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Gastrointestinal Permeability and Motility in Inflammatory Bowel Disease

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Abstract
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Synchronized motility, permeability and secretory (hormones and enzymes) events are integral to normal physiology. Smooth muscle syncytium operates with enteric nervous system (ENS) and endocrine signalling to accommodate, mix and control passage of ingested materials. The intestinal epithelial cells (IECs) drive digestion and absorption while repelling harmful compounds.

This thesis investigated GI barrier function (permeability, mucosal integrity), motility and hormonal patterns in inflammatory bowel disease (IBD) by: **1)** assessing GI motility using a wireless motility capsule (WMC, SmartPill®) and video capsule endoscopy (VCE, Pillcam®), **2)** investigation of intestinal fatty acid binding protein (I-FABP) as a biomarker of Crohn's disease (CD) activity, **3)** evaluation of small intestinal permeability in IBD, **4)** investigating meal-related motility using WMC and simultaneous hormonal (e.g., Ghrelin, GLP-1, GIP, PYY) patterns in IBD. Reference motility values of transit times for gastric emptying, small bowel, orocecal, small+large bowel, colon and whole gut were established. Software-generated estimates and visually determined values were nearly identical. Compared with VCE estimates (represents fasting conditions), the WMC records longer GET and SBTT. Variations in intra-subject reproducibility must be considered in clinical investigations. This data was then used to investigate IBD patients. I-FABP was primarily expressed in the epithelium of the small bowel and to lesser extent also in the colon and stomach. Circulating I-FABP was elevated in active CD with a magnitude comparable to TNFα. I-FABP lowers and rises again in parallel with TNFα and HBI during infliximab treatment. I-FABP can be used as a jejunum and ileum selective prognostic biomarker for monitoring disease activity. Increased small intestine mucosal barrier permeability to lactulose in both CD and UC was found. Sucralose can serve a dual purpose in quantifying small and large intestinal permeability. Small intestinal hyper-permeability was not revealed as a transporter dependent nutrient (riboflavin) malabsorption. Using the WMC, consistent motility disturbances in IBD were limited, as were differences in pH. However, disturbances within many individuals were found. As part of the investigation, defects in gut peptide and metabolic hormone meal responses were found, typically higher plasma levels. No clear associations between hormones and motility were found. Effects on hunger/satiety signaling in IBD are anticipated.

The present thesis shows the utility of the WMC and gut barrier tests in monitoring IBD patients.

Keywords: Gastrointestinal motility, intestinal permeability, leaky gut, Mucosal barrier, Ghrelin

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To whom who made me exist. To my parents & family

List of Papers

This thesis is based on the following papers, which will be referred to by their corresponding Roman numerals:

I.   Diaz Tartera HO*, Webb D-L*, **Al-Saffar A Kh,** Halim MA, Lindberg G, Sangfelt P, Hellström PM. Validation of SmartPill® wireless motility capsule for gastrointestinal transit time: Intra-subject variability, software accuracy and comparison with video capsule endoscopy. Neurogastroenterol Motil. 2017; 29:1-9.

II. **Al-Saffar A Kh*,** Hampus CM*, Gannavarapu VR*, Hall G, Li Y, Diaz Tartera HO, Lördal M, Ljung T, Hellström PM, Webb D-L. Parallel changes in Harvey-Bradshaw Index, TNFα, and intestinal fatty acid binding protein in response to infliximab in Crohn's disease. Gastroenterol Res Pract, vol. 2017, Article ID 1745918, 8 pages, 2017.

III. **Al-Saffar A Kh,** Halim MdA, Hall G, Hellström PM, Webb D-L. Concurrent small and large intestinal permeability in inflammatory bowel disease. *Manuscript.*

IV. **Al-Saffar A Kh,** Diaz-Tartera HO, Webb D-L, Hellström PM. Gastrointestinal motility and gut hormone profiles in inflammatory bowel disease. *Manuscript.*

* These authors contributed equally to the work

Related articles not included in this thesis:

Karimian N, Moustafa M, Mata J, **Al-Saffar AK**, Hellström PM, Feldman LS, Carli F. The effects of added whey protein to a pre-operative carbohydrate drink on glucose and insulin response. Acta Anaesthesiol Scand. 2018; 62 (5):620-627.

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Contents

Abbreviations

AUC	Area under the curve
C-18	Saturated 18-carbon chain on silica stationary phase
CCK	Cholecystokinin
CDAI	Crohn's disease activity index
CNS	Central nervous system
CRP	C reactive protein
CTT	Colon transit time
ENS	Enteric nervous system
FABPs	Fatty acid binding proteins
FC	Fecal calprotectin
GET	Gastric emptying time
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
HBI	Harvey Bradshaw Index
HC	Healthy controls
IBD	Inflammatory bowel disease
ICC	Interstitial cells of Cajal
ICJ	Ileo-cecal junction
IECs	Intestinal epithelial cells
IHC	Immunohistochemistry
IL-6	Interleukin-6
kDa	kilo Dalton
LPS	Lipopolysaccharide
MI	Motility index
MMC	Migrating motor complex
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSAID	Non-steroidal anti-inflammatory drugs
PYY	Peptide tyrosine-tyrosine
RCF	Relative centrifugal force
SBTT	Small bowel transit time
SLBTT	Small + large bowel transit time
TJs	Tight junctions
TLR4	Toll-like receptor 4
TNF-α	Tumour necrosis factor alfa

VCE	Video capsule endoscopy
WGTT	Whole gut transit time
WMC	Wireless motility capsule

1. Introduction

1.1 Gastrointestinal tract

1.1.1 Anatomy and histology

The gastrointestinal (GI) tract is a continuous tube from the oral cavity to the anus. The GI tract is surrounded by the peritoneal fold (mesentery) to maintain the GI organs in position and supports movements. Mechanically and electrically (gap junctions) connected smooth muscle layers (tunica muscularis) form the syncytium and give a tube shape to the GI tract (1). The serosa forms the outer lining of the GI tract. The outermost muscle layer is longitudinally oriented and the innermost is circularly oriented, relative to the tube axis. The longitudinal muscles in the colon are arranged to form band like structures (taenia coli), giving the colon a saccular appearance. The muscularis mucosa comprises a thin sheet of smooth muscles that comes next to the circular smooth muscle layer closer to lumen. The connective tissue (lamina propria) is situated intermediate between the muscularis mucosae and epithelial surface. Intestinal epithelial cells (IECs) line the mucosal surface and come in contact with the luminal milieu, interspersed with mucosal glands (2).

Life expectancy of IECs lasts 4 to 5 days with a given luminal shedding of 10^{10} IECs/day (3, 4). Stem cells reside in the base of the intestinal crypts, and give rise of different cell types forming the cellular diversity as they differentiate and climb along the crypt villus axis (5). Absorptive enterocyte type cells (alkaline phosphatase +) as well as secretory type cells (goblet, Paneth, enteroendocrine and tuft cells) dominate the cell population (6). Digestive enzymes are attached to the membrane of the IECs for in the cytoplasm digestion and for extracellular secretion. Mucous secreting (goblet) cells produce a thick mucus layer ("firmly" adherent) and a thin outermost layer ("loosely" adherent) in direct contact with lumen contents. These cells ultimately protect the underlying tissue from the harsh intraluminal environment. Enteroendocrine cells are visibly more opaque secretory cells that are sporadically dispersed throughout different segments of the GI tract. They secrete mediators, to the blood stream (endocrine) and to surrounding tissues (paracrine) or even autocrine, for hormonal actions and for local influence of various GI functions (1).

Interspersed within the GI tract layers are blood and lymph vessels as well as two types of nerve plexuses. The myenteric or Auerbach's plexus resides between the longitudinal and circular muscle layers. The Meissner's plexus resides in the submucosa. Intrinsic primary afferent neurons detect and convey luminal information to the submucosal nerve plexus. Interneurons connect the two plexuses to integrate the information in the system. Motor neurons regulate movements of the GI tract and are under the control of the interstitial cells of Cajal (ICC), which function as pacemaker cells (7). Collectively, these GI tract neurons constitute the "enteric nervous system" (ENS). The ENS is capable of carrying out many GI functions independently from the central nervous system (CNS). Although the ENS possesses considerable autonomy in regulating the GI tract, the CNS has a modulatory homeostatic dominance (8, 9). The ENS provides regulatory neurogenic inputs to the GI smooth muscle cells, which all have a basal intrinsic myogenic tone for contractile force (9).

1.1.2 Motility

Motility of the GI tract is the coordinated smooth muscle mechanical activity (rhythmic waves of contracting and relaxing) to ensure a proper functionality (accommodation, secretion, digestion, absorption, and elimination). The GI sphincters (circular oriented muscle aggregates) serve as check points that delimit passage of ingested materials in order to maintain proper digestion and absorption before storage and evacuation. The regulation of motility is under nervous and hormonal control reflexes (1). Although motility function in the GI tract is autonomously controlled by ENS, the CNS has a modulatory fine-tuning and monitoring control. Contractions in the smooth muscle cells initiate when calcium ions are released from the endoplasmic reticulum to bind calmodulin and promote myosin light chain kinase activation (myosin phosphorylation) (9).

GI smooth muscle cells autonomously generate electrical slow waves irrespective of their extrinsic nerve input. This unique electrophysiological event of the GI smooth muscle cells is governed by the ICC to exert an intrinsic control (1, 7). Many factors influence GI segment specific motility, consistent with the fact that motility plays an important role in glucose homeostasis, nutrient absorption and electrolyte balance (10). Of the motility functions of the GI tract, the migrating motor complex (MMC) is the best known. The MMC is a regulated recurring pattern of contractions generated in the fasting state (7, 10). The MMC comprises three phases. Phase I represents quiescence where no noticeable contractions occur. In phase II, irregular contractions originate in the lower part of the stomach and proximal duodenum, continuing distally along the GI tract. Finally, in phase III forceful high-amplitude downstream migratory contractions complete a single MMC cycle, returning to quiescence. The MMC occurs in repetitive sweeping cy-

cles, driving peristaltic waves that propel undigested food residues, cell debris and bacteria distally towards the colon (11). Pelvic ganglia project nerve fibers to the colonic and rectal myenteric plexuses (8). Activation by rectal distention (i.e., increased rectal pressure) retards colonic transit, whereas colonic distension results in small intestinal transit delay by way of neuronal reflex (9 and references therein).

