aziz 2008). It has been proposed that 5-HT plays a role in peripheral sensitisation. As mentioned above, there have been several studies demonstrating an increased number of EC cells and mast cells in patients with IBS. Data from animal studies suggests that mast cells are important in the development of visceral hypersensitivity (Ohashi, Sato et al. 2008).

In another experiment, TPH-1\(^{-}\) deficient mice failed to develop experimental colitis (Ghia, Li et al. 2009). Decreased availability, or complete lack of 5-HT is the common link in these two experiments. However, visceral hypersensitivity failed to develop in the former experiment despite the presence of general inflammation induced by TNBS, suggesting a direct role for 5-HT in peripheral sensitisation.

Capsaicin TRPV 1 (transient receptor potential vanilloid type 1) receptors have also been suggested as playing a role in the development and maintenance of visceral hypersensitivity in animal models even in the absence of inflammatory changes (Winston, Shenoy et al.\^)\textsuperscript{2001})

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2007). Significant increases in these receptors have also been reported in recto sigmoid mucosa of patients with both IBS-C and IBS-D, which directly correlate with pain symptoms in these patients (Akbar, Yiangou et al. 2008). However, all these changes in patients with IBS were accompanied by a significant increase in proinflammatory cells. Similarly, an increase in these receptors has been reported in patients with rectal hypersensitivity (Chan, Facer et al. 2003) without any proinflammatory changes. The mechanism and trigger for an increased expression of these receptors remains unknown but it has been proposed that this plays a role in peripheral sensitisation in altered gut brain axis in FGIDs (Farmer and Aziz 2009).

Increased inflammatory cells, EC cells, and decreased SERT with associated increased availability of mucosal 5-HT may all have pivotal roles in the development of peripheral sensitisation in FGIDs. Moreover, communication from lumen to the ENS and from the GI tract to the CNS is mediated by 5-HT through 5-HT receptors linked to afferent neurones. Similarly, downward modulation of these sensations from the CNS is mediated via serotonergic neurones besides others. Ultimately, changes in sensory perception, motility and secretions in the GI tract are also mediated via 5-HT. This central integrating role of 5-HT makes it a key component in gut brain axis and pathophysiology of FGIDs.

1.2.10 Psychopathology

In a systematic review, 54% to 94% of patients with IBS were reported to have at least one associated psychiatric disorder (Whitehead, Palsson et al. 2002), in particular major depression, anxiety and somatisation. Increased probability of sexual abuse history has also been reported in FGIDs (Drossman, Li et al. 1996), recently supported by a meta analysis (Paras, Murad et al. 2009). The association between a history of abuse and FGIDs was accepted even from earlier studies, however, there was certain scepticism about the selection bias and measurement processes leading to an over representation of this problem in these studies (Talley and Boyce 1996).
Similarly, somatisation symptoms, also sometimes classed as non-colonic symptoms, have been shown to be strongly associated with IBS (Whorwell, McCallum et al. 1986; Maxton, Morris et al. 1991). However, these are still not included in the diagnostic criteria for IBS due to lack of specificity. A population based study found an association between somatisation symptoms and IBS but not with FD. The presence of these somatisation symptoms in the IBS patients did not predict health seeking behaviour (Koloski, Boyce et al. 2006) contrary to the previous studies (Kettell, Jones et al. 1992; Herschbach, Henrich et al. 1999). These findings have been confirmed in another community based study, which has shown similar associations between somatisation symptoms and other psychological distress markers in patients with IBS but no relation to health seeking behaviour (Choung, Locke et al. 2009). Likewise, the history of sexual abuse has been linked to increased gastric sensitivity and altered accommodation in functional dyspepsia (Geeraerts, Van Oudenhove et al. 2009).

Increased prevalence of anxiety, depression and social dysfunction has been associated with functional constipation (Mason, Serrano-Ikkos et al. 2000; Nehra, Bruce et al. 2000). High prevalence (65%) of eating disorders (Nehra, Bruce et al. 2000), somatisation and less satisfaction with their sexual life has also been reported in women with constipation, with no difference for slow or normal transit constipation (Mason, Serrano-Ikkos et al. 2000). Another study has reported higher prevalence of anxiety and depression in patients with normal transit constipation compared with slow transit constipation, along with different coping mechanisms in these two subgroups of FC (Chan, Cheng et al. 2005).

How these psychological factors play a role in the pathophysiology of FGIDs is still not clear but studies have shown increased colonic motility in healthy volunteers, both after acute physical and psychological stress, with a more prolonged response to the latter stimulus (Rao, Hatfield et al. 1998). Another study reported significantly faster mouth to caecum transit time associated with psychological stress in healthy volunteers, without significant effect on gastric emptying, suggesting increased small bowel motility (Cann, Read et al. 1983). Other studies have shown that acute stress in animal models increases colonic motility, which can be reversed by corticotrophin releasing factor (CRF/CRH) antagonists,
suggesting a role for the CRF pathway in mediating stress related GI effects (Gue, Junien et al. 1991).

In experimental studies, a CRF analogue has been shown to increase intestinal motility in both healthy volunteers and patients with IBS. The response to exogenous CRF was more prolonged in patients with IBS, with a significantly higher rise in adrenocorticotrophic hormone (ACTH) levels, suggesting an exaggerated brain-gut response to CRF in patients with IBS (Fukudo, Nomura et al. 1998). Besides dysmotility, there are some studies to suggest that stress alters pain perception in patients with IBS (Dickhaus, Mayer et al. 2003). Data from animal and human studies available so far suggest that stress related GI dysmotility and altered visceral sensitivity is at least partly mediated through the endogenous corticotrophin releasing factor (CRF) pathway (Monnikes, Tebbe et al. 2001). Further indirect evidence for the role of the CRF pathway in IBS pathophysiology and stress comes from the development of CRF₁ receptor antagonists for the treatment of IBS and social anxiety disorders. Despite promising results in animal and physiological studies, results of clinical trials with various CRF₁ receptor antagonists in patients with IBS have not shown any clinical benefits (Moeser, Nighot et al. 2008).

Animal studies have also suggested that chronic stress alters immune functions in colonic epithelium with increased permeability and mast cell hyperplasia and activation (Santos, Yang et al. 2001). In another study, stress related GI dysmotility persisted despite hypophysectomy, suggesting a role for the autonomic nervous system in the effect of stress on the GI tract (Gue, Junien et al. 1991).

Decreased platelet SERT has also been reported in patients with unipolar depression (Alvarez, Gluck et al. 1999) and somatoform disorders (Belous, Ramamoorthy et al. 2001). Indeed this association has been reported in several other psychiatric conditions such as bulimia, schizophrenia and panic disorder, highlighting its non specificity (Marazziti, Placidi et al. 1989). However, this change in SERT activity is hypothesised to be a pre synaptic serotonergic dysfunction, which potentially may have a role in psychopathology. Some animal studies have suggested that stress increases 5-HT levels in selective areas in the
brain and plasma (Dunn 1988). There are some suggestions that the catecholamine pathways induce 5-HT release from platelets into plasma, increasing circulating levels of 5-HT in response to stress stimulus (Palermo, del Rosso et al. 1986). It has been proposed that increased 5-HT release in the hippocampus mediates adaptive changes in corticosteroid receptor expression and it is one of the adaptive mechanisms to deal with repeated stress (Robertson, Beattie et al. 2005). Acute stress stimulus in rats increases release of 5-HT from the EC cells in the ileocaecal mucosa and this phenomenon is inhibited by tegaserod, suggesting the possible role of stress on 5-HT release in the GI tract, and hence gut functions (Chi, Liu et al. 2005).

1.2.11 Genetics

Studies have reported an increased prevalence of IBS in first-degree relatives (Kalantar, Locke et al. 2003; Saito, Zimmerman et al. 2008), suggesting the possibility of an association between genetics and IBS. This is further supported by a study in twins, which suggested that the concordance rate of IBS is twice in monozygotic (MZ) twins (17.2%) compared with dyzygotic (DZ) twins (8.4%) (Levy, Jones et al. 2001). The same study also reported that a higher proportion of DZ twins (15.2%) who had IBS also had a mother with IBS, as compared to DZ twins (6.7%) who only had a co-twin with IBS. However, the overall percentage of mothers with IBS was the same in both MZ and DZ twin groups. This suggests that besides a possible role for genetics, there is also an important role for social learning in the development of IBS. On the other hand, a large study from the UK has reported equal prevalence of IBS both in MZ (17%) and DZ twins (16%) (Mohammed, Cherkas et al. 2005), although they also showed significant association between the presence of somatisation disorder and IBS. Another recent study in twins reported significantly higher concordance for extra intestinal symptoms and psychiatric disorders in MZ twins compared with DZ twins, suggesting an association between genetics and extra intestinal symptoms, but not IBS (Lembo, Zaman et al. 2009). Thus, although these epidemiological studies suggest a possible role for genetics in pathophysiology, they are far
from conclusive. Another approach to exploring the role of genetics in IBS has been to look for individual candidate genes, which might have an association with a particular pathophysiological factor in IBS.

There are several studies that have investigated possible links between individual genes and potential pathophysiological factors in IBS, such as altered levels of 5-hydroxytryptamine (5-HT) and pro-inflammatory cytokines. One study showed a reduced frequency of the genotype (-1082*G/G) linked to higher levels of interleukin-10 (IL-10) in patients with IBS (Gonsalkorale, Perrey et al. 2003). IL-10 is an anti-inflammatory cytokine and lower levels could potentially lead to a mild inflammatory state, which is one of the proposed mechanisms in the pathophysiology of IBS. However, a second study failed to replicate these findings (van der Veek, van den Berg et al. 2005). Other studies have shown the α2C Del 322-325 deletion (12bp deletion in the gene for α2C adrenoceptor subtype) to be associated with IBS-C and higher prevalence of somatic symptoms (Kim, Camilleri et al. 2004). Similarly, a few early epidemiological studies involving mutations in the mitochondrial DNA (Camilleri, Carlson et al. 2009) and sodium ion channelopathies (Saito, Strege et al. 2008) have also suggested a possible association between genetics and functional GI disorders.

The gene SLC6A4, which is responsible for the synthesis of the 5-hydroxytryptamine (5-HT) transporter protein (SERT), is one of the most extensively studied candidate genes in the pathophysiology of IBS. Two common single nucleotide polymorphisms (SNPs) in the SERT gene; are a variable number of 17bp tandem repeats located in intron 2 (VNTR2/ST in 2) and a 44bp deletion/insertion in the SERT promoter region, resulting in a short (ss or sl) or long (ll) allele (Heils, Teufel et al. 1996). The transcriptional activity of SERT mRNA is significantly lower when both the homozygous 5-HTTLPR (serotonin transporter linked polymorphic region) ss and ST/in2*10 polymorphisms, which in themselves lead independently to lower expression, are present together (Hranilovic, Stefulj et al. 2004). Such polymorphisms might be thought to be theoretically associated with higher levels of 5-HT.
Although an increase in the frequency of the 5-HTTLPR ss genotype in IBS-D has been demonstrated in two studies (Yeo, Boyd et al. 2004; Park, Choi et al. 2006), another study demonstrated an equal frequency of the ss genotype in IBS-D patients (35%) and healthy volunteers (33%), but decreased frequency of the ss genotype in IBS-C (20%)(Kim, Camilleri et al. 2004). Another study from our centre demonstrated slightly decreased frequency of the ss genotype in both IBS-D (16.5%) and IBS-C (14.3%) compared with healthy controls (23.9%), but this difference did not reach statistical significance (Niesler, Kapeller et al. 2009). This study is one of only a few studies that also looked at the effect of gender and showed that the decreased frequency of the ss genotype in IBS-D is probably due to a decrease in men but not women. A recent meta-analysis of eight studies did not find any association of SERT polymorphism to IBS (Van Kerkhoven, Laheij et al. 2007).

There is indirect evidence to suggest an effect of SERT polymorphism on the SERT transcription and thus, 5-HT activity. The ss genotype has been associated with less SERT transcription, resulting in reduced 5-HT reuptake, which leads to more 5-HT being available at the receptor level. Tegaserod, an agonist at the 5-HT₄ receptor, has been shown to be efficacious (85%) in ss compared with ll genotype patients with constipation, due its synergistic action with 5-HT. The ss genotype is thought to be associated with more 5-HT availability at receptor level due to decreased SERT transcription (Camilleri, Atanasova et al. 2002; Li, Nie et al. 2007). On the other hand, alosetron, a 5-HT₃ receptor antagonist, was more effective in slowing colonic transit in ll genotype patients with IBS-D, as theoretically it has to compete with lower amounts of serotonin at the receptor level (Camilleri, Atanasova et al. 2002).

Thus, although generally there may not be an association between SERT polymorphism and IBS, a patient having a particular SERT polymorphism, such as ss, might be more prone to particular alterations in GI functions. Moreover, a study has shown that the presence of ss SERT polymorphism is associated with a history of depression compared with IBS patients who did not have this polymorphism (Jarrett, Kohen et al. 2007). Indeed any alteration in
synthesis or metabolism, and consequently levels of 5-HT, a key neurotransmitter, can have significant effects on GI functions and psychopathology.

### 1.2.12 Serotonin Signalling

Altered levels of 5-HT in the GI mucosa have been reported in different clinical conditions as shown in Figure 3.3 (Spiller 2007). The mechanisms behind these altered levels may vary, but a common theme emerges, which suggests that the conditions associated with increased motility tend to have increased mucosal 5-HT content.

![Diagram showing abnormalities in serotonin levels in human diseases](image)

**Figure 1.9 - Medical conditions and 5-HT levels in GI mucosa (Spiller 2007)**

The first pilot data on altered serotonin signalling in IBS-D was published by Bearcroft et al. who demonstrated an increase in postprandial platelet poor plasma 5-HT, compared with healthy controls (Bearcroft, Perrett et al. 1998). Later studies showed that patients with IBS-D have both higher fasting baseline and postprandial PDP 5-HT levels, with the ratio of postprandial to fasting levels being no different from healthy volunteers (Atkinson, Lockhart et al. 2006). This data suggests that the response to meal ingestion in patients with IBS-D was similar to healthy controls. In addition, it was demonstrated that the ratio of 5-HIAA/5-HT was significantly decreased in IBS-D patients compared with controls, suggesting that
these patients may have a problem with the reuptake or metabolism of 5-HT. It has been proposed that the latter could also be as a result of decreased MAO activity (Spiller 2007).

Conversely, patients with IBS-C have been shown to have normal fasting 5-HT concentrations (Atkinson, Lockhart et al. 2006), which did not significantly increase during the postprandial period (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). The low plasma 5-HT levels were associated with low 5-HIAA levels but a normal 5-HIAA/5-HT ratio, suggesting a problem with 5-HT release in these patients. This is supported by a study demonstrating a significantly reduced 5-HIAA/5-HT ratio in enterochromaffin cells in rectal biopsies taken from IBS-C patients, which possibly would not be affected by 5-HIAA from any other sources in the body (Dunlop, Coleman et al. 2005). This reduced ratio was as a result of increased 5-HT and decreased 5-HIAA in the enterochromaffin cells. The study also reported decreased numbers of the enterochromaffin cells in patients with IBS-C.

In a study, which looked at the histology of the colonic mucosa from patients with colonic inertia (functional constipation), an increased number of EC cells (Baig, Zhao et al. 2002) in the left colon was reported. However, the majority of these cells demonstrated low density immunocytochemical staining, suggestive of less serotonin per cell (Zhao, Baig et al. 2000). Plasma 5-HT and 5-HIAA were not measured in these studies. Other studies have also reported decreased numbers of EC cells in idiopathic constipation (El-Salhy, Norrgard et al. 1999; Dunlop, Coleman et al. 2005). Unlike IBS-C, there are no known large published studies measuring plasma 5-HT and 5-HIAA in functional (idiopathic) constipation.

An increased 5-HT content with decreased SERT mRNA in the rectal mucosa has been demonstrated in patients with IBS (mainly IBS-D), with an inverse correlation between SERT mRNA and 5-HT content (Faure, Patey et al. 2010). However, there are studies with contradictory findings, reporting normal (Camilleri, Andrews et al. 2007) or increased (Kerckhoffs, Ter Linde et al. 2008) SERT mRNA in the GI mucosa in patients with IBS. The levels of TPH-1 were normal in these studies (Kerckhoffs, Ter Linde et al. 2008; Faure, Patey et al. 2010), which may indicate a problem with the release or reuptake, rather than altered synthesis of 5-HT.
Reduced SERT expression and binding capacity on platelets has also been reported in patients with IBS-D compared with healthy controls (Bellini, Rappelli et al. 2003) and this may possibly explain increased availability of PDP 5-HT in patients with IBS-D. These changes in SERT activity also correlated with symptom severity, suggesting that SERT dysfunction and possibly altered 5-HT signalling has a role to play in the clinical presentation of IBS (Bellini, Rappelli et al. 2003).