1.1.3 Gastric emptying

Gastric emptying involves cooperativity of the CNS, ENS and stomach smooth muscles. The duration of gastric emptying is called the gastric emptying time (GET). This is an important GI mechanism for regulating nutrient delivery to the small intestine for absorption and energy homeostasis. The pyloric sphincter controls gastric emptying of chyme to the duodenum (9). Mixing and churning contractions of the stomach degrades the coarse food bolus into more dispersed nutrients (chyme) that facilitate food assimilation. The emptying phase is initiated by the stomach. Potent duodenal inhibitory feedback signals modulate this process. This feedback mechanism (neuronal and hormonal) ensures optimal digestion and nutrient absorption for energy homeostasis (10). The initial gastric accommodation after food intake, and later, the gastric emptying process influence hunger and satiety by controlling the time of food appearance (fluid and solid particles of 1-2 mm) in the upper small intestine (11).

Proper food digestion and disintegration influence gastric accommodation and emptying rate. Gastric accommodation is the relaxation of the stomach musculature that allows for volume expansion in order to accept the incoming meal. These processes activate mechano- and chemo-receptors in the proximal part of the stomach. The vagal nerves transmit these afferent neuronal signals to the CNS, ultimately culminating in the perception of satiety, the state of feeling one has eaten enough. The complex signalling between the vagal nerves and CNS is bidirectional and is called vago-vagal reflex. By the time that gastric emptying initiates, yet another satiety mechanism starts and the mechano-sensitivity influence is overwhelmed by a nutrient-stimulated release of enteroendocrine hormones in the upper small intestine (1, 9, 12). The influence of GI motility on appetite is initiated in the stomach at the time of food ingestion and continues during a lag phase for about 30 to 60 minutes as the contents are degraded by the churning motility before the gastric emptying process starts (13). The inhibitory hormonal influences on gastric motility are mediated by cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). Gastric emptying rate is therefore regulated through dual mechanisms: neuronal (activating afferent vagal neurons) and hormonal. Abnormal responses in these two systems will influence GET and hence energy homeostasis (8, 9). There are conflicting opinions regarding the effect of leptin in prolonging

GET, from stating no effect (13), while others have stated that leptin and other hormones (GLP-1 and CCK) can activate gastric break mechanism to slow gastric emptying mind the slow effect in hour timescales (14). In addition, pancreatic amylin and insulin, likely through blood glucose changes, can slow gastric emptying (11, 14).

1.1.4 Gastroprokinetic mechanisms

In the fasted state, the hunger hormone ghrelin (growth hormone secretagogue receptor ligand) is secreted from the stomach and duodenum (15). Ghrelin promotes acid secretion, gastric motility and accelerates gastric emptying of solids and liquids (9, 16). The functionally related hormone, motilin, shares its action with ghrelin by enhancing GI motility. Motilin is secreted from the upper part of the small intestine and plays an important role in regulating antroduodenojejunal contractions. Together with ghrelin, motilin can initiate the housekeeping phase III of MMC that accelerates emptying to prepare the stomach for the next meal (17, 18). Motilin and ghrelin differ in the diversity of their sites of action. Motilin receptor agonists (e.g., the antibiotic erythromycin) have been used to accelerate gastric emptying in gastroparetic patients (19). Interdigestive contractions initiated by ghrelin and motilin are important for the integrity of GI motor functions.

After food intake, the stretch of the stomach muscles, dependent in part on food type (e.g., proteins), promotes gastrin secretion from the antral and duodenal mucosa. Gastrin promotes acid secretion, motility and activity of the pyloric pump to facilitate emptying (15). The duodenum then feeds back directly through ENS and prevertebral neurons as well as through the vagus nerve, to slow down the gastric emptying (14). Pharmaceutical agonists of motilin/ghrelin receptors are considered for treating delayed gastric emptying (i.e., gastroparesis) and other gastric emptying-related disorders.

1.1.5 Incretins

GI hormones that augment glucose-stimulated insulin secretion for glucose homeostasis are called incretins. This term was coined following observations that the same amount of glucose has a more pronounced insulinotropic effect if taken orally compared to the parenteral route (20). The two major incretins in humans are GIP and GLP-1. Other hormones such as peptide tyrosine–tyrosine (PYY), glucagon and amylin can also influence glucose homeostasis (13, 20). All of these hormones have different effects on gastric emptying to balance glucose homeostasis, where GLP-1 seems to have an outstanding role. Secretion of GLP-1 occurs in response to a meal, mainly together with PYY, from the enteroendocrine L-cells predominantly located in the ileum and colon (8). The GLP-1 main actions in glucose homeostasis are to decrease glucagon, amplify nutrient-stimulated insulin secretion, and

prolong GET and small bowel transit time (SBTT). These actions have the combined effect of optimizing nutrient absorption from the intestine, while reducing further food intake (8, 17, 20). The effects of GLP-1 have been shown to be dependent on intact vagal innervation (8). Previous work in our lab demonstrated: *i*) GLP-1 receptor immunoreactivity at myenteric neurons, but not muscle, throughout the human GI tract, *ii*) direct action of GLP-1 on human muscle relaxation in gut *ex-vivo* resections, and *iii*) *in vivo* suppression of motility index (MI) at near physiological infusion of GLP-1 (21). Infusions of GLP-1 or its analogues inhibit food intake and induce weight reduction. In clinical practice, due to these effects on gastric emptying and appetite, GLP-1 is used to treat diabetes type 2 and obesity (20, 22, 23). GIP at low doses can slightly decrease GET due to an effect on the pyloric sphincter (22). GIP has little effect on food intake (23). PYY is secreted from the L-cells in response to the presence of fat in ileum (9).

1.1.6 Gastric acid secretion and pH

Acid secretion during the cephalic phase is entirely mediated by vagal stimulation, while during the fed state (gastric phase) it responds to gastric distension via vagal and spinal reflexes. Neurocircuitry responses of acid secretion are under physiological and pathophysiological activation (8). In the fasted state, the stomach milieu acidity of healthy subjects falls in the range between pH 1.3 and 2.5 (12). Acidic pH in the stomach inhibits the microorganisms' entry to the GI tract and shapes the commensal microbiological taxa (24). Ghrelin influences acid secretion during the fasted state and night (15). In the stomach, the rate of pepsinogen converted to pepsin (pepsin precursor, protein digesting enzyme) is strongly regulated by pH; higher pH is associated with lower pepsinogen secretion. Pepsin is optimally active at pH 2.0 and is inactivated when pH exceeds 6.5 (25). A low gastric pH decelerates the gastric emptying rate (26). Acid that spills into the duodenum promotes closure of the pylorus by feedback through splanchnic nerves and reduces the gastric pressure, which prolongs GET (27). Woodtli 1995 reported that lowering duodenal pH prevents phase III interdigestive MMC despite normal plasma motilin cycling, concluding that duodenal pH regulates interdigestive MMC (28). Proteins and fats leaving the stomach bind to duodenal receptors and slow gastric emptying. Nutrients (e.g., glucose) appearing in small intestine promote GLP-1 and PYY secretion, which together with slowing of gastric emptying, also inhibit acid secretion rate (13). GIP secreted from the duodenum and upper jejunum inhibits acid secretion mediated via somatostatin secretion (29). More potently than GIP, CCK secreted from the jejunal mucosa into blood circulation can slow gastric emptying and reduce acid secretion (13).

1.2 Gastrointestinal barrier

The GI barrier covers an extended area and controls water and nutrient homeostasis. The physical and functional entities of the barrier cooperatively serve multiple tasks simultaneously. The barrier provides the physical and functional support to facilitate digestion, absorption and protection against harmful compounds (3).

1.2.1 Mucosal permeability and absorption

The GI barrier's function is to support water, electrolytes and nutrients influx from the luminal to the mucosal side. Simultaneous ingress of xenobiotics (e.g., endocrine disruptors), bacterial toxins and injurious by-products should be constrained by this barrier (3, 30). Permeability is regulated by the epithelial integrity, tight junction proteins, the mucous layer, immune modulation, GI vasculature, motility and microbiota (1, 2, 31). Tight junction heteromeric proteins regulate trafficking of molecules through the GI barrier. Enterocytes tightly adhere to the mucosal lamina propria layer and to each other by specialized macromolecules known to serve integral and signalling functions. Non-luminal enterocytes aspects are connected to one another longitudinally by tight junctions (apical part), adherens junctions and desmosomes (middle part) and at the basal aspect by hemi-desmosomes (30, 32). Transmembrane proteins (e.g., claudins, occludins), the associated connecting molecules (zonula occludins) and regulatory proteins form complex epithelial junctions (30, 33). Hyper-permeability can develop directly due to high fat diet, western life style and stress or indirectly by endotoxaemia-associated dysbiosis (34, 35). Aberrant barrier function is detected in different conditions (food allergy, obesity, irritable bowel syndrome) and diseases (diabetes, respiratory failure, microbial enteric infections, inflammatory bowel disease (IBD)) (30, 33, 36). There is no consensus whether this GI permeability change is incipient to, or an outcome of, IBD (37). Increased GI barrier permeability to xenobiotics and antigens promote low-grade inflammation and disease progression (30). Activation of toll-like receptor 4 (TLR4) by lipopolysaccharide (LPS) complexed with LPS binding protein promotes NF-kB activation. Downstream of NF-kB, TLR4 activation drives production of tumour necrosis factor alfa (TNFα) and interleukin-6 (IL-6), along with an associated inflammation and barrier damage (33). Increased GI permeability promotes enhanced translocation (i.e., aberrant selectivity) of luminal contents (potentially endocrine disruptors, for example) towards the blood. Permeability control through tight junction modulation is a target for diet, pharmaceutical therapeutics and other interventions (microbiota shaping) in disease processes, including those of IBD (32, 38, 39).

1.2.2 Intestinal permeability assessment

Barrier function assessment (GI permeability) is performed by orally ingested biomarkers, which are then recovered in blood or urine. Different biomarkers can be used *in vivo*, such as radioisotopes, fluorescent molecules, polyethylene glycol or ova albumin, but the most used molecules are sugars. Assessing para- and trans-cellular routes across the epithelial cell barrier of the small intestine is most commonly done using lactulose and mannitol sugars (40). An intact paracellular route should largely repel > 0.35 kDa molecules, while the transcellular route can allow passage of small molecules. Lactulose and mannitol are recovered in urine (0 to 6 h), representing the small intestine permeability (41). Sucralose is comparable to lactulose in size and is recovered in urine (6 to 24 h), representing large intestine permeability. Unlike lactulose, sucralose is resistant to fermentation by colonic microbiota and does not increase GI motility (42). Mannitol is absorbed transcellularly and is completely recovered in urine during the first 8 h. Lactulose is more slowly absorbed, which may last over 24 h (41, 42). Permeability assay confounders can be gastric dilution, GI motility, renal function and bacterial degradation. Bacterial degradation of lactulose and the influence on colonic motility (increase) can limit its use in patients. Limiting recovery time (6 h) and minimizes the dose, while excluding patients with renal insufficiency could be considered (32).