1.2.12.1 5-HT and 5-HIAA

There was a long held view that 5-HT in plasma was directly derived from activated platelets and was not measurable in normal circumstances. However, this was due to the technical limitations in measuring low levels of 5-HT. In last 30 years, liquid chromatography has been used to measure 5-HT, with several improvements to the technique over time (Kissinger, Bruntlett et al. 1981). Since the majority of 5-HT in whole blood comes from stored 5-HT in platelets, measuring it at a one point of time would not reflect dynamic changes due to release of 5-HT in the GI tract. 5-HT values in whole blood are 100 times higher than in PDP (Monaghan, Brown et al. 2009) hence, a technique with high sensitivity is required for measurement in PDP. High performance liquid chromatography (HPLC) methods have been used to assess plasma 5-HT levels, but the reported normal values of fasting PDP 5-HT vary significantly, from 0.77 ± .38 nmol/L (Beck, Wallen et al. 1993) to a median 5-HT level of 21.1 nmol/L (inter quartile range; 8.3, 19.9 nmol/L) (Atkinson, Lockhart et al. 2006). These differences perhaps reflect technical variation in sample collection, processing, and the inherent difficulty in measuring low concentrations of 5-HT in plasma. In fact, in the first pilot data of altered 5-HT signalling in IBS, the authors failed to detect any plasma 5-HT in the fasting stage in both IBS patients and healthy controls despite using similar methods of assessment (Bearcroft, Perrett et al. 1998).

Various techniques have been suggested to prevent sampling artefacts, as any activation of platelets leading to release of 5-HT would increase values significantly. The double spin
technique to separate platelets from plasma (Artigas, Ortiz et al. 1986), using EDTA to improve platelet stabilisation has helped, as has the immediate processing of samples, since delay may lead to a 3 fold increase in PDP 5-HT levels, perhaps due to platelet activation (Beck, Wallen et al. 1993). All these techniques have been implemented with consistent results in previous studies from our centre (Houghton, Atkinson et al. 2003; Houghton, Atkinson et al. 2007; Houghton, Brown et al. 2009). Studies involving assessment of dynamic changes in 5-HT levels over a period of time require large numbers of samples to be processed, which is difficult if the analytical method involves individual sample preparation and long processing time. Recently, a simple and robust liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for 5-HT has been developed and validated, in collaboration with our centre, which has cut time for preparation and analysis (Monaghan, Brown et al. 2009).

A few researchers have also used 5-HT and 5-HIAA in rectal mucosa to assess mucosal turnover of 5-HT in IBS (Dunlop, Coleman et al. 2005; Faure, Patey et al. 2010). Measuring other parameters along with 5-HT and 5-HIAA, such as TPH-1 expression, SERT expression and other transporter expression, gives a wealth of information about 5-HT turnover. However, this only provides a snap shot of metabolism of 5-HT rather than an assessment of dynamic changes reflected by plasma 5-HT/HIAA time profiles.

Since 5-HT is not metabolised in platelets, levels of 5-HIAA in blood or plasma are not affected by platelet activation. Urinary 5-HIAA does not reflect real time postprandial changes in 5-HT metabolism. In addition, difficulty in collecting several samples to get a time profile of 5-HIAA levels makes plasma 5-HIAA level assessment more appropriate. 5-HIAA in circulation comes from 5-HT metabolism and has a more consistent profile, with a gradual increase in postprandial state (Houghton, Atkinson et al. 2003) compared with postprandial 5-HT levels.

5-HIAA level measurement, along with 5-HT in PDP, is used to calculate the 5-HIAA/5-HT ratio to estimate 5-HT turnover. However, it is important to remember that this ratio is affected by several factors such as secretion and reuptake of 5-HT, its catabolism in
enterocytes and other sites, and the release of 5-HIAA in the circulation. Using this data, in conjunction with the estimation of platelet 5-HT content and changes from pre to post prandial levels gives information about 5-HT release from the EC cells, reuptake and its catabolism. This method has been used to study 5-HT signalling in IBS in several studies from our centre (Atkinson, Lockhart et al. 2006; Houghton, Atkinson et al. 2007; Houghton, Brown et al. 2009).

1.3 Aims and Objectives:

I. To determine whether there is a similar abnormality in 5-HT signalling in patients with functional constipation and IBS-C compared with healthy volunteers, as measured by platelet depleted plasma 5-HT concentrations under fasting and fed conditions.

II. To determine possible mechanisms of altered signalling in functional constipation by concomitantly measuring 5-HIAA concentrations, along with calculating the ratio of 5-HIAA: 5-HT.

III. To relate abnormal 5-HT signalling to the pathophysiology in patients with functional constipation and IBS-C, by assessing small and large bowel transit, and visceral sensitivity.

IV. To determine whether there are any differences between two functional sub-types of constipation on the basis of small and large bowel transit time and visceral sensitivity.
Chapter 2

Methods
2.1 Subjects

24 IBS-C, 12 FC patients and 24 healthy, premenopausal females, between the ages of 18 - 50 years were recruited. One IBS-C patient and one healthy volunteer did not complete the study because of personal reasons and one FC could not be contacted further to resolve some outstanding blood results as, she had moved out of the UK. Only 12 out of intended 24 patients with FC could be recruited during 24 months. Data from 23 healthy volunteers, 23 IBS-C and 11 FC patients was included in the final analysis.

2.2 Inclusion Criteria for IBS-C and FC patients:

Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to the diagnosis (Longstreth, Thompson et al. 2006) (see Appendix 1 for Rome III constipation module questionnaire).

2.2.1 A positive diagnosis of IBS-C according to the Rome III criteria:

1. Recurrent abdominal pain or discomfort associated with two or more of the following:
   a) Improvement with defaecation
   b) Onset associated with a change in frequency of stool
   c) Onset associated with a change in form (appearance) of stool

2. Hard and Lumpy Stools (Bristol Stool Scale 1 or 2) ≥ 25% and loose and mushy (Bristol Stool Scale 6 or 7) ≤ 25% of bowel movements.

Subjects with IBS-C were allowed to participate in the study only if their IBS was active at the time of participating in the study.

2.2.2 A positive diagnosis of Functional Constipation according to the Rome III criteria:

1. Must include two or more of the following
   a) Straining during at least 25% of defaecations
b) Lumpy or hard stools at least 25% of defaecations

c) Sensation of incomplete evacuation at least 25% of defaecations

d) Sensation of anorectal obstruction/blockage at least 25% of defaecations

e) Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor)

f) Fewer than three defaecations per week

2. Loose stools are rarely present without the use of laxatives.

3. Insufficient criteria for IBS

Above criteria are only applicable for spontaneous bowel movements.

2.2.3 Healthy volunteers with no major medical or psychiatric conditions.

2.2.4 Able to communicate well with the investigator and able to understand and comply with the requirements of the study. Understand and sign the written informed consent.

2.3 Exclusion Criteria

2.3.1 For IBS-C and FC patients:

1. Pregnant or breast-feeding.

2. Patients with BMI of <18 or >30.

3. Patients smoking >10 cigarettes a day or drinking more than recommended units of alcohol (18 units/week) or had a history of drug abuse.

4. Previous abdominal or pelvic surgery other than uncomplicated caesarean section or appendectomy or had undergone traumatic labour, which required instrumentation or episiotomy to facilitate delivery.

5. Patients with a recent or ongoing history of biliary colic/gall stones or pancreatitis or any other major illness or neurological/psychiatric condition.
6. Patients on antidepressants or other medications, which might affect the 5-HT system, gastrointestinal motility or visceral sensation such as prokinetics, 5-HT agonist or antagonists, serotonin re-uptake inhibitors or tricyclic antidepressants/analgesics.

7. Patients who were fully dependent on laxatives and would not be able to stop laxatives during the study period, or had features or a diagnosis of obstructed defaecation/megarectum.

8. Patients who could not understand the requirements and provide written informed consent for the study.

9. Participated in any drug trial within previous 30 days.

2.3.2 For Healthy Volunteers:

1. Subjects who had features suggestive of a functional bowel disorder as assessed using the Rome III criteria/questionnaire.

2. Pregnant or breast-feeding.

3. BMI of <18 or >30.

4. Smoking >10 cigarettes a day or drinking more than recommended units of alcohol (18 units/ week) or had history of drug abuse.

5. Participated in any drug trial within previous 30 days.

Study was approved by South Manchester Ethics Committee and all subjects provided written informed consent (ethics approval number 06/Q1403/201).

2.4 Power of the Study

Assuming a three-group comparison was the main objective, and ANOVA is used followed by appropriate multiple comparison tests, then the study had an 80% power to detect effect sizes of 1.0 or more between the two groups of size 23, and to detect effect sizes of 1.2 or
more between the two groups of size 11 and 23 (based on a simple t-test with 2% significance level to adjust for multiple testing).

2.5 Study Protocol

As previous data have shown that female sexual hormones and the menstrual cycle can influence the 5-HT system (Houghton, Brown et al. 2009), abdominal symptoms (Whitehead, Cheskin et al. 1990), and visceral sensitivity (Houghton, Lea et al. 2002), all physiological assessments were carried out either during the luteal phase (18th-27th day of menstrual cycle; high progesterone and oestrogen) of the menstrual cycle or any time of the cycle, if subjects were on contraceptives. See Figure 2.2 for visit schedule and study time line.

2.5.1 Assessment of Baseline Symptoms

All subjects completed a baseline symptom diary for 7-9 days, in which they scored the severity of their abdominal pain, bloating and bowel urgency every evening using a 6-point scale (0= none, 1= very mild, 2= mild, 3= moderate, 4= quite severe, and 5= very severe) and indicated the time and consistency (Bristol Stool Scale: 1= separate hard lumps, like nuts (hard to pass), 2= sausage shaped but lumpy, 3= like a sausage but with cracks on its surface, 4= like a sausage or snake, smooth and soft, 5= soft blobs with clear cut edges (passed easily), 6= fluffy pieces with ragged edges, a mushy stool, 7= watery, no solid pieces, entirely liquid) of any bowel movement (O'Donnell, Virjee et al. 1990).

Subjects were also asked to grade symptoms associated with each bowel motion such as straining, urgency and incomplete evacuation using the 6-point scale (0= none, 1= very mild, 2= mild, 3= moderate, 4= quite severe, and 5= very severe) (see Appendix 2 for symptoms diary 7-days).
All subjects also filled in the hospital anxiety and depression scale questionnaire at the beginning of the study (see Appendix 3 for HAD Scale questionnaire).

2.5.2 Blood Samples for 5-HT and 5-HIAA

Subjects were asked to stop cigarette smoking and advised not to do vigorous physical activity for 48 hours prior to the study. They were also asked not to consume alcohol or caffeine containing drinks 24 hours prior to the blood samples for 5-HT measurement.

After an overnight fast (minimum 8 hours), subjects attended the Neurogastroenterology Unit (Visit 2). A cannula was placed in one of the arm veins and nine millilitres of blood taken via an EDTA vacutainer for platelet count and 5-HT/5-HIAA analysis (9ml). Further 6 ml blood samples for 5-HT and 5-HIAA analysis were then taken at half-hourly intervals for 2 hours under fasting conditions and at half hourly intervals for a further 4 hours following ingestion of a standard carbohydrate rich meal consisting of 200g spaghetti (Heinz, Stockley Park, Uxbridge, UK), 2 medium slices of toast (Warburton, UK), a jam and fresh cream scone (Finest Cream Scone from Tesco, UK), and 200ml water (totalling 72.4g carbohydrate, 20.3g protein, 11.1g fat, calorie content of 493kcal) which was consumed within 10 minutes. All blood samples were processed immediately and platelet depleted plasma stored at -80°C. An aliquot of the first sample with platelet rich plasma was processed and sent for platelet counts in the central lab on the same day.

2.5.3 Platelet 5-HT Concentrations

The first blood sample was transferred to a tube containing 0.9 mL 3.12% trisodium citrate and initially spun only once at 900 rpm for 5 minutes. One aliquot of aspirated, platelet-rich plasma was then used to assess platelet count, whereas additional duplicate samples were stored at -80°C to be used later for 5-HT analysis in platelet rich plasma. The remaining
sample was processed by further centrifugation (as below) for analysis of platelet depleted plasma 5-HT/5-HIAA concentrations

2.5.4 Platelet Depleted Plasma 5-HT and 5-HIAA Concentrations

The collected blood samples were transferred to tubes containing 0.5ml of 3.12% trisodium citrate and centrifuged (room temperature) twice to ensure no platelet contamination of the supernatant; initially at 2500 rpm for 10 minutes and then at 4000 rpm for a further 10 minutes. Platelet depleted plasma was then aspirated and duplicate samples were stored at -80°C for later batch analysis. All samples were analysed blind to the subject status. The methodology of collecting, processing and storing blood samples has been validated for its accuracy and used in various other studies performed at our centre (Houghton, Atkinson et al. 2003; Atkinson, Lockhart et al. 2006; Houghton, Atkinson et al. 2007). 5-HT and 5-HIAA concentrations were measured in duplicate using liquid chromatography tandem mass spectrometry (Monaghan, Brown et al. 2009).

2.5.5 Assessment of Pre and Post-prandial Symptoms

Throughout the study, at hourly intervals (-2, -1, 0(i.e. immediately after meal), 1, 2, 3 and 4 hours), subjects were asked the question ‘How would you rate your abdominal discomfort/pain and bloating over the last one hour?’ and were asked to grade the severity of these symptoms using the 6-point scale as described above (see Appendix 4 for pre and postprandial symptoms diary).

2.5.6 Whole Gut (Colonic) Transit Time

On the first study day (visit 2), all subjects were given 3 sets of 24 radiopaque markers to take home with written instructions on how to take them. The three types of markers were
cut from polyethylene tubing (Portex Ltd) to be cylinders of identical mass. The external
diameters, internal diameters and length of the cylinders were: (i) 4.5 x 3 x 1.3mm; (ii) 3 x
2 x 3mm and (iii) 2 x 1 x 5mm. On the next day (day 1, at home) subjects were asked to
swallow the first set of 24 markers, the second set on day 2 (at home), and the third set on
day 3 (at home); each with 100ml of water at 08:30hrs, with the exact time being recorded
by the subjects. On day 4, after an overnight fast, the subjects came back to hospital at 8
am and a single abdominal x-ray was done by 08:30 hrs.

2.5.7 Oro-caecal Transit Time

After the abdominal x-ray, the patient came to the Neurogastroenterology unit for
assessment of oro-caecal (mouth to caecum) transit time. Subjects were given 1%
chlorhexidine mouthwash (Corsodyl, GSK Consumer Healthcare, UK). Patients were provided
with a standard meal consisting of 30 gms of dry potato flakes reconstituted with 150ml of
water (Smash, HL Foods Ltd, UK; 107 Kcal) and 120 gms of baked beans (HJ Heinz Co Ltd,
UK; 83 Kcal) to be consumed within 5 minutes with 50ml of water, and the mouthwash
repeated. Expiratory breath samples were taken twice, at a 10 minutes interval before
ingestion of the meal and at 10 minutes intervals thereafter for a maximum 8 hours for
analysis of breath hydrogen content using a breath hydrogen monitor containing an
electrochemical detector (Bedfont EC60 Gastrolyzer2).

2.5.8 Assessment of Rectal Sensorimotor Functions

Within 14 days of oro-caecal transit assessment and after an overnight (minimum 8 hours)
fast, subjects returned to the unit to have their rectal sensitivity assessed. However, four
subjects had their menstrual cycle early and assessment was delayed until the next luteal
phase of their menstrual cycle. Following a Fleet® phosphate enema (Laboratorios Casen-
Fleet SA, Zaragoza, Spain), a catheter (customised rectal barostat catheter (part no. C7-
2CBR-22F, MUI Scientific, Mississauga, ON, Canada), to which a polyethylene bag (Pillow
Type Rectal Barostat Balloon, part no. CT-BP600R; length, 22 cm; diameter, 15 cm; capacity
600 mL; MUI Scientific) was attached and was placed in the rectum. The balloon was then inflated gradually with 200ml of air using a syringe, to unfold it, and then deflated. The subjects were then allowed a 20-30 minute recovery period. To minimise the effect of any pressure from the abdominal viscera on the readings, subjects were placed in a semi prone position with the foot end of the bed raised by 15\(^\circ\). After a 20–30 min recovery period, the catheter was connected to a barostat (G&J Electronics Inc., Toronto, ON, Canada).

The basal operating pressure (BOP) was then assessed by increasing the pressure in the bag from 4 mmHg, in 2 mmHg increments, until respiratory artefact was observed (+2 mmHg), or 18 mmHg had been reached (16+2 mmHg). If respiratory artefacts were not seen by 18 mmHg, BOP was set at 12 mmHg. Following the identification of BOP, isobaric phasic distensions were performed (increments of 4 mmHg, 2 minutes with 2 minutes return to BOP in between), until a maximum pressure of 56 mmHg was reached (inclusive of BOP), or the patient reported a pain score of 3.

During each inflation from BOP, 60 seconds after commencement of the inflation, subjects were prompted to indicate on a standard proforma, whether they were experiencing either the sensation of ‘stool’ or ‘pain/discomfort’. For each of these, they were required to score the level of sensation using the scales below:

**Stool Sensation:** 0 = no sensation; 1 = 1st sensation; 2 = constant sensation/gas; 3 = feeling of a need to defaecate; and 4 = urgent need to defaecate.

**Pain/discomfort:** 0 = no pain/discomfort; 1 = mild but not sustained pain/discomfort; 2 = mild but sustained pain/discomfort; 3 = moderate pain; 4 = intense pain.

In an attempt to counteract for vigilance and make distension sequence unpredictable to the subject, tracking of the distension sequence was commenced when the subject first experienced moderate pain (score 3) (Whitehead and Delvaux 1997). Subsequent distensions were then adjusted up or down depending on the subject’s response to the previous distension; if the subject reported pain score 3 on the previous trial, the next distension pressure was decreased or kept the same; if the subject reported no pain or <3 on the previous trial, the next distension was increased or kept the same. In order to make the changes in the amount of distension unpredictable, a random numbers table was used.
to decide whether to increase the distension, or keep it the same on the next trial after a non-painful test trial. Similarly, a random numbers algorithm was used to decide whether to decrease the amount of distension, or keep it the same following a painful test trial (these are preinstalled in the barostat machine software). The threshold was defined as the average intensity over a series of trials on which one tracks (i.e. makes adjustments that are slightly above and slightly below) the threshold.

The distension test was stopped after 15 distension trials or after reaching the upper limit of 56 mmHg (inclusive of BOP) without pain sensation. None of the distension was stopped for pain >3. The distension pattern or timing was not revealed to the subject and communication during distension was kept to a minimum. Both the subject and the investigator could terminate the inflation for any reason by pressing the ‘panic button’ on the response controller.

2.5.9 Normal Sensory Reference Range

Reference for sensory threshold for pain (score of 3 = moderate pain) was derived by performing the rectal barostat test on 23 healthy volunteers.

2.6 Data and Statistical Analysis

2.6.1 Analysis of Symptoms

1. Abdominal Pain Score was calculated by averaging the pain score reported by subjects during the 7 day diary period.

2. Bloating Score was calculated by averaging the abdominal bloating score reported by subjects during the 7-day diary period.

3. Overall Abdominal Symptom Score was calculated by the sum of abdominal pain and bloating scores.
4. Bowel Frequency was calculated as the number of days bowels opened per week during the diary period, without the use of laxatives.

5. Average Stool Consistency was calculated by first calculating the average of stool consistency for each day, if bowels were opened more than once, and then the average of stool consistency over seven days by dividing the sum of the average daily stool consistency score by the number of days bowels opened during the 7-day diary period.

6. Straining score was calculated by dividing the sum of average straining associated with bowel motion/motions on each day, by the number of days bowels were opened during the 7-day diary period.

7. Urgency Score was calculated by dividing the sum of average urgency associated with bowel motion/motions on each day, by the number of days bowels were opened during the 7-day diary period.

8. Sense of Incomplete Evacuation Score was calculated by dividing the sum of average sense of incomplete evacuation score associated with bowel motion/motions on each day, by the number of days bowels were opened during the 7-day diary period.

9. Overall Symptoms Score for bowel movement associated symptoms was calculated by adding the individual symptoms score (straining, urgency and sense of incomplete evacuation score).

10. Pre-prandial Overall Symptom Score was calculated by the mean of abdominal pain and bloating score recorded prior to the test meal (two observations).

11. Post-prandial Overall Symptom Score was calculated by the mean of abdominal pain and bloating score recorded after the test meal (five observations).

12. Change in Symptoms was the difference between pre and post prandial overall symptom score.

13. HAD score was calculated for anxiety, depression and combined score. Scores >7 for individual anxiety or depression and a combined score of >14 was considered abnormal (Zigmond and Snaith 1983).
2.6.2 Analysis of 5-HT and 5-HIAA Results

1. Fasting 5-HIAA and PDP 5-HT was calculated as the mean of three fasting values taken over one and a half hours prior to meal.

2. Post prandial PDP 5-HT and 5-HIAA was measured by calculating AUC for all postprandial PDP 5-HT/HIAA values from samples taken over 4 hours after the meal.

3. Platelet rich plasma 5-HT was calculated from the platelet rich plasma sample and platelet counts were also done from this sample (first sample).

4. 5- HIAA/5-HT ratio was calculated from pre and postprandial 5- HIAA and PDP 5-HT concentrations. Fasting ratio was the mean of three pre-meal ratios and postprandial ratio was the mean of eight postprandial ratios.

The 5-HT and 5-HIAA measurement was done in the biochemistry department of central laboratories of University Hospital of South Manchester by senior biochemists Mr Phillip Monaghan and Ms Jo Adaway.

2.6.3 Analysis of Oro-caecal Transit Time

A rise in breath hydrogen of 10 parts per million (ppm) above the baseline and sustained for 20 minutes (i.e. 3 consecutive recordings) was taken as the arrival of the head of the meal in the caecum, based on principles first described by Levitt (Levitt 1969). If there was no rise in breath hydrogen after the meal for eight hours, the oro-caecal transit time was noted as >480 minutes and subjects were sent home. The 97.5\textsuperscript{th} percentile of oro-caecal time in healthy volunteers was taken as the upper limit of normal.
2.6.4 Analysis of Whole Gut (Colonic) Transit Time

1. The markers were counted on the x-ray and colonic (whole gut) transit time was calculated by using a modified protocol, based on the method first described by Metcalf and colleagues (Metcalf, Phillips et al. 1987), as used in previous studies (Agrawal, Houghton et al. 2009).

2. Segmental transit time was assessed by drawing an imaginary line joining the spinal process up to the fifth lumbar vertebra, and then two lines from the spinous process of the fifth lumbar vertebra, to pelvic outlets. The number of markers to the right and left of the central line joining the spinal processes were defined as right and left segmental colonic transit time and the number of markers in between two lines to pelvic outlet was defined as recto-sigmoid colonic transit time (Pomerri, Frigo et al. 2007) (see figure 2.1).

3. In addition, the 97.5th percentile of colonic transit time in healthy volunteers was taken as the upper limit of normal. Constipation patients with colonic transit time greater than 97.5th percentile of healthy volunteers were classified as slow transit constipation (STC) and rest as the normal transit constipation (NTC).

Figure 2.1: Abdominal radiograph of one of the volunteers with constipation on the 4th day of whole gut transit assessment. Spinal process and imaginary line from fifth lumbar vertebra to pelvic outlet serving as landmarks defining projection zones for right (R), left (L) and recto-sigmoid (RS) (Pomerri, Frigo et al. 2007).
2.6.5 Analysis of Rectal Sensorimotor Functions

The following measurements were derived using the Protocol Plus software package (G&J Electronics Inc. Toronto, Canada) from the rectal sensitivity study:

1. Visceral (pressure and volume) sensory thresholds were recorded for first sensation and stools sensation (for stool sensation score 3).

2. The pain threshold was calculated as the average pressure and volume over the series of tracking inflations commenced with the first reporting of pain score 3 (moderate pain) by the subject.

3. Static compliance was calculated as the volume/pressure relationship at 20mm Hg (Houghton, Lea et al. 2002; Lea, Houghton et al. 2003).

4. Dynamic compliance was calculated from the slope of the compliance curve (Houghton, Lea et al. 2002; Lea, Houghton et al. 2003).

5. Subjects with constipation (IBS-C and FC) were classified as either hypersensitive, normosensitive or hyposensitive, using a 95% reference range for pain threshold pressure for the healthy volunteers. Patients with constipation with pain threshold below 2.5th percentile were classified as hypersensitive and above 97.5th percentile were classed as hyposensitive.

6. Subjects with constipation (IBS-C and FC) were divided in terciles based on pain threshold pressure. Subjects with pain threshold below 33.3 percentiles were categorised with high visceral sensitivity and above 66.33 percentiles were categorised as low visceral sensitivity and between these groups as medium visceral sensitivity.
2.7 **Statistical Analysis**

Statistical software package SPSS version 15.0 was used to record data and for statistical analysis. Comparisons of baseline characteristics, symptoms, 5-HT/5-HIAA concentrations, transit times and rectal sensitivity thresholds for three groups (HV, IBS-C and FC) were made using one-way ANOVA, with Bonferroni corrections where appropriate, for post hoc multiple group comparisons or Kruskal-Wallis test and Mann-Whitney test with adjustment for multiple testing. Whereas, the independent t-test was used to make comparisons between two study groups and paired sample t-test or Wilcoxon Signed Ranks test to assess any changes in the same study group. Pearson or Spearman’s correlation and regression analysis were applied, where indicated.
Figure 2.2 Flow Chart for Study Visits

**V₁ Overnight Fasted**
- Blood -120 min ——— Questionnaire
- Blood -90 min
- Blood -60 min ——— Questionnaire
- Blood -30 min
  - Meal — 0 min (Sphegati, toast, cream cones)
  - Blood 30 min
  - Blood 60 min ——— Questionnaire
  - Blood 90 min
  - Blood 120 min ——— Questionnaire
  - Blood 150 min
  - Blood 180 min ——— Questionnaire
  - Blood 210 min
  - Blood 240 min ——— Questionnaire
- TAKE MARKERS HOME

**V₂ Overnight Fasted**
- Blood -120 min ——— Questionnaire
- Blood -90 min
- Blood -60 min ——— Questionnaire
- Blood -30 min
  - Meal — 0 min (Sphegati, toast, cream cones)
  - Blood 30 min
  - Blood 60 min ——— Questionnaire
  - Blood 90 min
  - Blood 120 min ——— Questionnaire
  - Blood 150 min
  - Blood 180 min ——— Questionnaire
  - Blood 210 min
  - Blood 240 min ——— Questionnaire
  - TAKE MARKERS HOME

**V₃ Overnight Fasted**
- ABD X ray - 30 min
- Chlorhexidine Mouthwash
- Hydrogen Breath Test - 10 min
- SBT Study Meal 0 min (Mashed potatoes and baked beans)
- Breath Test — EVERY 10 MIN
- Breath Test
- Breath Test
- Breath Test
- Breath Test
- Breath Test

**V₄ Overnight Fasted**
- Fleet Enema
- Barostat Testing

**Diary** for 7-9 days
Chapter 3

Results
3.1 Baseline Characteristics

Table 3.1 shows the baseline characteristics for height, weight and BMI in patients with IBS-C and FC and healthy volunteers. Age differed between the three groups (p=0.01), with the mean age of the FC patients being significantly greater compared with the healthy volunteers (p=0.009) but not with IBS-C patients (p=0.1). There was no significant difference in the mean age between IBS-C patients and healthy volunteers (p=0.1).

In addition, there were significant differences in anxiety and depression scores between the three groups (p<0.001). Both anxiety and depression scores were higher in IBS-C patients compared with healthy volunteers (p<0.001). The depression and anxiety scores were also higher in patients with FC compared with healthy volunteers but did not reach statistical significance (p=0.06 and 0.1, respectively). There was no difference in either score between the IBS-C and FC patients.

Table 3.1 Important Baseline Characteristics of the HV, Patients with IBS-C and FC Groups

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>27.4 (23.3, 31.4)</td>
<td>31 (26.6, 35.4)</td>
<td>38.1 (32.8, 43.4)*</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Height (meters)</strong></td>
<td>1.6 (1.6, 1.6)</td>
<td>1.63 (1.6, 1.7)</td>
<td>1.6 (1.6, 1.7)</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>61.7 (59.3, 64.2)</td>
<td>63.6 (59.8, 67.3)</td>
<td>63.9 (59, 68.9)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>23 (21.9, 24)</td>
<td>24 (22.7, 25.2)</td>
<td>23.2 (21.7, 24.7)</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>HAD Anxiety Score</strong></td>
<td>1 (0, 13)</td>
<td>8 (1, 13)*</td>
<td>5 (1, 9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HAD Depression Score</strong></td>
<td>0 (0.5)</td>
<td>3 (0,17)*</td>
<td>2.5 (0,6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aData expressed as mean (95% confidence interval for mean). bData expressed as median (range). *IBS-C and FC patients compared with healthy volunteers p <0.05.
3.2 Symptoms

3.2.1 Baseline Symptoms (during 7-day diary)

There was a significant difference in overall abdominal symptom score (pain and bloating) between the three groups during the 7 day diary period (<0.001). The overall abdominal symptom score was significantly greater in the IBS-C patients compared with both healthy volunteers (p<0.001) and FC patients (p<0.006) (see table 3.2). Similarly, there was significant difference in the overall abdominal symptom score between patients with FC and the healthy volunteers (p<0.001). The IBS-C patients reported greater abdominal pain compared with both FC patients and healthy volunteers (p<0.009 and p<0.001, respectively). Likewise, they also reported more bloating compared with FC patients and healthy volunteers (p<0.001). Similar to IBS-C patients, the patients with FC also reported more abdominal pain and bloating scores compared with healthy volunteers (p<0.001).

There was also a significant difference in the bowel frequency and the stool consistency (BSS) between the three groups (p<0.001 and p=0.007, respectively). The IBS-C patients opened their bowels less frequently and reported harder stools compared with the healthy volunteers (p<0.001 and p=0.009). Similarly, the FC patients opened their bowels less frequently compared with the healthy volunteers (p<0.001) and showed a trend towards lesser bowel frequency compared with the IBS-C patients (p=0.1). However, there was no significant difference in the stool consistency between FC and IBS-C patients, or between patients with FC constipation and the healthy volunteers (p=0.5 and p=1).

There was a significant difference in the overall bowel movement associated symptom scores (i.e. straining, urgency and sense of incomplete evacuation) (p<0.001) and the individual symptoms of straining (p<0.001) and sense of incomplete evacuation (p<0.001) but not sense of urgency between the three groups (see table 3.2).

Both IBS-C and FC patients had more overall bowel movement associated symptom score compared with the healthy volunteers (p<0.001). The IBS-C patients also reported higher overall abdominal symptom score compared with the patients with FC (p=0.04).
straining score was also significantly higher in both IBS-C and FC patients compared with the healthy volunteers (p<0.001 and 0.01, respectively). However, there was no difference in the straining score between IBS-C and FC patients (p=1).

Similarly, IBS-C and FC patients had a greater sense of incomplete evacuation compared with the healthy volunteers (p<0.001). There was no significant difference in sense of urgency scores reported by three subject groups (p=0.07).
Table 3.2 Baseline Symptoms Profile of the HV, Patients with IBS-C and FC Groups (7 Day Diary Period)

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Abdominal Symptom Score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2 (2,5.6)</td>
<td>7.8 (5.4,10.6)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>5 (2.1, 9)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal Pain Score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 (1,2.6)</td>
<td>3.7 (2.9,5)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>2 (1,4.4)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bloating Score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1 (1,3)</td>
<td>4.1 (2.6,5.7)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>2.4 (1.1, 4.6)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bowel Frequency (Bowel motions /week)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 (5, 7)</td>
<td>4 (1,7)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>3 (1,7)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average Stool Consistency (BSS)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.4 (1.9, 48)</td>
<td>2.2 (1,5)&lt;sup&gt;#&lt;/sup&gt;</td>
<td>2.2 (1,5)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

**Bowel Movement Associated Symptoms**

| Overall Symptoms Score<sup>b</sup> | 2 (1,5.6) | 9 (1.5, 12.2)<sup>#</sup> | 5.3 (1.4,9)<sup>*</sup> | <0.001 |
| Straining Score<sup>b</sup> | 0.5 (0,2.5) | 3.3 (0,5)<sup>#</sup> | 3 (0,5)<sup>*</sup> | <0.001 |
| Sense of Incomplete Evacuation Score<sup>b</sup> | 0 (0,1.8) | 2.9 (0,5)<sup>#</sup> | 2 (0,3.8)<sup>*</sup> | <0.001 |
| Stool Urgency Score<sup>b</sup> | 1.2 (1,2.7) | 2 (1,4.4) | 1.4 (1,2.1) | 0.07 |

<sup>b</sup>Data expressed as median (range).  ^ IBS-C patients compared with healthy volunteers p <0.01,  # IBS-C patients compared with FC patients p<0.04,  * FC patients compared with healthy volunteers p<0.01
3.2.2 Constipation Severity

A similar proportion of patients with IBS-C 43.5% (10/23) and FC 45% (5/11) reported severe constipation (based on average Bristol Stool Score <2 on 7-day diary).

3.2.3 Study Day Pre and Post-prandial Symptoms

There were significant differences in both pre and post prandial overall abdominal symptom (pain and bloating) scores, as well as change in this score with meal ingestion between the three groups (<0.001) (see table 3.3). The IBS-C patients had significantly greater pre and postprandial abdominal symptoms compared with healthy volunteers (p<0.001). Moreover, postprandial abdominal symptoms were higher in the IBS-C patients compared with FC patients (p=0.01) as well as a trend toward higher preprandial symptoms (p=0.1). On paired sample analysis, IBS-C patients reported a significant increase in abdominal symptoms after meal (p<0.001) but no significant change in symptoms score was observed in healthy volunteers or patients with FC.
Table 3.3 Study Day Pre and Post-prandial Symptoms

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-prandial Overall</td>
<td>0 (0,1.5)</td>
<td>1.5 (0,6) ^</td>
<td>0 (0,2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Symptom Score b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-prandial Overall</td>
<td>0 (0,1.2)</td>
<td>4.4 (0,9) ^#</td>
<td>1.2 (0, 3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Symptom Score b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in Symptoms</td>
<td>0 (-1.1,1.2)</td>
<td>1.6 (-1.4,7)*</td>
<td>0 (-2,3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>after Meal b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data expressed as median (range). ^ IBS-C patients compared with healthy volunteers p <0.001, # IBS-C patients compared with FC patients p=0.01.
*Change in symptoms from pre to postprandial state (p<0.001)

3.3 Platelet Depleted Plasma 5-HT Concentrations

There was no significant difference in either fasting or change in PDP 5-HT concentrations from the fasting to postprandial periods (p=0.1). However, there was a significant difference in postprandial PDP 5-HT concentrations between the three groups (p=0.02) (see table 3.4). The postprandial 5-HT concentration in patients with FC was higher compared with IBS-C patients (p=0.03). The fasting PDP 5-HT concentration tended to be higher in patients with FC compared with HV (0.2).