1.3 Integrity markers of GI relevance

1.3.1 Intestinal fatty acid binding protein (I-FABP)

Fatty acid binding proteins (FABPs) are a group of lipophilic proteins (MW ~15 kDa) within the cytoplasm of the mammalian cells that support lipid homeostasis and signalling mechanisms. Uptake and transport of fatty acids, cholesterols and retinoid or other hydrophobic ligands are the main known functions of FABPs. Cellular energy homeostasis (lipid metabolism) is a further proposed function (43). Other proposed functions are to support availability and transport of some vitamins (43, 44). Apart from intracellular trafficking and energy homeostasis, some of the FABPs have been shown to have extracellular functions, such as local and/or systemic inflammatory mediation, implying potential as therapeutic targets for immune-metabolic diseases (44, 45). Some FABPs serve as transporters for specific receptors, such as peroxisome proliferator activated receptor, thus promoting intracellular signalling mechanisms (46). FABPs can serve as diagnostic biomarkers for diseases like intestinal ischaemia, myocardial infarction, hepatitis C and liver transplantation rejection. As cells producing FABPs die and lyse, FABPs are released into blood circulation. Tissue damage in some cases correlates with FABPs levels in plasma or urine. These changes can occur

from near or below limits of detection in normal healthy individuals to clearly detectable levels after tissue damage (fourfold or higher). Following the trajectory levels during a treatment course can be useful to monitor a tissue healing process (44, 47, 48). IECs express three types of FABPs molecules, which are the liver, intestinal and ileal forms (L-FABP, I-FABP and Il-FABP). Expression levels depend on the GI segment, L-FABP and Il-FABP expressed mostly in the upper and lower segment respectively, while I-FABP is thought to be expressed throughout the GI tract (48). Long chain fatty acids and hydrophobic molecules are binding targets of these proteins with high affinity (45, 48, 49). The availability of fatty acid-FABPs complexes in the blood flow to the pancreas could modulate insulin secretion and glucose homeostasis. Support for this is seen in findings that circulating A-FABP enhances hepatic glucose production and insulin levels in knockout mice (44). Crohn's disease (CD) can manifest as lesions throughout the GI tract; ileocecal involvement is particularly common. Prior to this thesis, it has been unknown if circulating I-FABP changes with disease activity in CD. Such a relationship would be critical for use of I-FABP as a biomarker.

1.3.2 TNFα

The pro-inflammatory cytokine TNFα modulates multiple signaling pathways. In IBD, TNFα initiates innate immune response against microbial agents. TNFα links the innate and adaptive branches of immunity by recruiting specific cells to promote differentiation, secretion of cytokines and apoptosis (50). TNFα production in the ileo-colonic segment in IBD is strongest in plasma cells, with a lesser production in lymphocytes and macrophages. One study using IHC showed diffuse versus focal expression patterns in ulcerative colitis (UC) compared to CD (51). Extraintestinal manifestations of IBD are linked to elevated circulating TNFα levels, this being associated with activation of inflammatory cells. Systemic changes in innate immune function promote inadequately lower response in affected extraintestinal organs of patients (52). Acute enterocyte exposure to TNFα induces receptor overexpression associated with consequent elevated intracellular TNFα levels and shedding. Elevated cellular shedding over villus axis and villus atrophy is consequence of chronic exposure (53). TNFα is cooperatively produced by activated macrophages, and inflammatory and non-inflammatory enterocytes of IBD patients (54). Its recognized increase and involvement in IBD formed the basis for artificial anti-TNFα antibodies to become among the first monoclonal antibody based drugs (e.g., infliximab was discovered in 1988). Hence, TNFα remains an important analyte in IBD research.

1.4 Self-reporting assessment, objective indexes

1.4.1 Crohn's disease with the use of Harvey Bradshaw index for disease activity

Assessment of Crohn's disease patient's symptoms is performed prior to treatment initiation and is used to evaluate treatment responses. The Harvey Bradshaw index (HBI) is a self-assessment questionnaire (performed by the patient and medical doctor) for disease activity discernment (55). Although HBI is less cumbersome to perform compared to Crohn's disease activity index (CDAI) commonly used in clinical trials for assessing disease activity, they have strong correla-tion (56). However, both CDAI and HBI show poor correlation with mucosal inflammation (57). The dependence on functional assessments could lead to under or over treatment (58). Another level of confirmation is the endoscopy associated histopathological examination, although it is cumbersome (59). The subjective markers, C reactive protein (CRP) and/or fecal calprotectin (FC) are typically used as part of monitoring IBD disease activity (60, 61).

1.4.2 Ulcerative colitis with the use of Mayo clinic score for disease activity

An objective tool for UC disease activity is required for better patient as-sessment. The Mayo score is used widely for this purpose. The invasive ver-sion includes stool frequency, rectal bleeding, a physician's global assess-ment and a sigmoidoscopic assessment on a score scale from remission at 0 to 12 (62). The modified non-invasive 9-point scale omits endoscopy. Yet another modification is the 6-point scale. The 9- and 6-point scales correlate strongly with the invasive version (63). Clinical improvement is set to be ≥ 3 points reduction from the baseline (63, 64). Identification of UC patients in remission is important. This requires a score less than 2.5 on the full Mayo scale (63). For the long term outcome, endoscopy is not enough and mucosal healing (e.g., markers and/or histopathology) is required in defining remis-sion (65). Presence of microscopic disease activity, even with normal clini-cal and endoscopic findings (i.e., partial and full Mayo clinic score index), is common, which is why histopathology and subjective markers (CRP, FC) are required (62, 66).

1.5 GI monitoring system

1.5.1 Wireless motility capsule, SmartPill[®]

Functional GI disorders are increasingly prevalent due to different factors (e.g., stressful lifestyle, altered food habits) including disease (10, 67). Dis-ordered GI motility and increased sensitivity are common symptoms that

require investigation. Clinically available tests are mainly limited to esophagogastroduodenoscopy despite the limited relevance of this investigation for functional symptoms (68). Manometry, radiology, scintigraphy and ultrasound-based or biochemical methods are seldom used (69). Techniques, such as the wireless motility capsule (WMC), combine investigation of the whole GI tract using pH, transit times and MI as surrogate marker for health and disease (67, 70). The system includes a computer that runs dedicated software (MotiliGI v. 3.0), ingestible diagnostic sensing capsule device (26×13 mm) and data receiver. The capsule broadcasts real time data from the GI lumen under natural conditions over a period up to 5 days. Timestamped pressure, pH and temperature data are processed by the software into graphic form (67, 68). Test results that can be compared between healthy controls (HC) and patients with GI symptoms (71). The full range of clinical use has yet to be fully realized. Novel variants are being developed.

1.5.2 Video capsule endoscopy, PillCam®

Video capsule endoscopy (VCE) consists of an ingestible capsule that acquires intraluminal pictures that are transmitted to a wearable device, and used for detecting small intestinal abnormalities. One downside of this technique is the short battery life of 8-12 h, which is why colon imaging by VCE is not done (72). Researchers have utilized this technique to produce data of gastric and small bowel transit times in the fasted state, since it is required for imaging (73). Effects of gender, but not age, on transit times by this technique have been reported. Longer transit time was reported for females, although non-significant (74). This technique can obtain small intestinal transit time in IBD patients and others. Pharmacokinetic studies can utilize VCE transit data to individualize treatment protocols for disease activity (75).

1.6 Interventional therapeutics – infliximab

Anti-TNFα monoclonal antibodies form stable complexes with soluble and membrane bound TNFα. This induces and stabilizes remission in IBD. Disease activity is reduced in response to treatment and life quality is improved (76). In murine induced colitis models, anti-TNFα therapy promotes mucosal integrity and transport function restoration by reducing inflammatory activity despite the ongoing insult (77). Antidrug antibodies are thought to contribute to 30% of treatment failures. Adding to this, 50% of responders develop loss of response with time. For this reason, individualizing treatment by monitoring drug and antidrug antibodies is desirable (78). Although remarkable treatment advantages have been reported, drawbacks could be deleterious, and even fatal, in elderly and severely affected patients due to the

risk of developing malignancies like lymphomas (79). Heart failure, neurologic or liver diseases and malignancies are other comorbidities that pose risks with TNFα antagonist treatments. Risk benefit assessment is required to initiate treatment with these drugs (80).

2. Aims of the thesis

The doctoral study aims were to investigate dysregulated GI barrier function and identify altered motility and hormonal patterns in IBD patients. This should improve overall understanding of issues related to drugs and nutrient absorption and leaky gut uptake of harmful molecules (i.e., environmental contaminants) in patients with compromised GI physiology.

Specific aims by paper:

Paper I: To assess WMC (SmartPill®) software accuracy of derived transit times and reproducibility in healthy subjects. Compare fed versus fasted state transit time data obtained by WMC and VCE (PillCam®) and obtain reference transit data for local population.

Paper II: To investigate I-FABP as a biomarker in CD by examining *i*) distribution along the human GI tract, *ii*) I-FABP stability in biobanked samples, *iii*) levels in relation to current biomarkers, such as CRP, *iv*) changes with TNFα and HBI during anti-TNFα antibody (infliximab) therapy.

Paper III: To assess the small intestinal permeability function in patients with IBD (UC and CD). To determine the utility of sucralose as a probe to simultaneously quantify small and large intestinal permeability in healthy subjects and IBD patients by confirming results with lactulose and mannitol.

Paper IV: To assess the utility of the WMC technique to identify patient-specific GI motility disturbances in IBD. To identify disturbances in gut hormones involved in regulation of motility in IBD.

3. Study design

Paper I: reference data was derived from 73 healthy volunteers (46 males, 27 females) aged 19-74 years, in Uppsala and Stockholm, Sweden. A subset of 10 male subjects repeated WMC tests 2 weeks later and another 10 male subjects 4 weeks later. WMC transit data was compared to that of VCE (fed versus fasted state) using separate population of 70 healthy subjects (21 males, 49 females) aged 18-82 years. These subjects were drawn from referrals following positive findings of fecal occult blood tests or iron deficiency anemia with normal gastroscopies and colonoscopies, and subsequently negative VCE results. These subjects were deemed healthy for the purposes of this study and were un-medicated.