On paired sample sub-group analysis, patients with FC and IBS-C appeared to have no 5-HT response to meal ingestion whereas, the healthy volunteers showed a trend towards increase in PDP 5-HT concentration (p=0.1).
Table 3.4 Platelet Depleted Plasma 5-HT Concentrations

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>**Fasting PDP 5-HT (nmol/l)**b</td>
<td>23.9 (14.4, 51.5)</td>
<td>26.4 (8.9, 95.2)</td>
<td>30.6 (19.7, 64.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>**Postprandial PDP 5-HT (nmol/l)**b</td>
<td>28.2 (15.4, 66.4)</td>
<td>20.9 (9.6, 83.9)</td>
<td>30.4 (14.7, 67.8)*</td>
<td>0.02</td>
</tr>
<tr>
<td>**Change from fasting to postprandial PDP 5-HT (nmol/l)**a</td>
<td>3.9 (0.1, 7.8)</td>
<td>-2.7 (-5.3, 1)</td>
<td>-0.9 (-5.9, 4)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*a Data expressed as mean (95% confidence interval for mean). *b Data expressed as median (range). *FC patients compared with IBS-C patients (p<0.03)

3.4 Platelet Depleted Plasma 5-HIAA Concentrations

The fasting, postprandial and change from fasting to postprandial plasma 5-HIAA concentrations were similar among IBS-C and FC patients and healthy volunteers (see table 3.5, figures 3.1).
Table 3.5 Platelet Depleted Plasma 5-HIAA Concentrations

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting 5-HIAA (nmol/l)</td>
<td>21.1 (18.5, 23.8)</td>
<td>22.6 (19.9, 25.3)</td>
<td>18.9 (15.6, 22.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>Postprandial 5-HIAA (nmol/l)</td>
<td>19.3 (16.8, 21.8)</td>
<td>20.7 (18.3, 23)</td>
<td>18 (14.4, 21.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Change from fasting to postprandial 5-HIAA (nmol/l)</td>
<td>-1.7 (-2.6, -1.0)</td>
<td>-1.8 (-2.7, -1.0)</td>
<td>-0.9 (-2.3, 0.5)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Data expressed as mean (95% confidence interval for mean)

Figure 3.1 Fasting and postprandial plasma 5-Hydroxyindoleaceticacid (5-HIAA) concentrations in healthy volunteers (HV), patients with irritable bowel syndrome with constipation (IBS-C) and functional constipation (FC). Bar represents 95% CI for fasting and postprandial PDP 5-HIAA with horizontal line representing mean for the group.
3.5 5-HIAA/ 5-HT Ratio

The fasting 5-HIAA/ 5-HT ratio was lower in FC patients compared with both the IBS-C patients (p=0.01) and healthy volunteers (the latter did not reach statistical significance, p=0.2) (see table 3.6). However, there was no difference in the fasting 5-HIAA/5-HT ratio between IBS-C patients and healthy volunteers (p=0.8). Whereas, the postprandial 5-HIAA/HT ratio was higher in the IBS-C patients compared with both the healthy volunteers and FC patients (p=0.01 and p=0.007). There was no difference in the fasting and the postprandial 5-HIAA/5-HT ratio between the FC patients and healthy volunteers (p=0.2 and p=1).

Table 3.6 5-HIAA/ 5-HT Ratios in the HV and Patients with IBS-C and FC

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting 5-HIAA/5-</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HT Ratio</strong></td>
<td>0.9 (0.7, 1)</td>
<td>1 (0.8, 1.2)</td>
<td>0.6 (0.5, 0.7)#</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Postprandial 5-</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIAA/ 5-HT Ratio</strong></td>
<td>0.7 (0.6, 0.8)</td>
<td>0.9 (0.8, 1)#</td>
<td>0.6 (0.4, 0.7)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data expressed as mean (95% confidence interval for mean). ^ IBS-C patients compared with healthy volunteers p <0.01, # IBS-C patients compared with FC patients p<0.01,

3.6 Platelet 5-HT Concentrations

There was no significant difference in the platelet 5-HT concentrations between patients with IBS-C (5.9 nmol/10^9 platelets (4.9, 7.0)), FC (7.9 nmol/10^9 platelets (5.5, 10.3)) and the healthy volunteers (6.9 nmol/10^9 platelets (5.6, 8.2)) (p=0.1).
3.7 Transit

3.7.1 Oro-caecal Transit Time

The oro-caecal transit time was similar in the three groups (p=0.8). Using 97.5\textsuperscript{th} percentile of oro-caecal transit time in the healthy volunteers (480 minutes), only 4/23 (17.4\%) patients with IBS-C and 2/11 (18.1\%) patients with FC had delayed oro-caecal transit. (see table 3.7)

3.7.2 Whole Gut (Colonic) Transit Time

The colonic transit time was significantly different between the three groups (p<0.001) (see table 3.7) The IBS-C and FC patients had significantly slower colonic transit compared with the healthy volunteers (p=0.001). However, there was no difference in colonic transit time between IBS-C and FC patients (p=1.0).

The transit was particularly slower on the right side of the colon in IBS-C and FC patients compared with the healthy volunteers (p<0.001). In IBS-C and FC patients it was also delayed in the left colon compared with healthy volunteers however, it did not reach statistical significance in the latter (p=0.008 and p=0.1). There was no difference in the right and left segmental transit time between IBS-C and FC patients (p=1). The recto-sigmoid colonic transit time was similar in the IBS-C, FC patients and healthy volunteers (p=0.5).
### Table 3.7 Oro-caecal and Colonic (whole Gut) Transit Times

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oro-caecal Transit Time (minutes)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>297 (125,480)</td>
<td>290 (175,480)</td>
<td>300 (195,480)</td>
<td>0.8</td>
</tr>
<tr>
<td>Colonic Transit Time (hours)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38 (6,64)</td>
<td>65.5 (24,72)&lt;sup&gt;^&lt;/sup&gt;</td>
<td>71 (13,72)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right Segmental Colonic Transit Time (hours)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 (0,25)</td>
<td>16.5 (3,35)&lt;sup&gt;^&lt;/sup&gt;</td>
<td>21 (8,51)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left Segmental Colonic Transit Time (hours)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9 (0,54)</td>
<td>21 (2,55)&lt;sup&gt;^&lt;/sup&gt;</td>
<td>22 (1,45)</td>
<td>0.01</td>
</tr>
<tr>
<td>Recto-sigmoid Transit Time (hours)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.8 (10.4,19.1)</td>
<td>16.6 (11.7, 21.5)</td>
<td>12.4 (7.3, 17.6)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data expressed as mean (95% confidence interval for mean).  
<sup>b</sup>Data expressed as median (range).  
<sup>^</sup>IBS-C patients compared with healthy volunteers p <0.009,  
<sup>*</sup>FC patients compared with healthy volunteers p<0.001

### 3.7.3 Normal (NTC) and Slow (STC) Transit Constipation

Using 97.5th percentile (64 hours) of colonic transit time in the healthy volunteers, 19/34 (55%) patients had slow colonic transit, of which 12/23 (52%) patients in the IBS-C group and 7/11(64%) patients with FC constipation had slow colonic transit.

### 3.8 Rectal Sensory-Motor Functions

The BOP (mm Hg) and volume at BOP (ml) were similar in the three groups (p=0.9) (see table 3.8).
3.8.1 Rectal Sensory Thresholds: Pressure Thresholds

There was a significant difference in the pain threshold pressure (average of tracking pressures p=0.01) but no difference in the first sensation pressure (p=0.1) and the stool sensation pressure (p=0.1) between the three groups (see table 3.8 and figure 3.2). A multiple comparison between these groups showed IBS-C patients to have significantly lower pain thresholds compared with FC patients and a trend towards lower pain threshold compared with the healthy volunteers (p=0.02 and p=0.07 respectively). There was no significant difference in the pain threshold between the FC patients and the healthy volunteers (p=1.0).

Figure 3.2 Multiple comparisons of different sensory pressure thresholds using ANOVA between healthy volunteers (HV), patients with irritable bowel syndrome with constipation (IBS-C) and functional constipation (FC). Data expressed in bars is 95% confidence interval with horizontal line representing mean. # P =0.01 for pain threshold between HV, IBS-C and FC. * P= 0.05 between FC and IBS-C

3.8.2 Rectal Sensory Thresholds: Volume Thresholds

There was a significant difference in the stool sensation volume (average of tracking volumes readings) but no difference in the pain threshold volume and the first sensation
volume between the three groups (p=0.01, p=0.1 and p=0.2, respectively). The mean stool sensation volume was significantly less in IBS-C patients compared with healthy volunteers and FC patients (p=0.05). The mean stool sensation volume was comparable in FC patients and healthy volunteers (p=1.0). (see table 3.8, figure 3.5)

Figure 3.3 Multiple comparisons of different sensory volume thresholds using ANOVA between healthy volunteers (HV), patients with irritable bowel syndrome with constipation (IBS-C) and functional constipation (FC). Data expressed in bars is 95% confidence interval with horizontal line representing mean. # P = 0.02 for stool sensation between HV, IBS-C and FC, * P = 0.05 between FC and IBS-C, ^ P = 0.05 between IBS-C and FC
Table 3.8 Pressures, Volume Sensory Thresholds and Compliances between Healthy Volunteers and Patients with IBS-C and FC

<table>
<thead>
<tr>
<th></th>
<th>HV (n=23)</th>
<th>IBS-C (n=23)</th>
<th>FC (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BOP (mmHg)</strong></td>
<td>8.9 (7.9, 10)</td>
<td>9.1 (8, 10.2)</td>
<td>9.5 (7.9, 11)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Volume at BOP (ml)</strong></td>
<td>78.5 (60.7, 96.3)</td>
<td>77.3 (52.5, 102.2)</td>
<td>86.9 (41.1, 132.7)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>First Sensation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.3 (10.8, 13.7)</td>
<td>12.5 (10.8, 14.2)</td>
<td>15.6 (10.3, 20.9)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>120.8 (97.6,144)</td>
<td>115 (84.4, 145.7)</td>
<td>157.7 (82.8, 232.5)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Stool Sensation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.9 (22.6, 29.2)</td>
<td>21.3 (17.4, 25.3)</td>
<td>26.6 (18.1, 34.9)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>235.3(200.1,270.4)</td>
<td>168.7(124.1,213.3)^ #</td>
<td>252.4 (189.3,315.6)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Pain Threshold</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.6 (26.5, 32.8)</td>
<td>23.4 (19.8, 27)#</td>
<td>32.7 (24.5, 40.9)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>263.9 (231, 296.9)</td>
<td>217 (179.4,256.6)</td>
<td>262.9 (210.6, 315.1)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Static Compliance (ml/mmHg)</strong></td>
<td>10.2 (8.9, 11.5)</td>
<td>9 (7,10.9)</td>
<td>10.2 (8, 12.3)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Dynamic Compliance (ml/mmHg)</strong></td>
<td>7.9 (6.7, 9.3)</td>
<td>8.3 (6.9, 9.7)</td>
<td>8.6 (6.3, 10.9)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Data expressed as mean (95% confidence interval for mean).  
^ # IBS-C patients compared with healthy volunteers and FC patients p <0.05
3.8.3 Compliance

There was no difference in the static or the dynamic compliance between the three groups (p=0.5 and p=0.8) (see table 3.8, figure 3.4).

![Figure 3.4 Multiple comparisons of the static and the dynamic compliance using ANOVA between the healthy volunteers (HV), patients with irritable bowel syndrome with constipation (IBS-C) and functional constipation (FC). Data expressed in bars is 95% confidence interval with horizontal line representing mean.](image)

3.9 Constipation patients with High Vs Low Rectal Sensitivity

The pain threshold observed in the healthy volunteer with in 2.5th percentile (18mmHg) and 97.5th percentile (42mmHg) range was defined as normal for the purpose of this study.

Patients with constipation (IBS-C an FC) with a pain threshold below 18.0 mmHg (2.5th percentile) were classified as hypersensitive and those above 42 mmHg (97.5th percentile) as hyposensitive. Using these reference values, hypersensitivity was identified in 7 (30.5%) patients in the IBS-C group, but none in the FC group. Furthermore, 1 (4.5%) in the IBS-C group and 3 (27%) in the FC group were hyposensitive, with the remaining 15 (65%)
patients in the IBS-C group and 8 (73%) patients in the FC group classified as normally viscerally sensitive.

As the number of subjects in subgroups was too small, especially in the hyposensitive group to do any meaningful statistical analysis, subjects with constipation (IBS-C and FC) were divided in terciles based on their pain threshold pressure. The constipation patients with pain threshold below 33.3rd percentiles (20.04 mmHg) were categorised as patients with high visceral sensitivity (32.4%, 11/34) and above 66.3 percentiles (29.7 mmHg) were categorised as patients with low visceral sensitivity (32.4%, 11/34) and between these groups with medium visceral sensitivity (35.2%, 12/34).

The patients with constipation at either end of rectal sensitivity were compared to see any similarities and differences in the transit times and symptoms. There was no difference observed in transit times, abdominal symptoms or bowel movement associated symptoms (see table 3.9).
Table 3.9 Oro-caecal and Colonic Transit Times and Symptom Profile of Patients with Constipation with High Vs Low Rectal Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>High Sensitivity (n=11)</th>
<th>Low Sensitivity (n=11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oro-caecal Transit Times (minutes)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>290 (175,480)</td>
<td>345 (195,480)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Colonic Transit Time (hours)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65 (2,72)</td>
<td>69 (13,72)</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Overall Abdominal Symptom Score</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1 (6,8.2)</td>
<td>6.6 (5.1, 8.2)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Abdominal Pain Score</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 (2.8, 4)</td>
<td>3.1 (2.3, 3.9)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Bloating Score</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 (3.1,4.3)</td>
<td>3.4 (2.6,4.2)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Bowel Frequency ( Bowel motions /week)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 (3.2,5)</td>
<td>3.3 (2,4.6)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Average Stool Consistency (BSS)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 (1.6,3.2)</td>
<td>2.9 (1,9,3.9)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Bowel Movement Associated Symptoms**

<table>
<thead>
<tr>
<th>Overall Symptoms Score**&lt;sup&gt;b&lt;/sup&gt;</th>
<th>6.7 (5,3,8.2)</th>
<th>6.2 (4,1,8.2)</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straining Score**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 (1,9,3.2)</td>
<td>2.3 (1,2,3.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>Urgency Score**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 (1,4,2.6)</td>
<td>1.7 (1,2,3)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sense of Incomplete Evacuation Score**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 (0,9,3.1)</td>
<td>2.1 (1,2,3)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data expressed as mean (95% confidence interval for mean).<sup>b</sup> Data expressed as median (range).
3.10 Relationship between Baseline Symptoms and Sensory Thresholds/Compliances

3.10.1 Healthy Volunteers

As expected there was no correlation between symptoms (overall abdominal symptoms or individual abdominal symptoms such as pain or bloating reported during 7 day diary) and sensory thresholds (pain and stool sensation) likewise, no correlation between symptoms and either static or dynamic compliance.

3.10.2 Constipation Patients (IBS-C and FC)

There were positive correlations between the anxiety scores and the overall abdominal symptom scores (p=0.01, R=0.38, R² = 0.14) as well as between the anxiety scores and the individual symptoms of abdominal pain score (p=0.02, R=0.38, R² = 0.14) and bloating score (p=0.03, R=0.37, R²=0.13) in patients with constipation (IBS-C and FC). (See table 3.11 and figures 3.5 and 3.6)
Figure 3.5 Linear regressions between anxiety score (HAD Scale) and average abdominal pain score in patients with constipation (IBS-C and FC). Spearman correlation $p=0.04$
Likewise, depression scores also positively correlated with overall abdominal symptom scores (p=0.04, R=0.36, R²= 0.13) but there was only a trend towards positive correlation with abdominal pain (p=0.1, R=0.31, R² = 0.1) and bloating (p=0.1, R=- 0.39, R²=0.1) in patients with constipation (IBS-C and FC). However, there were no correlations between HAD anxiety or depression scores and sensory thresholds (pain and stool sensation) (see table 3.11).

There was no correlation between overall abdominal symptom or abdominal pain scores and pain threshold. Similarly, there was no correlation between overall abdominal symptom scores or individual abdominal symptoms (pain or bloating) and stool sensation threshold.

Figure 3.6 Linear regressions between anxiety score (HAD Scale) and average bloating score in patients with constipation (IBS-C and FC). Spearman’s correlation p=0.03
Table 3.10 Correlations Between HAD Scores/ Baseline Symptoms and the Pain Threshold in patients with constipation (IBS-C and FC)

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R Square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAD Anxiety Scores and Overall Abdominal Symptoms Scores</td>
<td>0.38</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>HAD Anxiety Scores and Abdominal Pain Scores</td>
<td>0.38</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>HAD Anxiety and Bloating Scores</td>
<td>0.37</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>HAD Depression Scores and Overall Abdominal Symptoms</td>
<td>0.36</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>HAD Depression Scores and Abdominal Pain Score</td>
<td>0.31</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>HAD Depression Scores and Bloating Scores</td>
<td>0.39</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>HAD Anxiety Scores and Pain Threshold (mmHg)</td>
<td>0.14</td>
<td>0.02</td>
<td>0.4</td>
</tr>
<tr>
<td>HAD Depression Scores and Pain Threshold (mmHg)</td>
<td>0.1</td>
<td>0.01</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Linear regression analysis with p values from Spearman’s Correlations

3.11 5-HT Signalling Correlations

3.11.1 Symptoms: 7-day Diary

There was no correlation between the fasting or post prandial PDP 5-HT and the abdominal pain or bloating scores reported by patients with constipation during 7 day diary period. Similarly, there was no correlation between fasting PDP 5-HT concentrations and the stool
consistency or the bowel frequency in patients with constipation. There was a negative correlation between the postprandial PDP 5-HT concentration and the bowel frequency during the 7 day diary period (p=0.03, R=0.4 and $R^2=0.1$), but no correlation with stool consistency. Although, a similar a trend towards week negative correlation was also seen between fasting PDP 5-HT concentration and bowel frequency during the 7 day diary period but not statistically significant (p=0.1, R=0.15 and $R^2=0.02$). (see table 3.11 and figure 3.7)

**Figure 3.7** Linear regressions between bowel frequency (number of days bowel opened during 7-day diary without laxative) and postprandial PDP 5-HT (nmol/l) in patients with constipation (IBS-C and FC). Spearman’s correlation p=0.03
Table 3.11 Correlations between PDP-5HT Concentrations and Baseline Symptoms During 7-day Diary Period

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R Square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting PDP 5-HT and Abdominal Pain Score</td>
<td>0.1</td>
<td>0.02</td>
<td>0.7</td>
</tr>
<tr>
<td>Fasting PDP 5-HT and Abdominal Bloating Score</td>
<td>0.1</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>Post Prandial 5-HT and Abdominal Pain Score</td>
<td>0.2</td>
<td>0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>Post Prandial 5-HT and Abdominal Bloating Score</td>
<td>0.03</td>
<td>0.001</td>
<td>0.6</td>
</tr>
<tr>
<td>Fasting PDP 5-HT and Stool Consistency</td>
<td>0.1</td>
<td>0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>Fasting PDP 5-HT and Bowel Frequency</td>
<td>0.1</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>Post Prandial PDP 5-HT and Stool Consistency</td>
<td>0.02</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Post Prandial PDP 5-HT and Bowel Frequency</td>
<td>0.38</td>
<td>0.14</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Linear regression analysis with p values from Spearman’s Correlations

3.11.2 Symptoms: study day

On the study day for plasma sample collection for 5-HT before and after meal, there was no correlation between fasting PDP 5-HT concentrations and preprandial symptoms or postprandial PDP 5-HT concentrations and postprandial symptoms on the study day. There was no correlation between the change in PDP-5-HT concentrations and change in symptoms after meal on the study day (see table 3.12).
3.11.3 Transit

There was no correlation between the fasting or post prandial PDP 5-HT or HIAA concentrations and oro-caecal or colonic transit times in patients with constipation. Moreover, grouping constipation patients based on colonic transit time, the fasting and postprandial PDP 5-HT concentrations were similar in patients with normal (NTC) and slow transit constipation (STC). However, there was decrease in PDP 5-HT after meal in patients with NTC compared with STC (p=0.05) (see table 3.13).

There was no difference in the fasting, postprandial or change from fasting to post-prandial 5-HIAA concentrations or 5-HIAA/5-HT ratio between patients with NTC and STC (data not shown here).

Table 3.12 Correlations between PDP 5-HT Concentrations and Symptoms on the Study Day

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R Square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting PDP 5-HT and pre-prandial symptoms</td>
<td>0.1</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>Post-prandial PDP 5-HT and post-prandial Symptoms</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Change in PDP 5-HT and change in symptoms after test meal</td>
<td>0.02</td>
<td>0.001</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Linear regression analysis with p values from Spearman’s Correlations
3.11.4 Rectal Sensory Thresholds

There were positive correlations between the pain threshold pressure and both fasting (R=0.5, R square=0.3, p=0.003) and postprandial PDP 5-HT concentrations (R=0.4, R square=0.2, p=0.01) in patients with constipation (IBS-C and FC). (see table 3.14 and figures 3.8 and 3.9)
Table 3.14 Correlation between PDP 5-HT Concentrations and Pain Threshold in Patients with Constipation (IBS-C and FC)

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R Square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting PDP 5-HT and Pain Threshold (mm Hg)</td>
<td>0.5</td>
<td>0.25</td>
<td>0.007</td>
</tr>
<tr>
<td>Post-prandial PDP 5-HT and Pain Threshold (mm Hg)</td>
<td>0.4</td>
<td>0.18</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Linear regression analysis with p values from Spearman’ Correlations

Figure 3.8 Linear regressions between fasting PDP 5-HT (nmol/L) and pain threshold (mmHg) in patients with constipation (IBS-C and FC). Spearman’s Correlation p=0.007
**Figure 3.9** Linear regressions between postprandial PDP 5-HT (nmol/L) and pain threshold (mmHg) in patients with constipation (IBS-C and FC). Spearman’s Correlation p=0.02
3.11.5 Fasting and Postprandial PDP 5-HT concentration in Constipation Patients (IBS-C and FC) with High Vs Low Sensitivity

There was a significant difference in the fasting (p=0.01) and in postprandial PDP 5-HT concentrations (p=0.02) in patients with high rectal sensitivity compared with low rectal sensitivity with latter group having higher fasting and postprandial PDP 5-HT concentrations.

Table 3.15 Fasting and Postprandial PDP 5-HT Concentration in Constipation Patients (IBS-C and FC) with High Vs Low Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>High Sensitivity (n=11)</th>
<th>Low Sensitivity (n=11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting PDP 5-HT (nmol/l)\textsuperscript{b}</strong></td>
<td>21.4 (15.2,27.5)</td>
<td>39.3 (24,54)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Postprandial PDP 5-HT (nmol/l)\textsuperscript{b}</strong></td>
<td>20.1 (16.1,24.2)</td>
<td>34.5 (19.9,49)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\textsuperscript{b}Data expressed as median (range). * Patients with high sensitivity compared with low rectal sensitivity  p<0.03.
Chapter 4

Discussion
4.1 Opening Remarks

This is the first study, investigating 5-HT signalling in plasma, along with physiological parameters in patients with functional constipation in comparison with healthy volunteers and IBS-C patients. The interesting and novel findings observed in this study are:

1. The FC patients had no 5-HT response to meal ingestion, as previously seen in patients with IBS-C, compared with healthy volunteers.

2. Patients with FC had abdominal and bowel movement associated symptoms as well as delayed colonic transit (whole gut transit), similar to that seen in IBS-C compared with healthy volunteers.

3. The mean pain threshold in patients with FC was similar to that seen in healthy volunteers, with more patients with hyposensitivity or insensitivity in this group compared with IBS-C and no patients with hypersensitivity.

4. Patients with FC had a shift towards higher fasting PDP 5-HT levels, unlike patients with IBS-C, compared to healthy volunteers.

5. This relatively higher PDP 5-HT levels in patients with FC were associated with normal/similar stool consistency compared with healthy volunteers, despite significantly delayed colonic transit as seen in patients with IBS-C. However, opposed to FC patients the latter group of patients had decreased stool consistency (harder stools) compared with healthy volunteers.

6. The platelet depleted plasma 5-HT concentrations in patients with constipation (IBS-C and FC) directly correlated with pain thresholds.

This study show that based on symptoms, IBS-C and FC patients have more similarities than differences. However, although patients with FC had a similar 5-HT response to a test meal, they had different fasting 5-HT levels and some different physiological findings on assessment of visceral sensitivity with barostat. All these have been discussed in detail.
4.2 Recruitment

A total of 24 healthy volunteers, 24 IBS-C and 13 FC patients were recruited into the study. Data from one subject from the healthy volunteers and two from the FC sub-group were excluded from the final analysis due to incomplete data and 5-HT assay problems. One subject with IBS-C had a surgery and was excluded from the final study analysis. It was particularly difficult to recruit patients with FC despite the study being advertised in regional newspapers, in local GP surgeries, pharmacies, the University of Manchester and the University Hospital of South Manchester for both public and student/staff volunteers. In an attempt to increase recruitment further, collaborative links with the Warrell Unit at the St’ Mary Hospital Manchester and GI Physiology Unit at the Royal London hospital, which are regional referral centres for ano-rectal physiology services were also set up, but again proved disappointing. Clinical notes were scanned on more than one thousand patients with constipation, who had undergone ano-rectal physiological testing at the Royal London hospital. All these patients had participated in other studies and have given consent to be contacted for any future studies. Only 16 patients were found to be potential volunteers on initial screening. However, on contacting them with further questionnaires, only one patient satisfied the recruitment criteria but did not agree to participate in the study.

Significant proportions of patients with severe constipation usually have identifiable causes with physiological or anatomical abnormalities (Bharucha, Wald et al. 2006). The majority of patients with FC identified from the FGID clinic and specialist constipation clinic at the University Hospital South Manchester and its affiliated hospitals had one or more co-existing exclusion criteria such as mega rectum, recto / vaginocele, perineal tear/injury/ nerve injury during labour etc. These patients could not be included in the study and some others who were referred as patients diagnosed with FC actually fulfilled Rome III criteria for IBS-C on detailed questionnaire and were recruited instead to the IBS-C group. These practical difficulties led to the low recruitment in the FC sub group.
4.3 Baseline Characteristics

A statistically significant age difference was observed between patients with FC and healthy volunteers in this study but not compared with IBS-C patients. The patients with FC were mainly recruited from the potential volunteers contacted through the public advertising. Moreover, the age range of the healthy volunteers and IBS-C was more evenly distributed, as they were recruited from a large pool of volunteers on the department database. A previous study in patients with FC and IBS-C, also encountered similar circumstances where patients with FC were significantly older compared with IBS-C (Wong, Palsson et al. 2010). There are very few studies, which have looked at the effect of age alone on the GI physiology, suggesting delayed colonic transit and decreased sensitivity. However, the patients studied were elderly, compared with controls in their 2\textsuperscript{nd} and 3\textsuperscript{rd} decade in that study, also as compared with patients and healthy volunteers in the present study (Varma, Bradnock et al. 1988). Moreover, in the present study, patients with FC were still within the age range of 18 and 55 years and the oldest patient in this group was only 46 years old. It is highly unlikely that this age difference would have any bearing on the findings of this study.

As expected, patients with IBS-C had higher anxiety and depression scores compared with healthy volunteers (\(p< 0.001\) and 0.003, respectively), supporting previous studies (Levy, Olden et al. 2006). Interestingly, the anxiety and depression scores for functional constipation lay between the IBS-C patient and healthy volunteers’ scores, and the FC patients were no more depressed / anxious than either IBS-C patients or healthy volunteers. This contradicts previous studies, which have shown increased prevalence of anxiety and depression in patients with FC (Mason, Serrano-Ikkos et al. 2000; Chang, Myung et al. 2003; Chan, Cheng et al. 2005).

Alternatively, looking at the data closely it also revealed that 8\% (2/23) of healthy volunteers and 18 \% (2/11) of patients with FC had anxiety score >7 (score >7 being abnormal) (Snaith and Zigmond 1986) and none had depression score >7, whereas 61\% (14/23) patients with IBS-C had either anxiety or depression or both scores >7. This would
suggest that anxiety/depression is perhaps more prevalent in patients with FC compared with healthy volunteers, but significantly less than in patients with IBS-C.

However, patients in the clinics with a significant psychiatric co morbidity, including anxiety or depression, were generally been treated with anxiolytics / antidepressants and these patients were excluded from this study because of possible interaction of these medications with the 5-HT signalling. Inclusion of these patients in the study might have shown increased difference in the anxiety and depression scores between the different study groups. Where appropriate, results in the present study were adjusted for differences in the age and HAD scores.

4.4 Symptom Profile

4.4.1 Abdominal Symptoms During 7-day Diary Period

Patients with IBS-C reported significantly more abdominal pain and bloating during the 7-day diary period compared with healthy volunteers. This might be expected, given that the abdominal pain is an essential diagnostic criteria for the diagnosis of IBS, and bloating is a common associated symptom of IBS (Longstreth, Thompson et al. 2006). The IBS-C patients also reported greater abdominal pain and bloating compared with FC patients. Similarly, overall abdominal symptom, abdominal pain and bloating scores were also higher in patients with FC compared with the healthy volunteers. Moreover, 45% (5/11) of patients with FC reported abdominal pain or discomfort on more than one occasion during the 7-day diary period compared with only 8.7% (2/23) of healthy volunteers. Similarly, bloating was reported by 63.4% (7/11) of patients with FC and by only 13% (3/23) healthy volunteers on more than one occasion during 7-day diary period. In contrast, 69% (16/23) of IBS-C patients reported abdominal pain and 91% (21/23) reported bloating on more than one occasions. These observations suggest that patients with FC also report more abdominal pain and bloating compared with healthy volunteers but less than in comparison to patients with IBS-C. These findings highlight similarities in symptoms but perhaps also the difference
in the severity of these symptoms in two functional sub-groups of patients with constipation. Several studies have tried to evaluate the similarities and differences in symptoms profile between patients with FC and IBS-C. Studies have highlighted that abdominal pain is also reported by patients with FC contrary to the Rome III criteria (Longstreth, Thompson et al. 2006). In one study 44.8% patients with FC reported abdominal pain or discomfort (Wong, Palsson et al. 2010). Similarly, in another study 74% subjects with FC reported bloating (Rao, Tuteja et al. 2004).

Both groups of constipated patients, IBS-C and FC opened their bowel less frequently compared with healthy volunteers. Patients with IBS-C also reported harder stools compared with the healthy volunteers. In fact, the patients with FC also showed a trend towards lesser bowel frequency compared with IBS-C patients (p=0.1) but surprisingly the stool consistency was no different in patients with FC compared with healthy volunteers and IBS-C patients (p=1 and p=0.2). The lack of difference in the stool consistency was despite the fact that similar proportions of patients with IBS-C (43.5%) and FC (45%) had severe constipation (average Bristol Stool Score <2 during 7 day diary period), as well as both IBS-C and FC having significantly slower colonic transit compared with the healthy volunteers (p=0.001). Previous researchers have suggested that stool consistency reflects/correlates with the whole gut transit time (O'Donnell, Virjee et al. 1990) and that perhaps stool consistency is a more appropriate means to define constipation (Longstreth, Thompson et al. 2006). The absence of any difference in the stool consistency between healthy volunteers and patients with FC despite the latter having significantly lower bowel frequency and prolonged whole gut transit time may be due to fewer subjects in the FC patient group. The majority of patients with FC in this study had an average stool consistency <3, but three patients did have average stool consistency of >4.5, which affected the overall mean stool consistency for the FC patient group.
4.4.2 Bowel Movement Associated Symptoms During 7-day Diary Period

As expected, both patients with IBS-C and FC reported more symptoms associated with the bowel movements compared with healthy volunteers. They reported more straining and sense of incomplete evacuation, supporting previous studies in patients with FC and IBS-C (Koch, Voderholzer et al. 1997; Schmulson, Lee et al. 1999; Longstreth, Thompson et al. 2006). The sense of incomplete evacuation has also been shown to be associated with dyssynergic defaecation (functional defaecation disorder; FDD) but is not very specific and is diagnostically unreliable (Rao, Kuo et al. 2009). However, in this study any patient with constipation and a diagnosis of FDD or with mega rectum/rectocele/pelvic floor dysfunction was excluded at the screening stage, so the sense of incomplete evacuation observed in this study was not related to a mechanical obstruction. IBS-C patients also reported higher scores for the sense of urgency, a symptom more commonly associated with IBS-D (Longstreth, Thompson et al. 2006; Camilleri, McKinzie et al. 2008), but also reported by patients with IBS-C (Caldarella, Milano et al. 2005; Longstreth, Thompson et al. 2006; Camilleri, McKinzie et al. 2008). Again, as observed in abdominal symptoms scores both IBS-C and FC patients reported more bowel movement associated symptoms compared with healthy volunteers in the study. Moreover, the IBS-C patient also reported more overall bowel movement associated symptoms compared with FC constipation patients.

4.4.3 Pre and Postprandial Symptoms

Similarly, on the study day for blood sample collection for 5-HT, patients with IBS-C reported more fasting and postprandial abdominal symptoms compared with healthy volunteers and FC patients. Moreover, IBS-C patients also reported worsening of symptoms after meal ingestion compared with healthy volunteers a finding consistent with previous studies (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). Patients with FC appeared to have postprandial symptoms scores much higher than the healthy volunteers but the difference was not statistically significant (p=0.2), probably because of the large variability.
in the data and fewer number of patients in this subgroup. On paired sample analysis IBS-C patients showed a significant increase in their symptoms from the baseline after meal (p<0.001) and a similar trend in patients with FC (P=0.2). The fact that the FC patients also had very high levels of fasting and fed PDP 5-HT concentrations is a novel finding as discussed later. The effect of a meal on abdominal symptoms in the FC patients has not previously been reported and this possibly highlights a difference severity of these symptoms that separates these patients from those of IBS-C.