Paper II: serum I-FABP levels were measured in 10 CD patients and 31 healthy subjects with normal GI permeability assessed by urinary recoveries of riboflavin, lactulose, mannitol and sucralose. CD patient samples were obtained from a biobank of pre- and post-anti-TNFα (infliximab) treatments to obtain intra-patient temporal data of I-FABP, CRP and TNFα. The TNFα levels were compared with another 61 healthy subjects used as upper reference cutoff. Infliximab infusions were carried out on day 0 (infliximab naive), week 2 and week 6, with blood tapped one week after each treatment. I-FABP, HBI, CRP and TNFα were tabulated for all time points. TNFα, CRP and I-FABP were compared to reference values established for HC. Healthy GI tissue specimens (stomach, jejunum, ileum and colon) were investigated for relative I-FABP expression levels by IHC.

Paper III: healthy control subjects (n = 25) and IBD patients (11 CD and 19 UC) were investigated for GI permeability function *in vivo*. Riboflavin, lactulose, mannitol and sucralose were ingested and urine was collected (0 to 6 h representing the small intestine and 6 to 24 h representing the colon). Urinary recovery of riboflavin (small intestinal transporter mediated absorption) was determined by intrinsic fluorescence. Recoveries of lactulose and mannitol (small intestinal paracellular and transcellular permeation, respectively) were quantified by NADPH and NADH coupled enzyme assays (81, 82), with modifications for smaller volumes and microtiter plates and plate readers as detailed in **Paper III**. Sucralose (established colon permeation probe) was quantified using HPLC with an evaporative light scatter detector. The laxative property and capacity for intestinal fermentation of lactulose delim-

its the tolerance and compliance for repetitive intra-subject monitoring. We hypothesized that sucralose would be a feasible replacement for lactulose without disturbing laxative properties for small intestinal paracellular permeability assessment. Therefore, sucralose recovery from the small intestine was measured and compared to lactulose as an additional endpoint and to confirm findings with lactulose measurements.

Paper IV: WMC data of pH, luminal pressure and MI from 10 UC and 10 CD patients was obtained and compared to age- and gender-matched controls. The WMC was ingested with a standardized 260 kcal (1088 kJ) mixed meal. Venous blood samples were also drawn at -10, 0, 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 minutes into the meal. The software (MotiliGI 3.0) generated a diagram showing pH, peak pressure amplitude and MI values of selected GI tract segments aligned according to time. The WMC recordings of pH were used to identify anatomical boundaries of the stomach, small intestine and colon. The stomach segment was defined by a low pH after test meal intake to an abrupt increase of pH by more than 4 pH steps. The small intestine was defined by an increasing pH to neutral levels, after which a sudden drop pH of more than 1.5 steps defined the ileo-cecal junction (ICJ). The colon segment was defined as time point of ICJ until the WMC exited from the body, which was indicated by temperature drop and signal loss. Data of pH, luminal pressure and MI were obtained and analyzed in 60 min period of the following segments: post-ingestion, pre- and post- pyloric, pre- and post-ICJ and the final 20 min period in the rectum segment prior to exiting the body. Plasma collected during the first 240 min was assayed for glucose, triglycerides, insulin, leptin and the GI peptide hormones active acyl-ghrelin, motilin, active GIP, active GLP-1 and total PYY. Results were compared to their age- and gender- matched HC.

4. Materials

4.1 Ethic approvals

Studies were approved by Regional Ethics Committee at Uppsala University and/or Karolinska Institutet. Subjects signed an informed consent prior to their participating in the study. Ethics approval numbers;

Paper I: approval was granted by the ethics review board at Uppsala University (Dnr: 2010/184/1).

Paper II: the ethical approval number is Dnr: 92:38 for CD patients (Karolinska Institute, Sweden). Healthy subjects were further covered under Dnr: 2012/323 (blood samples), 2010/157, and 2010/184 (surgical specimens for immunohistochemistry) to Uppsala University.

Paper III: study was approved by way of ethical approval Dnr: 2010/184/1 issued by the Regional Ethics Committee in Uppsala, Sweden.

Paper IV: approval was granted by the ethics review board at Uppsala University (Dnr 2010/184/1).

4.2 Human subjects

4.2.1 Human subjects, Paper I

Healthy volunteers (n = 73, 46 men, 27 women) aged 19 to 74 years (29 ± 1 years, mean ± SEM) in Uppsala and Stockholm, Sweden, were recruited. Subjects with the following GI issues were not included: acute or chronic abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions, diverticulitis, celiac disease, CD, UC or proctitis, previous GI surgery, implanted electromechanical medical devices or medications shown to influence GI motility and transit time (prokinetics, antidiarrheals, laxatives) as well as non-steroidal anti-inflammatory drugs (NSAID) and tricyclic antidepressants, selective serotonin re-uptake inhibitors or opioids. Additional exclusion criteria were: cardiovascular, endocrine, renal, or other chronic disease, children under 18 years of age, females during their menses

or pregnancy, tobacco for 8 h or alcohol (for 24 h) use before or during the monitoring period. A subset of 10 male subjects repeated WMC tests 2 weeks later and another 9 male subjects 4 weeks later. For PillCam® VCE studies, a separate population of 70 healthy subjects (21 males, 49 females) aged 18 to 82 years were examined. These subjects were drawn from referrals following positive faecal occult blood tests or iron deficiency anaemia, but with normal gastroscopies and colonoscopies and subsequently negative VCE results and otherwise normal findings. Exclusion criteria were small bowel pathology such as polyps, tumour, celiac disease, UC, CD, unspecific ulcers with or without blood in the lumen, unclear view of mucosa upon VCE leaving the stomach or small intestine, or retropulsion between small intestine and stomach, or colon and small intestine. The remaining subjects were included since they were deemed healthy for the purpose of this study and not taking medications (e.g., opioids). The last image time point of leaving the gastric and small intestine lumen, respectively, was recorded.

4.2.2 Human subjects, Paper II

CD patients (n = 10) were identified out of a biobank database containing a total of 47 CD patients with repeat visits that underwent infliximab therapy (Remicade®, 5 mg/kg body weight) between the years 2000 and 2005. Plasma samples corresponding to 6 patient visits with corresponding HBI data. Colonoscopy was performed to document inflammation in all patients. Serum samples from 31 healthy adult controls with normal gut permeability assessed by lactulose/mannitol ratio ≤ 0.7 (83), were included to establish a reference interval for serum I-FABP. Another 61 healthy adult controls, constituting an established in house TNFα reference interval was used for comparison against TNFα in the CD patient samples.

4.2.3 Human subjects, Paper III

Diagnosed IBD patients ($\geq$ six months) outside their flare-up period (19 UC, 11 CD) without concomitant diseases and 25 healthy control subjects were recruited. Riboflavin data obtained from a previous study of 12 healthy subjects was appended (83). Healthy subjects with one or more of the following criteria were excluded: those under 18 years of age and females during their menses or pregnancy, fever, food allergy, acute or chronic abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions, diverticulitis, celiac disease, proctitis, previous GI surgery, implanted electromechanical medical devices or medications shown to influence GI motility (prokinetics, antidiarrheals, laxatives) as well as NSAID, tricyclic antidepressants, selective serotonin re-uptake inhibitors or opioids. A history of cardiovascular, endocrine, renal or other chronic disease was also basis for exclusion.

Energy drinks and tobacco (for 8 h) or alcohol (for 24 h) were not consumed before or during the monitoring period.

4.2.4 Human subjects, Paper IV

Patients, 10 UC and 10 CD, were compared to their 20 age- and sex-matched healthy volunteers (aged 19 to 74 years). Healthy volunteers were devoid of the following GI disorders: acute abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions, diverticulitis, celiac disease, previous GI surgery, implanted electromechanical medical devices or medications known to influence GI motility and transit time (prokinetics, antidiarrheals, laxatives), as well as NSAID, tricyclic antidepressants and selective serotonin re-uptake inhibitors or opioids. Additional exclusion criteria were: cardiovascular, endocrine, renal, or other chronic disease, individuals under 18 years of age, menstruation or pregnancy, tobacco (for 8 h) or alcohol (for 24 h) use before or during the monitoring period.

5. Methods

5.1 SmartPill® establishing reference values, Paper I

5.1.1 SmartPill® WMC and monitoring system

The SmartPill® WMC (Given Imaging Ltd) is a 4.5 g indigestible single-use, 26 × 13 mm cylindrical capsule. Data is transferred to the accompanied wearable external data receiver and displayed and analyzed using MotiliGI version 3.0 computer software (Given Imaging) (84). This WMC records temperature (range 25 to 49 °C), pH (range 0.05 to 9.0 units) and pressure (range 0 to 300 mmHg). The capsule contains a battery that provides power for at least 5 days, which drives a radio transmitter that broadcasts real time data to the receiver (85). Visual analysis of the WMC data for GET, SBTT, colon transit time (CTT) and whole gut transit time (WGTT) was performed by one investigator (HDT) and was separately confirmed (blinded to software or other investigator's result) by another (PMH).

5.1.2 Standardized Meal

In order to align WMC motility recordings with actual meal-associated transit times, subjects first received a standardized mixed test meal consisting of two egg whites, buttered toast and jelly (260 kcal or 1088 kJ: 3% fat, 21% protein and 76% carbohydrate of which ~3% was fiber; % dry weight). The validated SmartPill® clinical protocol was originally specified in kcal, but kJ is the current SI unit for meal energy content. The meal used in this study corresponds to the specified SmartBar® (Given Imaging).

5.1.3 SmartPill® WMC test

After the test meal, the capsule was ingested with 100 ml water. Subjects were ambulatory, but encouraged to sit. Six hours after capsule ingestion, they returned to normal daily activities, including *ad libitum* feeding (86). To standardize test conditions and facilitate interpretation, strenuous activities, such as sit-ups, abdominal crunches, exercise or prolonged aerobic activity (>15 min) were not allowed (70, 86). The data receiver was carried in a sling around the neck daytime and in bed nighttime until passage of the capsule.

At 144 hours post-ingestion, subjects returned the data receiver, and the data was downloaded to a computer (85).

MotiliGI 3.0 software was used to generate graphs for visual assessment and summary reports of software computed transit times in hours and minutes. WMC data was analyzed visually for GET, SBTT, CTT and WGTT by two investigators (AKhAS, HDT) and confirmed by another (PMH). Comparisons with software calculations were done by yet another (DLW). Visually derived GET, SBTT, CTT and WGTT results from WMC were compared to software calculated values by the MotiliGI 3.0 software for optimal alignment. The pH profiles were analyzed by one investigator (AKhAS) and confirmed by another (PMH). The MI was calculated as Ln (sum of amplitude × number of contractions +1) (87). GET was defined as the time from ingestion (transition from room temperature to 37 °C) of the WMC to pyloric passage (abrupt pH rise >3 units from gastric baseline to pH >4). SBTT was defined as the time between passage of the WMC into the small bowel and entry into the cecum (rapid pH drop >1.5 units) upon traversing the ICJ. CTT was defined as the time between cecal entry and exit from the body (abrupt pH drop of at least 1.5 pH units and temperature drop to room temperature or signal loss). Exit from the body during defecation was documented in the subjects' diaries as abrupt loss of signal (85). WGTT was defined as the time between WMC ingestion and body exit (rise from room temperature to body temperature and subsequent return to room temperature) (70, 88).