In summary, as in previous studies this study showed patients with IBS-C had higher anxiety and depression scores, decreased bowel frequency and harder stool. They also had significantly more, abdominal and bowel movement associated symptoms as well as meal related symptoms, compared with the healthy volunteers. This study also showed that patients with FC have a similar profile of symptoms to IBS-C, but did not show statistical significance on certain measures, perhaps due to the smaller number of patients studied in this group. It is also possible that the constipation in patients with FC, did not show any significant differences because they lie in between patients with IBS-C and healthy volunteers, in terms of severity of these symptoms or have some physiological differences, hence the difference in symptom profile, which separates them from IBS-C patients.

4.5 5-HT Signalling

There was no significant difference in fasting and postprandial PDP 5-HT concentrations in patients with IBS-C compared with healthy volunteers. These findings are consistent with previous studies, which showed fasting PDP 5-HT concentration in patients with IBS-C was similar to healthy volunteers (Atkinson, Lockhart et al. 2006) and there was no significant increase in PDP 5-HT concentration in IBS-C patients after the test meal compared with healthy volunteers (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). The majority of 5-HT is derived from the EC cells in the GI tract (Nilsson, Dahlstrom et al. 1987), and is released in response to a meal (Richter, Stockmann et al. 1986). Dunlop et al have
shown that in patients with IBS-C although, they have an overall 5-HT concentration in the rectal mucosa that is similar to controls, they had higher 5-HT levels per EC cell and this was associated with decreased plasma 5-HT response to a test meal. This perhaps suggests a problem with the release of 5-HT in these patients (Miwa, Echizen et al. 2001; Dunlop, Coleman et al. 2005). Another study suggested however, increased total mucosal 5-HT (not per EC cell) in patients with IBS-C compared with healthy controls but normal 5-HIAA levels, again supporting the hypothesis of a problem with the release of 5-HT; hence normal levels of the 5-HT metabolite; 5-HIAA (Miwa, Echizen et al. 2001). These difference in 5-HT profiles in the present study are unlikely to be as a result of platelet activation, as previous studies have shown that β-thromboglobulin levels are not elevated (Houghton, Atkinson et al. 2003) and the method used was same for all subjects and biochemists doing analysis were blinded to the subject diagnoses.

Other factors such as decreased reuptake of 5-HT by SERT for metabolism, due to the presence of the s/s or s/l allele of SERT promoter gene (Kim, Camilleri et al. 2004; Niesler, Kapeller et al. 2009) or reduced transporter protein expression (Coates, Mahoney et al. 2004) may also have a role in 5-HT profiles seen in this study. These factors may influence plasma 5HT levels but the presence of polymorphisms in the study groups was unfortunately not explored. However, meta analysis of SERT polymorphism in IBS showed no association between different SERT polymorphism and IBS sub-types (Van Kerkhoven, Laheij et al. 2007).

The fasting and post prandial 5-HIAA concentrations in patients with IBS-C were similar to that in healthy volunteers (p=0.3 and 0.4, respectively) contrary to one previous study, which showed that patients with IBS-C had lower plasma 5-HIAA concentration compared with healthy volunteers (Atkinson, Lockhart et al. 2006). This discrepancy from previous study finding may be due to the relatively lower concentration of 5-HIAA in all our study subjects compared with the previous studies (Atkinson, Lockhart et al. 2006; Houghton, Brown et al. 2009). The method used in this study to measure 5-HIAA concentration was slightly different compared with previous studies; although the assays used in this study,
that is liquid chromatography tandem mass spectrometry (LC-MS/MS), was validated against higher performance liquid chromatography (HPLC) used in previous studies (Monaghan, Brown et al. 2009). Slight decreases in 5-HT concentration has been reported on storage at -20°C but these samples were stored at -80°C and there are no reports on stability issues for samples for HIAA. The concentration of 5-HIAA in the plasma might be affected by several other factors such as transporter protein activity affecting reuptake of 5-HT, release of 5-HT by platelets increasing the substrate for metabolite 5-HIAA, as well as by the monoamine oxidase enzyme activity, which metabolises 5-HT to 5-HIAA in different cells.

The fasting 5-HIAA/HT ratio was similar in IBS-C patients and healthy volunteers but the postprandial ratio was higher in IBS-C compared with healthy volunteers. A previous study reported that the fasting 5-HIAA/5-HT was lower in IBS-C compared to healthy volunteers, but the postprandial ratio were similar between two groups (Atkinson, Lockhart et al. 2006).

Looking at the previous studies, which have measured both PDP 5-HT and 5-HIAA concentrations simultaneously; it would appear that interpreting data from the 5-HIAA and 5-HT ratio might be difficult. One previous study showed that 5-HIAA concentration were consistently higher in comparison with PDP 5-HT concentrations in healthy volunteers (Atkinson, Lockhart et al. 2006); whereas, another study using a similar method showed 5-HIAA concentration were lower or similar to that of PDP 5-HT concentrations in healthy controls (Houghton, Atkinson et al. 2003). These differences in 5-HIAA concentration relative to 5-HT concentrations would affect the interpretation of plasma 5-HIAA/5-HT ratio in this study. The mucosal 5-HIAA/5-HT ratio has been suggested to be more of an appropriate measure of 5-HT turnover (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006), which would be expected to be reduced with impaired release of 5-HT in patients with IBS-C, as shown in a previous study (Dunlop, Coleman et al. 2005).

Coates et al showed no difference in 5-HT release from mucosal EC cells in patients with IBS-C compared with healthy volunteers (Coates, Mahoney et al. 2004). However, this study mechanically agitated the cells, which may not mimic a physiological stimuli, such as a meal in vivo. This study also suggested decreased reuptake of serotonin in patients with IBS-C
with decreased SERT transcript protein and expression in the mucosa, which may not play a significant role, if 5-HT release is reduced in first place in these patients. Alternatively, this decreased SERT transcription reported by Coates et al could be reflecting the down regulation of SERT expression in response to decreased release of 5-HT in patients with IBS-C.

The overall ratio in the IBS-C patients from fasting to post prandial did not change (1 and 0.9, respectively) in this study, as seen in the previous study (Atkinson, Lockhart et al. 2006). The lower 5-HIAA/ 5-HT ratio after the meal in IBS-C patients compared with healthy volunteers in the present study was mainly due to only a modest increase in PDP 5-HT concentrations after meal in healthy volunteers and overall lower 5-HIAA levels as discussed earlier. The healthy volunteers in the present study only showed 14% increase in the mean PDP 5-HT concentration compared with 33.9% increase seen in a previous study (Atkinson, Lockhart et al. 2006). The two previous studies had used the same meal with jam scones from Mark and Spencer, which had to be replaced with jam scones from Tesco's in this study due to practicality of accessing meals in time for the study. Moreover, Tesco's made minor changes in the jam scone product half way through the study. Although, the calorie content and constituent ratio of carbohydrate, fats and protein were comparable in these different jam scones from Mark and Spencers and Tesco's products, it is still plausible that these slight variations might have an effect on 5-HT stimulation/release. Since, previous studies had hypothesised problem with release of 5-HT in response to test meal in patients with IBS-C (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). Hence, this variation in the test meal in the present study is unlikely to have had an effect on the postprandial 5-HT response in patients with IBS-C. However, this change in the meal, which possibly may not have stimulated 5-HT release very well could have affected the 5-HT response, leading to a relatively modest rise in PDP 5-HT seen in healthy volunteers this study.

In this study, fasting and post prandial PDP 5-HT concentrations in patients with FC were no different from healthy volunteers, these findings were similar to those seen in patients with IBS-C in a previous study (Atkinson, Lockhart et al. 2006) and also observed in this study.
More interestingly, the patients with FC had relatively higher fasting and postprandial PDP 5-HT levels compared with both healthy volunteers (28 % and 7.8 % higher, respectively) and patients with IBS-C (15.9 % and 45.4 % higher, respectively). Apart from higher postprandial PD 5-HT concentrations in patients with FC compared with the healthy volunteers, these differences were not statistically significant, again may be due to the fewer patients in FC patient group, they are none the less an interesting observation. Both patients with IBS-C and FC have constipation as a common feature, the fasting levels PDP 5-HT concentration in patients with IBS-C and healthy volunteer are similar (23.9 nmol/L and 26.4 nmol/L, respectively), where as these levels were 25.3 % higher in patients with FC (30.6 nmol/L). Nevertheless, in terms of response to meal ingestion, there was no significant difference seen in change in PDP 5-HT concentration in patients with FC compared with healthy volunteers, similar to patients with IBS-C.

The absence of a significant difference in 5-HT response to meal ingestion in FC patients compared with healthy volunteers was may be due to the fact that there were fewer subjects in FC patient group and relatively, a suboptimal response seen in healthy volunteers in the present study. The significant difference in PDP 5-HT concentration observed between healthy volunteers and IBS-C patients in this and previous studies were mainly driven by an increase in PDP 5-HT concentration with meal ingestion in healthy volunteers. In addition, the 5-HT response to the test meal in patients with FC showed a trend similar to that seen in patients with IBS-C in this and previous studies, such that there was a decrease in PDP 5-HT concentration but only by 2.5% (34.2 nmol/L and 33.3 nmol/L, fasting and postprandial) as opposed to 10% decrease seen in patients with IBS-C. One study, which looked at the 5-HT content of the rectal mucosa in patients with chronic constipation, showed that the relative percentage of EC cells was the same in chronic constipation and controls but that both the 5-HT/weight of mucosal tissue, and TPH-1 enzyme activity were increased. The patients also had increased release of 5-HT under basal and stimulated (mechanical agitation) conditions but normal SERT transcription levels suggesting perhaps these patients have increased 5-HT synthesis and release but this may lead to 5-HT receptor desensitisation or relative down regulation of SERT due to continuous availability of serotonin (Costedio, Coates et al. 2009).
More interestingly, this study also looked at patients who were on opiates with and without opiate induced constipation. There was no difference in EC cells, 5-HT content, TPH-1 activity or SERT mRNA levels compared with controls, indicating a primary 5-HT signalling abnormality in patients with constipation rather than secondary abnormality due to constipation. Another small study reported increased 5-HT and normal TPH-1 activity with decreased SERT in the rectal mucosa of female patients with slow transit constipation (Guarino, Cheng et al.). The only published study (in Chinese, only abstract available in English) on the association between SERT polymorphism and slow transit constipation, which showed increased prevalence of s/s allele in patients with slow transit constipation (Ding, Lu et al.). Another study has shown an increased s/s polymorphism in patients with IBS-C (Sikander, Rana et al. 2009). The s/s polymorphism would be associated with decreased SERT transcription with potentially increased 5-HT availability.

There was no difference in fasting and postprandial 5-HIAA concentration between patients with FC and healthy volunteers; a finding similar to that seen in patients with IBS-C with no change in 5-HIAA concentration after meal. The fasting 5-HIAA/5-HT ratio in patients with FC was lower compared with IBS-C patients (p=0.01) as well as a trend towards a lower ratio compared with healthy volunteer (p=0.1). The postprandial ratio was similar in healthy volunteers and patients with IBS-C and FC. Interestingly, the 5-HIAA/5-HT ratio in patients with FC actually did not show any change from fasting to fed state (0.6 and 0.6, respectively). In patients with FC, the 5-HIAA/5-HT ratio showed a similar trend to that seen in patients with IBS-C, in the previous study (Atkinson, Lockhart et al. 2006).

Platelet 5-HT levels were comparable in healthy volunteers and IBS-C patients, as reported in a previous study (Dunlop, Coleman et al. 2005). One study has reported higher platelet 5-HT concentration in patients with IBS-C compared with healthy volunteers (Atkinson, Lockhart et al. 2006). At physiological levels, the plasma 5-HT levels might be enough to maintain steady levels in platelets even with possible reduced SERT activity in these patients.
In summary, in this study the IBS-C patients showed a decreased response to meal ingestion compared with healthy volunteers, a finding consistent with previous studies. It also showed for the first time that FC patients have a similar response. Moreover, the patients with FC had relatively higher fasting PDP 5-HT concentration compared with both healthy volunteers and IBS-C patients. This did not show statistically significant difference, this may be again due to fewer patients in FC group but if validated in a larger group of patients, it would be an important finding in terms of 5-HT signalling. The 5-HIAA/5-HT ratios are difficult to interpret but consistently shown no real change in concentrations of either of the two after meal in both sub-groups of functional constipation. This is first study to indicate that despite different fasting (baseline) PDP 5-HT profiles in two sub-types of functional constipation (IBS-C and FC) both share the same feature of a non-significant 5-HT response to meal ingestion.

4.6 Transit Times

4.6.1 Oro-caecal Transit Time

Oro-caecal transit time was similar in the three groups (p=0.8). In previous studies, both delayed and normal oro-caecal transit time have been reported in patients with constipation. The oro-caecal transit time has been shown to be significantly delayed (Cann, Read et al. 1983; Altomare, Portincasa et al. 1999; Soares, Lederman et al. 2005; Agrawal, Houghton et al. 2009) or normal (Jorge, Wexner et al. 1994; Benninga, Buller et al. 1996) in patients with constipation with functional bowel disorder. However, the latter two studies had used lactulose as a substrate for the breath test, which is commonly used as a laxative and might accelerate the transit resulting in reduced transit time (Miller, Parkman et al. 1997). Another study using wireless motility capsule found normal small bowel transit in patients with constipation (Rao, Kuo et al. 2009) finding similar to observed in this study.

A previous study, which reported increased oro-caecal transit time in patients with IBS-C using the same protocol as in this study had similar mean and 95% CI values for healthy
volunteers [287 (259, 315)] and IBS-C patients [331 (295.8, 366.2)] (Agrawal, Houghton et al. 2009). Moreover, they had similar proportion of patients in IBS-C (17%) with slow oro-caecal transit as observed in this study in patients with IBS-C (17.4%) and FC (18%). Nevertheless, unlike that study, the difference in oro-caecal transit time in patients with IBS-C and FC failed to show statistical significance compared with healthy volunteers. In the present study, the oro-caecal transit time was similar in patients with slow transit and normal transit constipation. This would suggest that the delayed whole gut transit in these patients with constipation (IBS-C and FC) appears to be because of delayed colonic transit only.

4.6.2 Whole Gut (Colonic) Transit Time

In accordance with previous studies (Agrawal, Houghton et al. 2009; Cook, Talley et al. 2009) colonic (whole gut) transit time was longer in both IBS-C and FC patients compared with the healthy volunteers ($p = 0.001$ and <0.001, respectively). Both right and left segmental transit time were delayed in both IBS-C and FC patients as observed in a previous study (Agrawal, Houghton et al. 2009). However, Agrawal et al also reported delayed recto sigmoid transit time, which in present study was normal in both IBS-C and FC patients ($p=0.5$). None of the patients with constipation in this present study exhibited slow colonic transit due to an isolated delay in recto sigmoid segment, a finding usually observed in patients with outlet obstruction (Pomerri, Frigo et al. 2007). This suggests the delayed colonic transit in patients with constipation (IBSC and FC) seen in this study was not due to the outlet obstruction. However, it must be borne in mind that not all studies have shown that delay in the recto sigmoid transit is associated with outlet obstruction (Zarate, Knowles et al. 2008). The proportion of IBS-C patients (55%) with slow colonic transit were similar to that seen in a previous study (Agrawal, Houghton et al. 2009). This present study showed that 64% patients with FC had slow colonic transit, which was comparable with patients with IBS-C group (55%) but higher than reported in previous studies (13%-27%) (Koch, Voderholzer et al. 1997; Cook, Talley et al. 2009). The different proportions of patients with
slow transit constipation in previous studies might be due to the fact that they all included patients with pelvis floor dysfunction or evacuation disorder, and some also did not exclude patients with IBS-C. Moreover, slow transit constipation is also considered to be almost exclusive to female patients (Knowles, Scott et al. 2003). Previous studies, which reported lower prevalence of slow transit constipation, included both men and women, which is different to present study, which only included female subjects. The higher proportion of FC patients with slow transit constipation observed in this study may be explained by the different selection criteria used in this compared with previous studies.

In summary, both IBS-C and FC patients have delayed colonic transit but normal oro-caecal transit, suggesting similar or overlapping pathophysiology between the two functional subtypes of constipation. The lack of isolated delayed recto-sigmoid transit perhaps suggests that the constipation in these two groups was not due to obstructive defaecation.

4.7 Visceral Rectal Sensitivity

4.7.1 Pain Thresholds

Patients with IBS-C had a trend (p=0.07) towards reduced pain thresholds compared with healthy volunteers, supporting some (Steens, Van Der Schaar et al. 2002; Awad, Camacho et al. 2006; Kanazawa, Palsson et al. 2008) but not all (Camilleri, McKinzie et al. 2008) previous studies. The author of the latter study hypothesised that their healthy controls were unusually sensitive, such that 10th percentile for pain threshold was only 13 mmHg, while other studies have found the 10th percentile for pain threshold (32mmHg) to be much higher in healthy volunteers (Bouin, Plourde et al. 2002) (Lembo, Munakata et al. 1994). In present study, a 2.5th percentile (18mmHg) for pain threshold in healthy volunteers was used as lower cut off for normal and 10th percentile was 22.2 mmHg still much higher compared with observed by Camilleri et al as mentioned above. Similarly another study observed the 5th percentile for pain threshold in healthy volunteers was 24 mmHg (Lea, Houghton et al. 2003). Moreover, Camilleri et al used a ramp method of distension, in which
subjects are aware of a gradually increasing pressure threshold, which might lead to volunteers reporting lower pain threshold compared with phasic distension, perhaps due to anticipation, a response bias.