5.1.4 PillCam® SB VCE

The PillCam® SB VCE (Given Imaging) is a cylindrical indigestible capsule that measures 26.3 × 11.4 mm (2.9 g) and obtains two images per second for approximately 8 h. Subjects had only liquid food the evening before, and no oral intake from midnight until morning of examination. No bowel preparation or prokinetics were used. Images were transmitted to a recording belt and later downloaded to a viewing station for clinical review (89). GET was defined as the time between the first gastric to the first duodenal image. Time between first duodenal and first cecal images was defined as SBTT (90).

5.2 I-FABP potential marker for monitoring infliximab treatment, Paper II

5.2.1 Blood Samples

Blood samples were drawn on days 1, 14, and 42 immediately before infliximab infusion and on follow-up visits, each one week after infusion (Fig. 1).

On the first blood draw for serum, immediately before infusion 1 (Inf1), CD patients were naive to infliximab. This time point was used as a baseline to normalize data. To verify a drug effect in relation to circulating I-FABP, TNFα and CRP levels were measured. A 50X protease inhibitor cocktail solution was prepared by dissolving a SigmaFast tablet (Cat# S-8830, Sigma-Aldrich, St Louis, MO, USA) in 2.2 ml deionized H2O and adding 5.5 µL of 10 mM peptidyl peptidase-4 inhibitor KR-62436 (Cat# K4264, Sigma-Aldrich) in DMSO along with a separate 68 mM 10X EDTA stock (91). After vortexing, the 50X cocktail was pipetted immediately into blood tubes to 1X final concentration. Inhibitors in SigmaFast (protease target, final µM concentration) in plasma/serum were: AEBSF (serine proteases, 2000), Bestatin (aminopeptidases, 130), E-64 (cysteine proteases, 14), Leupeptin (serine/cysteine proteases, 1), Aprotinin (serine proteases, trypsin and human leukocyte elastase, 0.2-0.3), Phosphoramidon (thermolysin/collagenase, 1) and Pepstatin A (acid proteases, e.g., pepsin, renin, cathepsin D, 10). Final concentration of KR62436 was 0.5 µM and EDTA was 6.8 mM. Because this cocktail was not added at the time of blood draw in the case of the biobanked infliximab infusion and follow-up samples, it was added along with final 1X EDTA (for comparisons to plasma) prior to thawing in order to minimize degradation during or subsequent to thawing and to permit identical chemical composition as with all the recently obtained samples (i.e., controls) they were compared against, into which this cocktail was added at the time of blood draw.
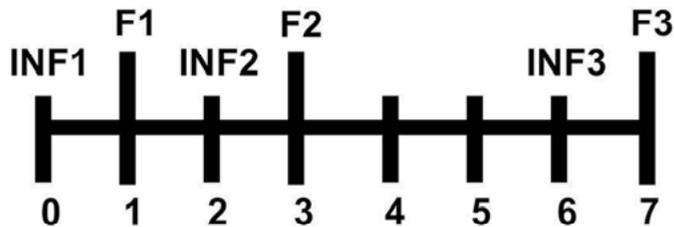
Figure 1. Time points in weeks (week 0 to 7) for the 3 consecutive infliximab infusions (Inf1, Inf2, Inf3) and weekly follow-ups (F1, F2, F3). At each visit, serum for TNFα, I-FABP and CRP was obtained along with HBI data.

5.2.2 Protein expression

5.2.2.1 I-FABP Enzyme Linked Immunosorbent Assay

Serum I-FABP was measured by commercial research sandwich ELISA kit (catalog number HK406-2, Hycult Biotech, Uden, The Netherlands) according to the product insert using 20-fold sample dilution, absorbance (450 nm) was read on a plate reader (TECAN infinity M200). TNFα and IL-6 were measured using "V-PLEX" sandwich ELISA kits (Meso Scale Discovery,

Rockville, MD, USA). The V-PLEX kits are validated kits manufactured for high reproducibility between lots. IL-6 was included because it is thought to drive CRP production and release from the liver. Serum CRP was measured using CRP Vario 6K26 assay (Sentinel CH. SpA, Milan, Italy) on an Architect analyzer (Abbott Labs, IL, USA) at the Department of Clinical Chemistry, Uppsala University Hospital.

5.2.2.2 Immunohistochemistry

Paraffin-embedded transmural sections (4 μm thickness) of normal human stomach (cardia, corpus, and fundus), small intestine (jejunum and ileum), and colon were non-pathological surrounding tissue obtained from donors undergoing different GI surgeries (colectomy or others, e.g., bariatric surgery). One negative and two positive (incubation with Ab) slides were included for each GI segment from different subjects. Immunostaining was performed using alkaline phosphatase-FastRed detection of rabbit polyclonal primary antibodies against human I-FABP (catalogue number HP9020, 1:50 dilution, Hycult Biotech). Western blotting has been shown to yield a single band with this antibody (92). Staining assessment was done independently by (AKhA and VRG), which was scored from 0 to ++++ for number of positive epithelial cells and for intensity of staining (red colour). Visual scoring was translated in a linear fashion to relative concentrations by comparison against different concentrations of dye solutions quantified by absorbance on a plate reader (TECAN infinity M200). The concentration difference between one visual score and the next was found to be approximately fourfold. These results were then used to calculate I-FABP relative abundance along with the GI tract using the literature findings for GI tract surface area of the epithelium (93), and I-FABP expression in duodenum (94).

5.3 Mucosal permeability in health and IBD, Paper III

5.3.1 Chemicals, reagents and clinical chemistry

Reagents were purchased from Sigma-Aldrich unless stated otherwise. Standard clinical chemistry analyses were carried out at the Department of Clinical Chemistry, Uppsala University Hospital, Uppsala, Sweden.

5.3.2 Mucosal permeability assessment

5.3.2.1 Probe ingestion and urine collection

Consumption of tobacco, coffee, juices and energy drinks for 8 h or alcohol for 24 h were not allowed prior to ingesting permeability test probes. Patients and healthy control subjects were fasted for 4 h (water permitted) before ingesting permeability probes: riboflavin 50 mg (Freeda Vitamins Inc,

Long Island City, NY, USA), lactulose 10 g (15 mL 0.67 mg/mL solution; Meda AB, Solna, Sweden), mannitol 5 g (D-Mannitol) and sucralose 5 g (food grade, Guardian Wholesale, Phoenix, AZ, USA) with 500 mL water after emptying the urinary bladder (collected as baseline), and with only *ad libitum* water for the next 4 h. Urine was collected from 0 to 6 h (small intestine) and then 6 to 24 h (colon) into two separate opaque containers.

5.3.2.2 Urine processing

Collected urine samples were stored at 4 °C until delivered to Gastroenterology lab at Uppsala University for analysis. Volumes were measured and a 50 mL aliquot was drawn from each sample and centrifuged at 2500 relative centrifugal force (RCF) for 10 min at 4 °C. Supernatant (15 µL) was drawn for riboflavin measurement. Remaining supernatant was stored at -20 °C until lactulose, mannitol and sucralose analyses. At that time, supernatants were thawed, vortexed and centrifuged at 2500 RCF for 10 min at 4 °C, and 1 mL was aliquoted for sucralose measurement and 4 mL aliquots were extracted using C-18 solid phase cartridges (Extra Super sep C-18 500 mg cartridges, LIDA) for measuring permeability lactulose and mannitol by enzyme assays.

5.3.2.3 Permeability probe measurements

Riboflavin was measured by intrinsic fluorescence (83). Lactulose and mannitol were assayed by NADPH- and NADH-coupled enzyme assays (81, 82). Sucralose measured by C8-HPLC connected to an evaporative light scatter detector (ELSD). All analytes were measured in duplicate. Four parametric curve fitting was used to obtain lactulose and mannitol concentrations from enzyme assays raw data relative to their respective standard curve. Standard curve of serial sucralose diluted stock concentration in pooled blank urine was prepared. The standard curves data were obtained by HPLC-ELSD, analyzing each standard concentration in duplicate. Area under the curve was generated for each standard concentration and used in calculating raw data to obtain sucralose concentrations from actual samples. Recovered analyte weight was calculated by multiplying concentrations (g/L) by urine volumes (L) for corresponding time intervals. Dividing recovered weight by ingested dose (g) yielded percent recovery. Lactulose or sucralose percent recovery was divided by percent recovery of mannitol to yield lactulose/mannitol or sucralose/mannitol ratio. Detailed procedures can be found in Supplementary section of **Paper III**.

5.4 Gastrointestinal motility and hormonal patterns in health and IBD

5.4.1 SmartPill® WMC and monitoring system

This was stated in methods of **Paper I**

5.4.2 Gut peptide hormones, leptin, insulin, glucose and triglycerides

Venous blood samples were drawn into 6 ml plasma tubes from an antecubital vein at -10, 0, 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 minutes in relation to ingestion of the standardized mixed meal. Immediately, 160 µl of protease inhibitor cocktail detailed in section 5.2.1 without EDTA was added. Plasma tubes were vortexed and centrifuged (2500 RCF for 10 min at 4 °C). Plasma was aliquoted and stored in -80 °C until analysis of insulin, glucose and triglycerides as well as gut peptide hormones ghrelin, GIP, GLP-1, PYY and also the adipokine leptin. Plasma glucose and triglycerides were measured at the Department of Clinical Chemistry, Uppsala University Hospital; this was done to confirm meal responses in all subjects. Sandwich ELISA kits (Meso Scale Discovery, Rockville, MD, USA) were used to measure hormones; ghrelin as a single-plex and all other hormones as a 5-plex.

6. Statistical analysis

Results are presented as mean ± standard error of mean (SEM) or median (Med) and interquartile range (Q1-Q3) as indicated. The significance level was set at $P < 0.05$.

6.1 Paper I

Reproducibility of transit times within the same subjects repeated after 2 or 4 weeks were presented as Bland-Altman plots and coefficient of variation (CV%), thus revealing limits of agreement between measurements on different occasions for the same subject in the same state of health. Averages of initial and 2 or 4 week repeats of WMC tests were assigned to horizontal axes. Initial transit times minus transit time from week 2 or 4 were assigned to vertical axes. Visually derived GET, SBTT, CTT and WGTT results from WMC were compared with values calculated by the MotiliGI software. Agreement between the two methods was assessed using Pearson's correlation coefficient (r) and presented graphically as Bland-Altman plots. Normal distribution of values was examined using the Kolmogorov-Smirnov test with 95% confidence interval for the WMC and VCE results. Comparison between WMC and VCE results for GET and SBTT were plotted as bar charts rather than Bland-Altman plots because the data was obtained from separate groups of subjects under different conditions (fed vs. fasted).

6.2 Paper II

The paired t-test was used to compare the difference in the levels of I-FABP and TNFα between the infusion and follow-up days. Mann-Whitney U-test was used to compare the I-FABP levels in CD patients versus HC. Statistical analysis was done using the SigmaPlot software (ver. 11.0). Power analysis was done using the "R" software (http://www.r-project.org) to calculate optimal sample size.

6.3 Paper III

The paired t-test and Mann-Whitney U-test were employed using the Sig-maPlot software (ver. 11.0).

6.4 Paper IV

Statistical differences were calculated using the non-parametric Wilcoxon signed-rank test employing SigmaPlot software (ver. 11.0).

7. Results & Discussion

7.1 Reference motility data, Paper I

The SmartPill® WMC recorded pH, pressure and temperature data relative to meal ingestion. This was used to assess GI motility in 73 healthy control subjects. Transit times for all GI regions could be identified visually (Table 1, **Paper I**). Visual versus software generated results were compared to determine software reliability. Inability of the software to generate GET was 5.5%, which was lower than reported (11%) previously (85 and references therein). In the ICJ, where the boundary between small intestine and colon can be detected as a transient pH fluctuation, there was 13.7% failure related to software detecting this pH change which was within the range (5-15%) by others (85, 88). No other serious events were identified, such as ingestion problems, retention or undetected leaving the body. Segment detection failure was addressed through visual confirmation. Individual variation in transit times was pronounced in term of two strong outliers where their GET values on the high end of GET values to constitute gastroparesis (15.72 and 16.15 h). Table 1 **Paper I** shows data without (a) and with outliers (b). The percent of subjects in which GET was elevated so far above the main dataset was 2.7 %, which was lower than the 20% reported in a study of fiber diet intervention in health (GET was between 18.5 and 20.4 h) (84). The observed lag phase elongation could be due to food particles size (12, 13). Low gastric pH and acid spill in the duodenum could also delay the GET (26, 27). An early start of hormonal control by duodenum could also influence appetite initiated delayed stomach emptying (9, 13).

In this study, the recruitment of male subjects was more than female, which might have influenced the significance level. Females GET was longer than males, although not significant (p = 0.191) with the two male outliers included. Removing outliers changed statistics, hence difference not significant (p = 0.081). Statistics on duplicate sample size achieved significance (p < 0.05). As regards other transit times (SBTT, CTT and WGTT, Table 2 **Paper I**), these were on average longer in females, albeit with no statistical difference. Although removing the two outliers from data set (n = 71 in **Paper I**) reduced the group number difference and changed the statistics near threshold of significance (WGTT, p = 0.088 vs. 0.056). Hormonal influence on bowel movements could be the reason since post-lag gastric emptying and CTT were reported to be longer in females, although a different method was used.

The authors (Degen and Phillips, 1996) have attributed this to the individual variation not to menstrual hormones (95). In a recent study, slower GET, SBTT and CTT were reported in females, yet the method was also different (96). In study of constipation using WMC, healthy females showed slower GET and WGTT compared to males (97). In a rat study, estrogen increased nitric oxide production, cyclic guanosine monophosphate and inhibited acetylcholine-induced contractions of smooth muscle in period and pregnancy. According to the authors, this might explain pregnancy associated functional GI disorders in females (98).

Reproducibility within the same individual (n = 19), that is, intra-subject variability, over time (2 and 4 weeks) referenced to the initial measurements were determined to have a CV% of 20.0 and 42.4%. Bland-Altman plots of transit times show considerable variation within the same individuals of all transit readouts (Fig. 1A-D, **Paper I**). There was no pattern suggesting any tendency to drift over time (2 compared to 4 weeks) after the initial reference measurements. Many studies with different techniques have shown variations in transit times within the same subject (95, 96, 99). This is apparently a physiological phenomenon that is method independent.

7.1.2 Visual and software disagreement

The MotiliGI 3.0 software failure in determining pH rise upon exiting the stomach and in ICJ contributed to lower correlation in SBTT ($R^2 = 0.28$) than other transit times. Correlations for GET, CTT and WGTT were 0.78, 0.96 and 0.99 respectively (Fig. 2, **Paper I**). Representative examples of disagreements between visually and software derived WMC transit times can be found in Fig. 3, **Paper I**.

7.1.3 Wireless Motility Capsule (fed state) and Video Capsule Endoscopy (fasted state) in gastric and small bowel transit

In age and gender matched subjects undergoing VCE, fasted state GET was 0.71 ± 0.08 h, with 28 cases (40%) documented with GET being < 0.5 h. The time of ICJ passage was also identified to quantify SBTT, which was 4.15 ± 0.13 h.

Obtained VCE data of GET (0.71 h) was ~5 fold lower (p < 0.001) than WMC (3.11 h) data, while SBTT was ~1.3 fold lower (p < 0.05) Fig. 4 **Paper I**.

Orocecal transit time (OCTT) is considered to be an important parameter in pharmacokinetics, in that it establishes the time available for most drugs to be absorbed. In WMC and VCE recordings, this can be approximated by summing GET and SBTT. OCTT calculated in this way in the fed state with a mixed meal (WMC, 8.71 h) was 1.8 fold longer than in the fasted state

(VCE, 4.86 h). It has been reported that food ingestion prolongs GET, causing absorption delay of various drugs (100). Vagal afferents stimulated by activation of mechanoreceptors as well as modulation of motility by incretins (e.g., GLP-1 action as an ileal-brake when lower GI tract is exposed to unabsorbed nutrients) delay transit times. This affects time available for drug absorption (9, 11, 12, 101). Females had (WMC) fed state OCTT, (9.12 h) that was about 35 min longer than men (8.47 h). This difference might be important to take into account when pursuing medications with unusually slow absorption.

7.2 I-FABP marker of monitoring infliximab treatment, Paper II

7.2.1 Crohn's disease patient characterization

Two Crohn's disease patients (20%) reported normal HBI at onset of the study (infusion 1, Inf1), however their CRP (20 and 8.1 mg/L) and TNFα (2.32 and 3.03 ng/L) levels were high (Table 1 Paper II). This could be explained due to the poor correlation with the ongoing inflammation that leads to under-estimating treatment (61, 62, 102). The need for a GI specific biomarker was stressed.

7.2.3 I-FABP parallels TNFα and HBI in CD

In CD patients, TNFα levels averaged 1.6-fold higher than in the HC (2.34 ± 0.22 vs 1.48 ± 0.06 ng/L, $P < 0.001$) Fig. 2a, Paper II. Among healthy subjects (n = 61), the TNFα reference interval was estimated to be 0.51 - 2.26 ng/L (5 - 95% percentile).

I-FABP levels in serum of CD patients were 2.5-fold higher than in the HC (2.07 ± 0.23 vs 0.84 ± 0.13 µg/L, $P < 0.001$). The reference range was established in-house (intervals 0.24, 1.45, and 2.43 µg/L, the 5, 75 and 95% percentile) Fig. 2b, **Paper II**. In 9 and 2 of the 10 CD patients, I-FABP was initially above this 75% and 95% percentiles, respectively. For comparison, 8 and 5 patients had TNFα above the 75% and 95% percentiles, respectively (Table 1, **Paper II**).

In control experiments, plasma I-FABP values were consistently two- to fourfold lower than values in serum tapped in parallel from same test subjects. In spike recovery experiments, the protease inhibitor cocktail used here yielded higher values in both plasma and serum. Immediate addition of this cocktail to blood from controls at the time of draw protected I-FABP from degradation. With no protection, serum I-FABP was 15% lower (n = 6, $P < 0.05$), suggesting that losses can occur even during first hour that typically elapses from blood draw until freezing. This apparent protection against

proteolytic degradation was only partial; I-FABP declined in plasma and serum to 70% and 50% after storage at room temperature for 24 h (p = 0.0001). Spiking experiments yielded similar results. No losses were identified in relation to repetitive freeze-thaw itself.

Reduced TNFα levels at follow up 1 (F1) versus infusion 1 (Inf1) as well as at the subsequent infliximab follow ups versus their respective infusion day. The drug effect was clearly identifiable with significance P = 0.001 using ANOVA on ranks (Fig. 3, **Paper II**). This was transient, returning to levels of Inf1 day by the time of the next infusion. HBI followed the same trend, although peaks and troughs were less pronounced. The mean I-FABP level was also lower at F1 than Inf1 (P = 0.019), but not reaching significance at F2 and F3 relative to Inf2 and Inf3 (P = 0.420 and 0.229, respectively). No statistical significance was found (P = 0.180) between Inf1 and F3 (Fig. 3a, **Paper II**). This could be due to the concomitant IECs lysis and lack of adjustment for the infusion dose of infliximab to TNFα levels in blood. Power analysis indicated a sample size of 25 to be optimal to reach significance for the range of values obtained in the present dataset. Combining all data (all three infusion and follow-up pairs) to reach n = 30, statistical significance was reached using a paired t-test (P = 0.014).

CRP concentrations declined gradually during infliximab treatment (F1 vs Inf1, P = 0.037) Fig. 3b, **Paper II**. A significant decrease was found at F3 versus to Inf1 (P = 0.041). In all 30 cases, TNFα declined on follow-up. In 18 cases (60%), I-FABP also declined, as did IL-6 and CRP. In 12 cases (40%), neither I-FABP nor CRP declined, which was also the case for IL-6 in 10 of these patients. TNFα immunoreactivity would be expected to be lower in samples in which infliximab was present (78, 80). This was seen on average in **Paper II**. Despite differences in TNFα between patients, their circulating TNFα was only ~1.6-fold higher than healthy subjects. The decline in I-FABP in which there is no masking interference from infliximab was of the same magnitude. Chronic IECs exposure to TNFα promotes elevated shedding over villus axis and consequent elevated I-FABP (53). At Inf1, TNFα immunoreactivity was reduced to about the same level as healthy subjects. As compared to CRP or IL-6 (or TNFα), I-FABP apparently can compete rather well as a disease activity biomarker.