Likewise, although patients with FC in this study reported similar pain thresholds compared with healthy volunteers, supporting works from a previous study (Liu, Chen et al. 2008), other investigators have reported increased pain thresholds in patients with FC (De Medici, Badiali et al. 1989; Gladman, Dvorkin et al. 2005). These differences may be due to different patient recruitment criteria and the fact that patients with obstructive defecation and those who had pelvic surgery were incorporated in these studies. They also used volume rather than pressure thresholds. De Medici et al also noted that the subgroup of constipated patients with features of obstructive defecation had further reduced rectal sensitivity. Previous anorectal/ pelvic surgery or spinal injury is more commonly associated with reduced rectal sensitivity (Gladman, Scott et al. 2003). Moreover, volume thresholds used in these studies can also be affected by confounding factors such as presence of megarectum. A significant proportion of patients with constipation were from our functional GI disorders/ constipation clinics at UHSM hospital, which are tertiary referral clinics for north west of England. Most of these patients had been extensively investigated for their symptoms prior or after referral to these clinics and we excluded any patient, who had been diagnosed with megarectum or pelvic nerves injury.

Mean volume for stool sensation was significantly lower in patients with IBS-C compared healthy volunteers supporting increased visceral sensitivity in these patients. However, previous validation studies of the use of the barostat to measure visceral sensitivity have suggested that pressure rather than volume thresholds are a more reliable method to assess visceral sensitivity (Whitehead and Delvaux 1997; Cremonini, Houghton et al. 2005). Moreover, pressure thresholds for pain have been found more reproducible compared with other sensations, such as first sensation, desire to defaecate etc. (Whitehead and Delvaux 1997; Bouin, Plourde et al. 2002; Cremonini, Houghton et al. 2005).
The present study shown that 30% of IBS-C patients had visceral hypersensitivity (low pain threshold, below 2.5th percentile for HV), which is consistent with an earlier study from our centre (28%) (Agrawal, Houghton et al. 2008). One patient with IBS-C (4%) was hyposensitive (higher pain threshold, greater than 97.5th percentile), which contrasts with Agrawal et al who reported 28% patients with IBS-C were hyposensitive. This could be perhaps explained by the fact that the subjects studied by Agrawal et al were much older and included both male and female volunteers. Moreover, a significant proportion (38%) of patients with hyposensitivity in by Agrawal et al’s in their study had undergone hysterectomy, compared with only 8% in hypersensitive and normosensitive patients. Both older age and previous surgery are known to affect anorectal physiology and sensitivity (Varma, Bradnock et al. 1988; Smith, Varma et al. 1990; Prior, Stanley et al. 1992). Beside this, the upper limit of distension in the protocol in the study by Agarwal et al was 48 mmHg as compared with 56 mmHg in the present study. This would result in lower mean value on tracking thresholds, consequently reduced upper limit of normal pain threshold (38 mmHg). This perhaps resulted in the relatively greater proportion of patients diagnosed with hyposensitivity. Minor differences in barostat protocols and definitions of the normal range can result in widely dissimilar results in terms of visceral hypersensitivity being reported in only 20% (Camilleri, McKinzie et al. 2008) of patients with IBS to as high as 94% (Mertz, Naliboff et al. 1995).

The present study also found (3)27% patients with FC had visceral hyposensitivity comparable with other studies: 23% (Gladman, Scott et al. 2003), 24% (Vasudevan, Scott et al. 2007). Other studies have reported a higher prevalence (68%) (Gladman, Lunniss et al. 2006) but this might be because they have included patients of all age groups including those with obstructed defecation/faecal incontinence and have mainly used volume rather than pressure to record sensory thresholds. Moreover, other studies have not applied strict cut-off criteria of 2.5th and 97.5th percentile to define the normal range, as used in this study.
There are no published studies reporting rectal sensitivity in FC, which has excluded obstructive defaecation and other confounding factors such surgery from their study groups.

4.7.2 Compliances

There was no difference in static and dynamic compliance between healthy volunteers and patients with IBS-C and FC supporting previous reports in the literature (Penning, Steens et al. 2001; Agrawal, Houghton et al. 2008; Camilleri, McKinzie et al. 2008; Park, Baek et al. 2008). On the other hand, some investigators have reported difference in compliance between in IBS patients and healthy volunteers (Steens, Van Der Schaar et al. 2002; Zar, Benson et al. 2006), the latter study only found difference in compliance in patients with IBS-D but not IBS-C compared with healthy volunteers. Although, the method of measuring compliance has been well described in the literature, the variation in results especially inability to find any difference in compliance, perhaps is explained by the volume component of compliance. Since, the air is subject to compression and slight variation in length/shape of bag has greater effect on volume component than the pressure. These factors lead to inter subject rectal compliance assessment been more prone to error than a change in compliance in the same subject (Whitehead and Delvaux 1997).

4.7.3 Constipation Patients with High and Low Rectal Sensitivity

Combining patients with IBS-C (23) and FC (11) revealed that (23) 68% were normosensitive, 7 (21%) hypersensitive and 4 (12%) hyposensitive. The number of patients in hyposensitive group was too small in comparison with normosensitive and hypersensitive patients with constipation to do any substantial analysis. There was no significant difference between constipation patients with high and low rectal sensitivity in their symptom profile during 7-day diary.
4.8 Correlations

4.8.1 Anxiety and Depression

There was a strong positive correlation between anxiety and depression scores on HAD questionnaire and abdominal symptoms reported by the patients. The constipation patients with higher anxiety and depression scores reported more severe abdominal pain and bloating during baseline 7-day diary period. However, the anxiety and depression scores had no correlation with pain and stool sensation thresholds, which were assessed by more objective physiological test. Increased somatisation in patients with IBS has been associated with reporting multiple symptoms (Talley, Dennis et al. 2003; Chan, Cheng et al. 2005; Choung, Locke et al. 2009). Perhaps, that is why patients with constipation with higher anxiety and/or depression scores reported more severe symptoms. This study shows that the functional constipation patients have relatively more abdominal pain and bloating compared with healthy volunteers, but with presence of anxiety and/or depression in patients with IBS-C, the perceived severity of these symptoms might be worse compared with those with less anxiety and depression. Moreover, increased visceral sensitivity (lower pain threshold) also correlated with higher bloating score (p=0.06), confirming a previous study in patients with IBS-C, which reported that patients with bloating alone had lower pain threshold than those with bloating and distension (p=0.06) (Agrawal, Houghton et al. 2008). These observations suggest that perhaps physiological testing with the right testing protocol it might be possible to be able to differentiate between perceived/reported symptoms severity and truly increased visceral sensitivity. Objective abdominal distension was not assessed in the present study.
4.8.2 5-HT and Symptoms

There was no correlation between fasting or postprandial PDP 5-HT or 5-HIAA and abdominal pain, abdominal bloating or over all abdominal symptoms. On the study day for blood sample collection for 5-HT, there was no correlation between fasting PDP 5-HT and fasting (pre-prandial) symptoms and postprandial PDP 5-HT and postprandial symptoms, confirming observations made in previous studies (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). In these studies, IBS-C patients had similar fasting and postprandial PDP 5-HT concentrations, where as IBS-D patients had higher fasting and postprandial PDP 5-HT concentrations. Despite these two dissimilar profiles of 5-HT, both IBS-C and IBS-D patients had reported significantly higher baseline and postprandial symptoms compared with healthy volunteers. In current study, there were no significant differences in base line or postprandial PDP 5-HT concentrations between patients with constipation and healthy volunteers, but patients with IBS-C reported more baseline and postprandial abdominal symptoms compared with healthy volunteers. These observations from current and previous studies would suggest, perhaps 5-HT does not play major part in manifestation of abdominal pain and bloating in functional patients with constipation.

4.8.3 5-HT and Transit

There was no correlation between fasting or postprandial PDP 5-HT concentrations, and oroecaecal, colonic transit time or stool consistency. However, the postprandial PDP 5-HT concentrations had a negative correlation with bowel frequency over 7-day diary period, as did fasting PDP 5-HT concentration and bowel frequency although only a trend towards negative correlation with bowel frequency (p=0.2). However, Dunlop et al have previously found a weak negative correlation between PDP 5-HT and whole gut transit time (Dunlop, Coleman et al. 2005). 5-HT4 agonists have also been shown to improve the bowel frequency in patients with chronic constipation (Evans, Clark et al. 2007; Quigley, Vandeplassche et al. 2009) although the effect is usually very modest.
4.8.4 5-HT and Visceral Sensitivity

There was a positive correlation between fasting, as well as postprandial PDP 5-HT concentrations and pain threshold i.e. hyposensitive patients had the highest levels of fasting and fed PDP 5-HT. This positive correlation continued to exist even when the hyposensitive patients were excluded from the analysis. In addition, the patients with constipation with low rectal sensitivity in this study had higher fasting and post prandial PDP 5-HT concentrations compared with patients high rectal sensitivity. These observations might suggest that IBS-C and FC is the same disorder and that patients lie at different levels within the spectrum of the disorder. In this and some of the previous studies (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006) no correlation was seen between 5-HT and abdominal symptoms in patients with IBS; thus the observation that 5-HT directly correlates with visceral sensitivity is an interesting and novel observation, suggesting that patients perception of pain is also influenced by additional factors other than visceral sensation, such as psychopathology.

Visceral hypersensitivity is more prevalent in patients with IBS-D compared with IBS-C (Prior, Maxton et al. 1990; Camilleri and Ford 1998; Agrawal, Houghton et al. 2008) but still a significant proportion of patients with IBS-C also have visceral hypersensitivity (Agrawal, Houghton et al. 2008). This is despite the fact that both IBS-D and IBS-C patients have opposite 5-HT profiles compared with healthy volunteers, as discussed above. One previous study has suggested a link between increased visceral sensitivity (hypersensitivity) and increased number of EC cells in patients with IBS-D (Park, Rhee et al. 2006), another study found no change in visceral perception after giving Citalopram (SSRI), which increased levels of plasma 5-HT (Kilkens, Honig et al. 2005). Moreover, alosetron a 5-HT3 receptor antagonist did not affect (reduce) visceral sensitivity (Delvaux, Louvel et al. 1998; Thumshirn, Coulie et al. 2000; Mayer, Berman et al. 2002). These observations would suggest that 5-HT alone, especially at the level of gut might play only a limited role in visceral sensitivity as suggested by one of the studies that showed an improvement in pain perception in patients with IBS after 3 weeks of treatment with alosetron (Mayer, Berman et
al. 2002) but not due to reduced visceral sensitivity. Alternatively, the positive correlation between 5-HT levels and pain threshold, and higher concentrations of PDP 5-HT in patients with low rectal sensitivity in present study could suggest that continually increased levels of 5-HT may lead to desensitisation of 5-HT receptors. Supporting desensitisation hypothesis, in experimental study, the SERT knockout mice had diarrhoea as main symptom due to increased motility but these mice also had intermittent constipation, believed to be due to desensitisation of 5-HT receptors because of the excessive serotonin in the absence of an effective clearance mechanism (Chen, Li et al. 2001).

4.8.5 5-HT and Stool Consistency

In this study, patients with FC had relatively higher fasting and postprandial PDP 5-HT concentration compared with healthy volunteers. Moreover, the hyposensitive patients with constipation, of which the majority were from the FC group, had higher fasting and post prandial PDP-5-HT concentrations compared with hypersensitive patients. These patients also had normal stool consistency. Whether these two observations are related is unclear from this study, as no correlation was found between 5-HT concentration and stool consistency. However, 5-HT is understood to mediate intestinal secretion (Mourad, O'Donnell et al. 1995; Budhoo, Harris et al. 1996). Tegaserod, a 5-HT$_4$ receptor agonist, has been shown to improve intestinal secretion in animal studies (Fang, Liu et al. 2008) and in vivo it appears to improve both stool frequency and consistency in both patients with IBS-C and constipation (Evans, Clark et al. 2007). It is conceivable that despite delayed whole gut transit time and decreased bowel frequency in patients with FC, the stool consistency was comparable with healthy volunteers because of higher circulating 5-HT in these patients, which might have positively affected intestinal secretion contributing to softer stools.
Chapter 5

Conclusions and Future Directions
5.1 Conclusions

The Rome III criteria recognise IBS-C and FC as two distinct diagnostic entities, which are mutually exclusive and one cannot be diagnosed in the presence of the other (Longstreth, Thompson et al. 2006). However, Wong et al. showed that if this condition of mutual exclusivity is removed, the majority of patients with IBS-C (89.5%) would fulfil the Rome III criteria for FC and 43.5% of FC patients would also fulfil diagnostic criteria for IBS-C (Wong, Palsson et al. 2010). Some longitudinal studies have also shown that there is a fair degree of switchover between diagnoses of these patients. One third or more of the FC patients would fulfil the diagnostic criteria for IBS-C after 12 months follow up and vice versa (Halder, Locke et al. 2007; Wong, Palsson et al. 2010). This would suggest that the same patient could be recruited/ studied in two separate studies as a Rome III defined different sub-types of patients, again supporting the hypothesis that these subtypes of functional constipation may just be at different levels of the spectrum of the same disease. Wong et al concluded that the symptom based Rome III criteria were inadequate and suggested physiological studies, such as transit measurements might be able to better differentiate between these two functional subtypes of constipation.

This is the first study to explore how patho-physiology associates with 5-HT signalling in patients with FC and whether this differs from patients with IBS-C and healthy volunteers. The patients with FC in this study had a similar abdominal symptom profile and bowel movement associated symptoms to IBS-C during the 7-day diary period, although the latter group of patients tended to report more severe symptoms. The anxiety and depression scores were also higher in IBS-C patients compared with both healthy volunteers and patients with FC, FC patients been somewhere between the two. Anxiety and depression scores also showed positive correlations with abdominal pain and bloating, suggesting that
some of the severity of symptoms in these patients might be exacerbated by anxiety and depression. However, even after adjusting for anxiety and depression as a confounder, the IBS-C patients had significantly more severe abdominal symptoms compared with healthy volunteers and patients with FC. The whole gut transit time was significantly delayed in both groups of constipated patients (IBS-C and FC) compared with healthy volunteers, suggesting that these two subtypes of functional constipation may share similar symptom and transit problems, and that only the severity of symptoms differentiates between them.

On rectal barostat assessment, the pain threshold tended to be lower in patients with IBS-C compared with healthy volunteers, confirming findings from previous studies (Steens, Van Der Schaar et al. 2002; Awad, Camacho et al. 2006). The FC patients had comparable pain thresholds to healthy volunteers, and there no hypersensitive patients in this group. A quarter (27%) of patients with FC however, had rectal hyposensitivity or insensitivity. Apart from the abdominal bloating symptom, the rectal sensitivity did not correlate with abdominal or bowel movement associated symptoms in patients with constipation. These findings, along with the fact that there was no difference in rectal compliance between patients with constipation (IBS-C and FC) and healthy volunteers in this study, suggests that perhaps there are mechanisms such as changes in afferent pathways or central sensitisation for altered visceral sensitivity other than change in compliance alone, as suggested in some previous studies (Gladman, Dvorkin et al. 2005). Again these observations suggest that both FC and IBS-C form part of the same disorder, with different patients located at different levels of the spectrum of disease; but also that those with hyposensitivity with elevated 5-HT might be a distinct sub-group.

5-HT is known to play a wide variety of roles in gastrointestinal functions such as sensation, motility and secretion (Holstein and Cederberg 1984; Grider, Kuemmerle et al. 1996; Gershon 2004; Spiller 2008). SERT offers an effective mechanism of clearing 5-HT released by the EC cells into the surrounding tissues to avoid desensitisation of 5-HT receptors (Chen, Pan et al. 1998). This study has shown that IBS-C patients and healthy volunteers have similar baseline and postprandial PDP 5-HT concentrations, with IBS-C patients showing no
significant 5-HT response to meal ingestion compared with healthy volunteers, confirming findings of previous studies (Dunlop, Coleman et al. 2005; Atkinson, Lockhart et al. 2006). This is the first study to report, a similar lack of the 5-HT response to meal ingestion in patients with FC compared with healthy volunteers. The lack of 5-HT response to meal ingestion in patients with IBS-C and FC is unlikely to be secondary to constipation as both appeared to have different baseline profile of 5-HT. Costedio et al showed that chronic constipation patients have higher 5-HT synthesis and release compared with healthy volunteers, whereas no change was observed in the 5-HT profiles in patients on opiates with or without constipation (Costedio, Coates et al. 2009), suggesting no compensatory changes in 5-HT signalling in response to constipation. In this study, FC patients had relatively higher concentrations of fasting and postprandial PDP 5-HT compared with healthy volunteers and patients with IBS-C. This appeared to be related to the fact that FC patients with hyposensitivity had the highest concentrations of fasting and postprandial PDP 5-HT. More interestingly, this is the first study to report a positive correlation between pain threshold and both fasting and postprandial PDP 5-HT concentrations in patients with constipation. 5-HT is believed to be one of the mediators of visceral sensation, and according to some studies, increased availability of 5-HT is associated with visceral hypersensitivity in patients with IBS-D (Park, Rhee et al. 2006), whereas others found no relationship between increased 5-HT and visceral sensitivity (Ladabaum and Glidden 2002). However, the positive correlation observed between PDP 5-HT and pain threshold in the present study would mean higher concentrations of 5-HT are associated with visceral hyposensitivity / decreased sensation. The patients with FC also had significantly delayed colonic transit compared with healthy volunteers, even with relatively higher 5-HT concentrations. This provides further support to the hypothesis proposed in earlier studies suggesting that perhaps persistent increased concentrations of 5-HT would lead to 5-HT receptor desensitisation (Costedio, Coates et al. 2009; Sikander, Rana et al. 2009). If this correlation were validated in larger studies perhaps along with mucosal 5-HT and 5-HIAA assessments, it would open new understanding in mechanisms of different subtypes of constipation. However, the FC patients also had normal stool consistency despite slow transit compared with healthy
volunteers. This could possibly be as a result of excessive 5-HT stimulation the intestinal secretions. These explanations might well seem contradictory and the normal stool consistency observed in this study might just be a type II error, due to relatively small number of patients in FC group or a selective desensitisation of certain types of 5-HT receptors.