7.2.4 I-FABP in the human GI tract

Immunohistochemistry showed selective I-FABP immunoreactivity confined to the epithelium of the stomach (cardia, fundus, and corpus), small intestine (jejunum and ileum), and colon (Fig. 4, **Paper II**). Expression of I-FABP in all GI segments confirms prior observations (94). This portion of **Paper II** extended on those findings by comparing jejunum to ileum and by estimating relative abundances in human different GI segments. There were also difference in the exact segments studied in that **Paper II** study examined IHC of stomach, small intestine

(jejunum and ileum), and colon that were not explored in the earlier publication using mRNA expression in mouse small intestine. The strongest immunoreactivity (i.e., highest protein expression) occurred equally in the jejunum and ileum, followed by the colon. Immunoreactivity to I-FABP was confined to the mucosal epithelium, with no observable staining in other layers (lamina propria, smooth muscle, enteric neurons, blood vessel endothelium, etc.). Estimations of the relative abundances of I-FABP by GI segments are provided in Table 2, **Paper II**. When relative total GI surface area of each segment was taken into consideration, roughly 45 to 48% of I-FABP was determined to be present equally in jejunum and ileum, with 1.6 to 4.5% occurring in the colon.

The potential of I-FABP as a biomarker stems from its specificity and selectivity for IECs and its distribution in segments unobtainable by conventional endoscopic methods (65, 102). The potential to monitor therapeutic responses in the context of mucosal healing (i.e., improved barrier integrity) using blood or urine samples is attractive.

7.3 Elevated small and large intestinal permeability in Crohn's disease and ulcerative colitis, Paper III

Urinary percent recoveries, (median and Q1-Q3) of riboflavin lactulose, mannitol and sucralose are shown in Table 1. Values were above the lower limits of quantification for the respective methods.

In the small intestine, the fold increase in % recovery of lactulose medians in UC and CD versus HC was 1.3 and 1.9, while in sucralose % recovery was 1.2 and 2.8 respectively. The fold increase in % recovery of sucralose in the large intestine was 1.4 and 2.1 (UC and CD). Lactulose and sucralose % recoveries were positively correlated in IBD patients ($R^2 = 0.62$, p < 0.001) and Controls ($R^2 = 0.7$, p = 0.001), with overall correlation of $R^2 = 0.6$, p < 0.0001 (Fig. 2 **Paper III**). Small and large intestine sucralose % recoveries also showed a positive correlation ($R^2 = 0.62$, P = 0.001) in IBD patients (Fig. 3 **Paper III**).

The strong positive correlation between % recoveries of lactulose and sucralose implies similar information can be acquired with more resolution (i.e., wider dynamic range) using sucralose. A decrease in total sugar marker ingestion in the *in vivo* permeability test might be beneficial. This will increase the compliance of the patients to undergo repetitive tests for monitoring the healing process (i.e., barrier restoration) and reduce cost. Sucralose exposure (5 g) was half that of lactulose (10 g) considered sufficient for acquiring the small and large intestinal permeability data simultaneously. The unabsorbed load of lactulose passing the small intestine and reaching the

large intestine provokes microbial fermentation, gas production, increased motility and inconvenience (32).

Table 1. Percent urinary recoveries of *in vivo* permeability probes (mannitol, lactulose and sucralose) for healthy controls and IBD patients (ulcerative colitis and Crohn's disease)

Markers recovery (%)	Healthy controls n = 25		Ulcerative colitis n = 19		Crohn's disease n = 11	
	Med	**Q1 - Q3**	**Med**	**Q1 - Q3**	**Med**	**Q1 - Q3**
Riboflavin **0-6 h**	4.90	3.30 - 6.60	17.61[**]	11.70 - 28.6	19.64[**]	9.40 - 28.0
Mannitol **0-6 h**	7.70	5.80 - 9.80	5.80	4.71 - 7.43	4.12	3.39 - 9.15
Lactulose **0-6 h**	0.44	0.22 - 0.58	0.59	0.38 - 0.69	0.84[**]	0.59 - 0.97
Sucralose **0-6 h**	0.17	0.10 - 0.24	0.32[**]	0.16 - 0.70	0.44[**]	0.31 - 0.74
Sucralose **6-24 h**	0.21	0.16 - 0.28	0.30[*]	0.20 - 0.66	0.41[**]	0.37 - 0.75
Lactulose/Mannitol	0.06	0.04 - 0.08	0.13[**]	0.07 - 0.18	0.22[**]	0.17 - 0.25
Sucralose/Mannitol	0.02	0.02 - 0.03	0.03[**]	0.03 - 0.11	0.10[**]	0.08 - 0.16

Values are given as medians and interquartile range (Q1-Q3); Statistical evaluation with Mann-Whitney U test; * p < 0.05; ** p < 0.01; h,

hours. The 0-6 h time interval represents upper GI permeability, while 6-26 h interval represents colon permeability.

The significance (Table 1) levels were more consistent for in the recoveries of sucralose compared to lactulose, implying sensitivity in discriminating functional permeability of the small and the large intestine. The disease process in Crohn's disease is known to involve different segments in the GI tract (37, 102), which is not the case in ulcerative colitis. However, the results of significance were higher in the CD group, the increase in the small and large intestinal % recoveries of sucralose both for UC and CD implying more common shared symptoms between them. This means an ongoing inflammatory process in the small intestine (site of absorption) in UC and CD (62). Xenobiotics and other harmful compounds (e.g., environmental contaminants) would likely be more prone to leak into blood stream from enhanced permeability in the duodenum, jejunum and ileum. It is noteworthy that sucralose, being itself a chlororganic, can be regarded as a surrogate for estimating absorption of endocrine disrupters, since many of these are chlororganics. This raises the possibility that IBD or other patients with compromised GI barrier may be particularly sensitive to the effects of such environmental agents.

Riboflavin % recovery was not lower than HC on average it was, if anything, higher. This suggested that despite hyper-permeability observed herein, transporter mediated nutrient absorption, at least as measured by riboflavin recovery, remains largely intact in most IBD patients.

7.4 Gastrointestinal deviated motility and hormonal patterns in IBD, Paper IV

7.4.1 Stomach acid profile

There were differences in luminal pH of CD patients compared to their matched HC. The postpyloric duodenal cap segment minimum pH was higher (p < 0.01), as was the case in the post-ICJ segment (p < 0.01), where also the maximum pH was higher (p < 0.05), and in the colon (p < 0.05), Table 1 **Paper IV**. Regarding motility parameters, the CD patients' GI tract experienced lower motor activity, both in terms of peak amplitude and contraction frequency postprandial, which clearly showed that the postprandial MI was markedly lower in CD in comparison to their matched HC (Table 2 and Fig. 1 **Paper IV**).

In UC patients, the pH differences versus their matched HC were less compared to results of same calculations applied to CD data. In the postpyloric region and in the small intestine, higher pH profiles were found, (Table 3 **Paper IV**). This implies either lower acid spill into duodenum from the stomach, or higher bicarbonate pancreatic secretion associated retrograde migration reflux (9). Reduced stomach acid production could be due to impaired vagal reflex (8) or duodenal inflammation associated CCK release (9).

7.4.2 Gastrointestinal transit time

GI transit time as measured by the WMC detection of pH changes in various regions of the GI tract showed that, although CTT and WGTT were slightly longer in CD, neither these, nor other transit times, were statistically different in CD or UC from their HC (Fig. 2 **Paper IV**). The motility patterns most affected were those of the pyloric region and small intestine, the latter having more vivid contractions at a lower frequency. Involvement of the upper GI tract has been reported in 15 - 20% of IBD patients (103, 104). MI data verified reduced motility in the small intestine in UC (Fig. 1 **Paper IV**). The lower contraction amplitudes in colon might be explained by distention (Table 4 **Paper IV**). Although the colon is commonly recognized GI segment to be involved with symptomatic lesion findings in UC, altered motility patterns were found in the upper GI tract of these patients. Small intestinal hyper-permeability was found in both CD and UC (**Paper III**). Delayed SBTT could be explained by colon distension associated neuronal reflex activation (9).

There were minor differences in transit times between patients with CD or UC, even though all patients had increased bowel movements more than five times a day. Thus, the motility increase (increased MI) primarily in the small bowel and colon is not directly related to the passage of intestinal contents in moderate disease activity. Postprandial motility was dampened throughout

the gut in CD, but relatively less in UC. In a study using scintigraphy, the gastric emptying in patients with non-obstructive CD and no upper GI involvement showed no significant differences compared to healthy subjects. The subgroup analysis revealed symptomatic patients to have a significantly delayed gastric emptying relative to healthy subjects (105). Disturbances in gastric emptying were found in another study on IBD patients suggested possibility of excessive CCK release could explain the deviation (106).

The small bowel represents a common anatomic site of CD lesions, while less involvement of the terminal ileum in UC (107). Inflammation was found to impair contractile activity of human small intestine *in vitro* (108), even with minimal inflammatory lesions (109, 110). Patients with CD or UC may have symptoms not only related to the active phase of disease, but also to a low-grade inflammation with symptoms correlated to autonomic dysfunction, small bowel motility or visceral sensations (111). Postprandial gastro-duodenal motor activity assessed in a fasted state study with manometry on 35 inactive CD patients showed 74% having abnormal motility, characterized by contractions reduction and increased single or clustered propagated contractions (112).

Experimental evidence of the inflammation potential effects on colonic motility were studied (113-116). These effects were observed even after inflammatory process resolving (117). Motility assessment by combined manometry and scintigraphy confirmed motility reduction in the proximal colon of UC patients with an increased propagated contractile activity that should hasten the transit of colonic contents (118).

7.4.3 Gut and metabolic peptide hormones

In plasma analysis of the GI and metabolic hormones show that the meal-related ghrelin decrement was preserved in CD but blunted in UC patients as compared to their respective HC. The area under the curve (AUC) of CD was not different from controls whereas in UC, the AUC was markedly higher ($p < 0.001$).

Ghrelin plasma levels were not so pronounced in the CD compared to UC group. The drop in response to the light meal was significant in CD patients (0 vs. 60 min), although at the threshold compared to their healthy matched and the pattern shows different nadir (10 to 30 min). This could explain the transit time comparability to healthy in this group.

In UC patients the, postprandial drop of ghrelin plasma levels was not significant (0 vs. 60 min) as compared to their healthy matched controls (Fig. 3. **Paper IV**). Our data confirm others' data of elevated ghrelin levels (119) but conclusions on the physiological impact of this finding could not be drawn since several other gut peptide hormones with different actions on appetite and motility act in concert after release. The anti-inflammatory properties of ghrelin may have some role in IBD (120).