5.2 Limitations of the Study

The protocol used in this study demanded very strict criteria for inclusion and exclusion of patients, and the enormity of the challenge to recruit such patients was only realised after starting the study. Moreover, even when subjects fulfilled the study recruitment criteria, many were unwilling to volunteer because of the invasive nature of the physiological assessments and time commitment required to participate in the study. Despite best attempts to improve recruitment, including an amendment and approval to run the study at a second site (GI Physiology Unit at the Royal London Hospital), the desired number of FC patients could not be achieved, this significantly underpowered the study. Moreover, slight variations in the test meal (i.e. change from Mark & Spencer to Tesco scone) might have influenced 5-HT release and thus the results.

Lastly, the present the study only assessed some of the pathophysologies that might underlie these two sub-types of functional constipation. The presence or absence of certain associations only reflects on possible mechanisms. Small subgroup analyses were undertaken to seek trends and should thus be viewed with caution till further studies can be undertaken.
5.3 Future Directions

5.3.1 Recruitment in the FC Group

Firstly, it is important that more FC patients be recruited to this study to confirm the results so far obtained, as they may have important implications when defining sub-groups with in patients with constipation.

5.3.2 5-HT Signalling Change: Primary or Secondary

The present study is one of several which has shown plasma 5-HT to associate with a particular sub-groups of functional bowel disorder and /or physiology but whether changes in 5-HT signalling in these studies are primary or a compensatory mechanism secondary to constipation or diarrhoea is unclear. Previous observations that the time to peak 5-HT and 5-HIAA concentration is similar in both IBS-D and IBS-C patients implies that the difference in 5-HT concentration is not solely due to changes in transit (Atkinson, Lockhart et al. 2006). This together with the fact that changes in transit are generally modest in IBS but that there is often a complete lack of 5-HT response to meal ingestion in constipation would support 5-HT been at least partly responsible for the changes in motility. However, it might be interesting to add a third constipation group to the present study whose constipation was precipitated by a surgery or pelvic injury during prolonged labour/childbirth etc. to determine whether this group of constipated patients have normal fasting /fed 5-HT concentrations, and response to meal ingestion. Such a difference might indicate that a reduced 5-HT response to meal ingestion might be a marker of functional constipation.

5.3.3 Role of SERT Polymorphism on 5-HT Signalling

Several studies have been published exploring associations between the SERT polymorphism and functional conditions, such as IBS and constipation, and the results so far have been inconclusive. These studies were based on the hypothesis that the SERT polymorphism results in a short allele and long allele, which in turn affects synthesis of SERT protein. SERT
is responsible for reuptake of 5-HT for metabolism, and hence its increased or decreased availability could affect 5-HT concentrations/availability to act on receptors. To date, there are no human studies to support any effect of this polymorphism *in vivo*, on plasma 5-HT. Studying SERT polymorphisms along with PDP 5-HT concentrations in healthy volunteers, IBS and FC would help to determine whether these SERT polymorphism do actually modulate the availability of 5-HT in these patients. Studying such association, in particular functional bowel disorders sub-groups are difficult because of the heterogeneous nature of these sub-groups and the fact that there may be overlap of patients across the sub-groups.

An additional, study that might be interesting would be to investigate how plasma 5-HT concentrations vary over time. It is well known that IBS subtypes can switch over time, and determining whether there is a change in plasma 5-HT profile associated with these changes in IBS sub-types, may give further insight into role of 5-HT signalling in pathophysiology of IBS.
Publications and Presentation During this Study Project


5. Oral presentation. Reduced 5-Hydroxytryptamine (5-HT) Signalling Determines Stool Consistency and Transit in Functional GI Patients with Constipation. Accepted for Young Investigators Meeting, April 2010, FBG Group, Tucson, USA.

6. Poster presentation. Reduced 5-Hydroxytryptamine (5-HT) Signalling Determines Stool Consistency and Transit in Functional GI Patients with Constipation. DDW 2010, New Orleans, USA.

7. Poster presentation. Are Functional Constipation and Constipation-Subtype Irritable Bowel Syndrome Distinct with respect to 5-Hydroxytryptamine Signalling and Motor-Sensory Function? (BSG ) & DDW 2011, Chicago, USA.
References


**Appendix 1 - Rome III Constipation Module Questionnaire**

<table>
<thead>
<tr>
<th><strong>Constipation Module</strong></th>
<th>1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?</th>
<th>No</th>
<th>Never (\rightarrow) Less than one day a month</th>
<th>One day a month</th>
<th>Two to three days a month</th>
<th>One day a week</th>
<th>More than one day a week</th>
<th>Every day</th>
<th>Skip to question 9</th>
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<tbody>
<tr>
<td></td>
<td>2. For women: Did this discomfort or pain occur only during your menstrual bleeding and not at other times?</td>
<td>No</td>
<td>Yes</td>
<td>Does not apply because I have had the change in life (menopause) or I am a male</td>
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<td>3. Have you had this discomfort or pain 6 months or longer?</td>
<td>No</td>
<td>Yes</td>
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<td>4. How often did this discomfort or pain get better or stop after you had a bowel movement?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
<td></td>
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<td></td>
<td>5. When this discomfort or pain started, did you have more frequent bowel movements?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
<td></td>
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<td></td>
<td>6. When this discomfort or pain started, did you have less frequent bowel movements?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
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<td>7. When this discomfort or pain started, were your stools (bowel movements) looser?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8. When this discomfort or pain started, how often did you have harder stools?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
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<td></td>
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<td></td>
<td>9. In the last 3 months, how often did you have fewer than three bowel movements (0-2) a week?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
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<td></td>
<td>10. In the last 3 months, how often did you have hard or lumpy stools?</td>
<td>Never or rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Most of the time</td>
<td>Always</td>
<td></td>
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</tbody>
</table>
11. In the last 3 months, how often did you strain during bowel movements?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

12. In the last 3 months, how often did you have a feeling of incomplete emptying after bowel movements?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

13. In the last 3 months, how often did you have a sensation that the stool could not be passed, (i.e., blocked), when having a bowel movement?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

14. In the last 3 months, how often did you press on or around your bottom or remove stool in order to complete a bowel movement?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

15. In the last 3 months, how often did you have difficulty relaxing or letting go to allow the stool to come out during a bowel movement?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

16. Did any of the symptoms of constipation listed in questions 9-15 above begin more than 6 months ago?
   - No
   - Yes

17. In the last 3 months, how often did you have loose, mushy or watery stools?
   - Never or rarely
   - Sometimes
   - Often
   - Most of the time
   - Always

**C3. Functional Constipation**

**Diagnostic criteria**

1. Must include two or more of the following:
   a) Straining during at least 25% of defecations  
      *At least once. (question 11>1)*
   b) Lumpy or hard stools at least 25% of defecations  
      *At least once. (question 18>1)*
   c) Sensation of incomplete evacuation at least 25% of defecations  
      *At least sometimes. (question 12>0)*
   d) Sensation of anorectal obstruction/blockage at least 25% of defecations  
      *At least sometimes. (question 13>0)*
   e) Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor)
F3: Functional Defecation Disorders

The diagnostic criteria define FDD solely in terms of laboratory tests. However, the following questions may identify probable cases who would require further investigation to confirm diagnosis. A response of at least ‘often’ to any of these questions identifies a probable case of FDD:

- Straining during bowel movements (question 11>1)
- Feeling of incomplete evacuation (question 12>1)
- Sensation of blocked stools (question 13>1)
- Manual maneuvers to facilitate defecation (question 14>1)

AND criteria for functional constipation are fulfilled

AND onset of constipation symptoms began more than 6 months previously.

Yes. (question 16=1)

Diagnostic Criteria for IBS (Exclusion Criteria for Constipation)*

Recurrent abdominal pain or discomfort* at least 3 days/month in last 3 months associated with two or more of criteria #1 - #3 below:

- Pain or discomfort at least 2-3 days/month (question 12>2)
  - For women, does pain occur only during menstrual bleeding? (question 2=0 or 2)
  - Improvement with defecation
  - Pain or discomfort gets better after BM at least sometimes (question 4>0)
  - Onset associated with a change in frequency of stool
    - Onset of pain or discomfort associated with more stools at least sometimes (question 5>0), OR
    - Onset of pain or discomfort associated with fewer stools at least sometimes (question 6>0)
  - Onset associated with a change in form (appearance) of stool
    - Onset of pain or discomfort associated with looser stools at least sometimes (question 7>0), OR
    - Onset of pain or discomfort associated with harder stools at least sometimes (question 8>0)

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

Yes. (question 3=1)
Appendix 2- Symptoms Diary 7-days

Please read Instructions Carefully

1. Please complete this diary at the end of each day.

2. Between first visit (screening) and your next due visit, if you develop any illness no matter how trivial such as loose stools, common cold etc. please contact unit as soon as possible.

3. Between first visit (screening) and your next due visit, if you are started on or you take any new medications, even if it’s simple medication like a laxative or pain killer please inform the unit.

It is important to contact the unit because the things mentioned in 2 & 3 may affect the results of your tests. If we believe they will affect the results; then we will simply delay the tests until you are well or have stopped the medications.

Contact –
Dr Chander Shekhar
Clinical Research Fellow
Neurogastroenterology Unit
Wythenshawe Hospital
Phone 0161 291 4191/88/89
### SYMPTOM DIARY

**Day 1 of 7**

<table>
<thead>
<tr>
<th>Todays Date</th>
<th>None</th>
<th>Very Mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Quite Severe</th>
<th>Very Severe</th>
</tr>
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<tbody>
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</table>

1. How would you rate your abdominal pain/discomfort over the last 24 hrs?

2. How would you rate your abdominal bloating over the last 24 hrs?

3. How would you rate your urgency over the last 24 hrs?

4. How many bowel movements you had over the last 24 hrs?

5. Please refer to the Bristol Stool Scale and indicate the type number (i.e. 1 to 7) which best describes your stools today. Please indicate also the time you passed the stool. Remember to tell us about each stool you pass. If you pass more than 3 stools please list these at the end of the page.

   **Stool 1**
   - Time: [__:__] (h:mm)
   - Bristol Stool Scale

   **Stool 2**
   - Time: [__:__] (h:mm)
   - Bristol Stool Scale

   **Stool 3**
   - Time: [__:__] (h:mm)
   - Bristol Stool Scale

6. Please indicate how difficult (i.e. straining) it was to pass each of these stools, as well as the 'urgency' and the 'feeling of incomplete emptying/evacuation' by ticking the box below for each stool you have passed. If you have passed more than 3 stools, please provide details at the end of the page or attach a piece of paper with the day clearly stated at the top.

   **Stool 1**
   - Straining
   - Urgency
   - Incomplete evacuation

   **Stool 2**
   - Straining
   - Urgency
   - Incomplete evacuation

   **Stool 3**
   - Straining
   - Urgency
   - Incomplete evacuation

7. I confirm that I have not taken any laxatives

---

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Bristol Stool Chart

Type 1  Separate hard lumps, like nuts (hard to pass)

Type 2  Sausage-shaped but lumpy

Type 3  Like a sausage but with cracks on its surface

Type 4  Like a sausage or snake, smooth and soft

Type 5  Soft blobs with clear-cut edges (passed easily)

Type 6  Fluffy pieces with ragged edges, a mushy stool

Type 7  Watery, no solid pieces. Entirely Liquid
APPENDIX 3 – Hospital Anxiety and Depression Scale

PART 6: HAD SCORE

8. The following questions refer to your general well-being over the last few weeks. Please place a tick in the box appropriate to the answer which most accurately describes how you feel.

Tick only one box in each section

<table>
<thead>
<tr>
<th></th>
<th>a) I feel tense or wound up:</th>
<th>b) I still enjoy the things I used to enjoy:</th>
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<tbody>
<tr>
<td></td>
<td>Most of the time</td>
<td>Definitely</td>
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<tr>
<td></td>
<td>A lot of the time</td>
<td>Not quite so much</td>
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<tr>
<td></td>
<td>From time to time, occasionally</td>
<td>Only a little</td>
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<td></td>
<td>Not at all</td>
<td>Hardly at all</td>
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<th></th>
<th>c) I get a sort of frightened feeling as if something awful is about to happen:</th>
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<tr>
<td></td>
<td>Very definitely and quite badly</td>
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<td></td>
<td>Yes but not too badly</td>
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<td>A little but it doesn’t worry me</td>
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<td>Not at all</td>
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<th>d) I can laugh and see the funny side of things:</th>
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<td></td>
<td>As much as I always could</td>
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<td></td>
<td>Not quite so much now</td>
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<tr>
<td></td>
<td>Definitely not so much now</td>
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<td>Not at all</td>
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<th>e) Worrying thoughts go through my mind:</th>
<th>f) I feel cheerful:</th>
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<tbody>
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<td></td>
<td>A great deal of the time</td>
<td>Not at all</td>
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<td></td>
<td>A lot of the time</td>
<td>Not often</td>
</tr>
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<td></td>
<td>From time to time, but not too often</td>
<td>Sometimes</td>
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<td></td>
<td>Only occasionally</td>
<td>Most of the time</td>
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<th>g) I can sit at ease and feel relaxed:</th>
<th>h) I feel as if I’m slowed down:</th>
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<td>Definitely</td>
<td>Nearly all the time</td>
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<td></td>
<td>Usually</td>
<td>Very often</td>
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<td></td>
<td>Not often</td>
<td>Sometimes</td>
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<td>Not at all</td>
<td>Not at all</td>
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<th></th>
<th>i) I get a sort of frightened feeling like ‘butterflies’ in the stomach:</th>
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<tbody>
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<td></td>
<td>Not at all</td>
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<td></td>
<td>Occasionally</td>
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<td>Quite often</td>
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<td>Very often</td>
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<th>j) I have lost interest in my appearance:</th>
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<tbody>
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<td></td>
<td>Definitely</td>
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<tr>
<td></td>
<td>I don’t take so much care as I should</td>
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<td></td>
<td>I may not take quite as much care</td>
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<tr>
<td></td>
<td>I take just as much care as ever</td>
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<th>k) I feel restless as if I have to be on the move:</th>
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<td>Very much indeed</td>
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<tr>
<td></td>
<td>Quite a lot</td>
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<td>Not very much</td>
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<td>Not at all</td>
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<th>l) I look forward with enjoyment to things:</th>
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<td></td>
<td>As much as ever</td>
</tr>
<tr>
<td></td>
<td>Rather less than I used to</td>
</tr>
<tr>
<td></td>
<td>Definitely less than I used to</td>
</tr>
<tr>
<td></td>
<td>Hardly at all</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>m) I get sudden feelings of panic:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very often indeed</td>
</tr>
<tr>
<td></td>
<td>Quite often</td>
</tr>
<tr>
<td></td>
<td>Not very often</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
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</tbody>
</table>

For office use only

A

TOTAL HAD SCORE: D

10
Appendix 4- Pre and Post-prandial Symptoms Diary

5-HT and Barostat Study in IBS-C/FC/HV
Screening No. __________ Category - IBS-C/FC/HV

<table>
<thead>
<tr>
<th>SYMPTOM DIARY</th>
<th>DURING Blood Sampling VISIT 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Todays Date:</td>
<td>Time: __________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No.</th>
<th>Question</th>
<th>None</th>
<th>Very Mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Quite Severe</th>
<th>Very Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>How would you rate your abdominal pain/discomfort over the last HOUR?</td>
<td>☐</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td>2</td>
<td>How would you rate your abdominal bloating over the last HOUR?</td>
<td>☐</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td>3</td>
<td>How would you rate your urgency over the last HOUR?</td>
<td>☐</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td>4</td>
<td>If stool has been passed over the last hour please indicate how difficult (i.e. straining) it was to pass, as well as the 'urgency' and the 'feeling of incomplete emptying/evacuation' by ticking the boxes below.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Stool Straining:</td>
<td>None</td>
<td>Very Mild</td>
<td>Mild</td>
<td>Moderate</td>
<td>Quite Severe</td>
<td>Very Severe</td>
</tr>
<tr>
<td></td>
<td>Urgency</td>
<td>☐</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td>Incomplete evacuation</td>
<td>☐</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td>6</td>
<td>If stool has been passed over the last hour please refer to the Bristol Stool Scale and indicate the type number (i.e. 1 to 7) which best describes your stool and time when stool was passed.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stool 1 Time: ________<strong>:</strong> __ (h:mm) Bristol Stool Scale: __________</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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