GIP in plasma showed a preserved postprandial release pattern in both CD and UC as compared to controls, however with a markedly increased AUC for GIP in CD ($p < 0.001$), as well as in UC ($p < 0.003$), Fig. 4 **Paper IV**. In CD patients the plasma GIP levels were higher, although the increase (~ 3 fold in CD and HC) to the meal (0 to 50 min) was not comparable to their significance (p= 0.035 vs. < 0.001). The GIP levels in UC patients were comparable to their matched controls and the increase (~2.5 vs. ~5) was significant ($p = 0.002$ vs. 0.001) to the meal. This GIP differences seem to have little impact on motility responses in line with previous experience by others (22), since no significant delay was obvious in the GET. Increased GIP AUCs could mean hypersensitivity due to inflammation in the duodenum (119), or increased nutrient dumping in duodenum and failure of pylorus closure due to the high pH of chyme. Villus atrophy (increased IECs shedding) associated malabsorption could delay nutrient clearance from the luminal side; this will provoke more stimulation of duodenal receptors (53), due to excessive CCK release (9, 13, 106). This is more pronounced in CD, since known small intestinal involvement with inflammation which was aligned with elevated GIP AUC in CD.

The postprandial AUC in GLP-1 was only numerically different from controls in CD, whereas in UC the AUC was higher than in controls ($p < 0.005$) (Fig. 5 **Paper IV**). The active GLP-1 release was less increased in CD as in compare to UC. The meal response of GLP-1 (0 vs. 30 min) was higher (~1.6 vs. ~1.5) in both CD ($p < 0.05$) and UC ($p < 0.01$) versus their matched controls ($p < 0.01$). The study by (Moran GW. *et al.* 2013) showed similar numerical increase, although with a relatively small sample of CD patients (119). Commensurate with their findings we found no clear cut increase of GLP-1 release in CD patients, whereas in UC there was a marked release with preserved pattern. In UC, the AUC increase was consistent with colonic site of inflammation, while this did not reach significance in CD ($p < 0.054$ compared to match controls $p < 0.005$). These increments had pronounced effects (mostly longer) in CD opposite to UC on their SLBTT compared to their matched controls values. There was a second peak increase in GLP-1 release in CD (120 min), indicating delayed delivery of nutrients in their distal ileum and colon (Fig. 5 **Paper IV** left panel).

The release pattern of PYY was disrupted in both CD and UC with only a minimal or no peak in after food intake, concomitant with a markedly increased AUC in CD ($p < 0.001$) and UC ($p < 0.042$) as compared to their respective control groups (Fig. 6 **Paper IV**). PYY release showed a different release pattern with rather a slow fall of plasma levels over the nearest hours which was compatible with earlier findings except no signs of nausea or bloating were recorded (119), including high plasma levels in CD as compared to normal controls. Conversely in UC, plasma PYY levels were lower than in the matched controls. Interestingly at 120 min, the increase of PYY release in CD had a similar pattern compared to GLP-1

indicating a PYY delay in the CD group.

The metabolic hormone leptin showed post meal fall in plasma levels unlike their matched controls. In CD the total hormone release as reflected by the AUC was greater compared to controls ($p < 0.001$). Conversely, in UC patients the plasma levels were lower than in controls ($p < 0.001$). However, data were not corrected for body weight or fat mass. The small leptin decrease occurring from 0 to 40 min in both CD and UC (but not to their matched controls) could be unique to a light meal. This decline from baseline was 19% in CD and 14% in UC). For example, redistribution of circulating leptin without stimulating leptin secretion would yield such a result. Earlier reports showed that elevated leptin levels in chemical models for induced intestinal inflammation in mice was correlating with inflammation (121). Conversely, no significant difference in leptin was found between UC and HC. Hyper-permeability in both small and large intestine demonstrated in CD and UC (**Paper III** of this thesis) implies higher ingress of endocrine disruptors through the intestine toward blood. The hydrophobic nature of these molecules promotes their deposition in adipose tissue where affecting leptin profile is a potential end result (122).

8. Conclusions

Paper-II

Under meal-stimulated conditions, the WMC acquired data of pH, pressure and temperature were utilized to generate reference values for GET, SBTT, OCTT, CTT, SLBTT and WGTT in the local population. These values can be utilized to evaluate deviated motility patterns in patients. The software-generated estimates and visually determined values are nearly identical. Compared with VCE estimates obtained under fasting conditions, WMC recordings in fed state had longer GET and SBTT. These differences between WMC and VCE are consistent. The WMC motility events are representative of a typical mixed meal (vagal and hormonal meal responses). Variations in intra-subject reproducibility imply that false positive results can occur and must be considered in clinical investigations.

Paper-II

I-FABP is primarily located in the epithelium of human jejunum and ileum, where lesions are common in CD, and to considerably lesser extent throughout colon and stomach. Circulating I-FABP is elevated in active CD with a magnitude comparable to TNFα. I-FABP lowers and rises again in parallel with TNFα and HBI during successive cycles of infliximab treatments and weekly follow-ups. I-FABP has requisite features expected of a jejunum and ileum selective prognostic biomarker for the purpose of monitoring CD patients' disease activity in terms of treatment outcomes and relapse-remission cycles. One implication of these findings is that some circulating gut peptide levels might be altered, since several of produced and secreted from entero-endocrine cells are situated at the jejunum and ileum.

Paper-III

Sucralose as a dual purpose probe to quantify both small and large intestinal barrier permeability without use of lactulose is realistic and feasible. Increased small intestine mucosal barrier permeability of lactulose in both CD and UC was discovered. This finding is reproducible using sucralose. Small intestine hyper-permeability is not necessarily revealed as nutrient malabsorption in the small intestine, since there is no detectable defect in riboflavin absorption, which is dependent on transporters confined to the duodenum and jejunum.

Paper-IV

Methods and reference values established using WMC in **Paper I** were used in motility investigations of moderately active IBD. Motility disturbances can be investigated using WMC in both CD and UC. Consistent motility disturbances among groups of patients are limited, as are group differences in pH. However, disturbances within individuals can be found. As part of the WMC investigation, group effects on gut peptides and metabolic hormones exist. No determinant associations between hormone levels and motility could be identified in this paper, although there are deviations from healthy, generally toward higher AUCs. Effects of deviated hormone patterns on hunger/satiety signaling in IBD are anticipated. The WMC data described in this thesis can be useful diagnostic tool for individualized therapy, in that it reveals meal associated disturbances that are unlikely to be discovered by other forms of investigation. Effects of deviated gut peptides pattern might be related to defects in the small intestinal, absorptive capacity (prolong provoking of hormone secretion, Paper IV) and epithelial barrier as described in **Papers II** and **III** of this thesis. Beyond pH use in GI landmarks identification, lowering stomach acidity can be considered in the treatment protocols of IBD together with small intestinal barrier stabilizers.

9. Popular scientific summary (populärvetenskaplig sammanfattning)

9.1 English summary

The gastrointestinal (GI) tract functions to continuously provide nutrients to maintain homeostasis in the body. Synchronized events of motility, permeability and secretory (hormones and enzymes) should be integrated to achieve this. The smooth muscle syncytium operate with the integrated enteric nervous system (ENS) and endocrine signaling to accommodate, mix and control the passage of the ingested materials for digestion. The intestinal epithelial cells (IECs) serve to maintaining digestion and absorption while repelling harmful compounds.

This thesis investigated a dysregulated GI barrier function (*in vivo* permeability and mucosal integrity), altered motility and hormonal patterns in patients with inflammatory bowel disease (IBD; Crohn's disease, CD; ulcerative colitis, UC) by 1) assessesing GI motility using a wireless motility capsule (WMC, SmartPill®) and video capsule endoscopy (VCE, Pillcam®), 2) investigation of I-FABP as a biomarker in CD in terms of I-FABP distribution along the human GI tract, I-FABP stability in biobanked samples, relationship to disease activity, paralleling to TNFα and Harvey-Bradshaw index (HBI) under treatment with anti-TNFα antibody (infliximab), 3) evaluation of intestinal permeability in IBD, 4) assessing the utility of the WMC technique in obtaining motility data across all GI segments in IBD patients along meal-related GI motility and hormonal patterns in IBD.

WMC was found to safely and reliably acquiring motility patterns through recording pH, pressure and temperature relative to time from the gut. This resulted in reference values for gastric emptying time, and transit times of small bowel colon and whole gut. These values were further used in evaluating IBD patients. Software-generated estimates were nearly identical to visually determined values. Compared with VCE estimates obtained under fasting conditions, the WMC records longer gastric emptying time and small bowel transit time. Variations in intra-subject reproducibility must be considered in clinical investigations.

I-FABP was primarily located in the epithelium of the small bowel and to lesser extent also in the colon and stomach. Circulating I-FABP was elevated in active CD with a magnitude comparable to TNFα. I-FABP lowers and rises again in parallel with TNFα and HBI during infliximab treatment and

follow-up. I-FABP can be used as a jejunum and ileum selective prognostic biomarker for monitoring of disease activity and treatment monitoring in CD patients.

Increased small intestine mucosal barrier permeability of lactulose in both CD and UC was discovered, where sucralose had the dual purpose of quantifying both small and large intestinal permeability as a replacement for lactulose. Small intestinal hyper-permeability was not revealed as a nutrient malabsorption in the small intestine, since there was no defect in riboflavin absorption, which is dependent on transporters confined to the duodenum and jejunum.

Using the WMC, consistent motility disturbances in IBD were limited, as were differences in pH. However, disturbances within individuals were found. As part of the investigation, effects on gut peptide and metabolic hormones were found with a general pattern of maintained meal responses, but at higher plasma levels. No clear associations between hormone levels and motility were found, but effects on hunger/satiety signaling in IBD are anticipated.

Taken together, the present thesis work shows the utility of the WMC, as well as the permeability testing for probing pathophysiological functions along the GI tract. In this context, I-FABP can be used to trace disease activity in the small bowel, whereas gut permeability testing provides information on increased activity in IBD selectively in the small and large bowel. The WMC together with signaling hormonal release provides information of endocrine aberrations in IBD along with motility disturbances and anticipated effects on appetite.

9.2 Svenska sammanfattning

Mag-tarmkanalens glatta muskulatur är funktionellt integrerad med enteriska nervsystemet och endokrin signalering för ackommodation, blandning och passage genom mag-tarmkanalen. De intestinala epitelcellerna upprätthåller digestion och absorption, samtidigt med barriärfunktion mot skadliga agens.

Avhandlingen utvärderar patofysiologiska barriärmekanismer vid inflammatorisk tarmsjukdom (IBD) beträffande permeabilitet, förändrad motilitet och hormonella mönster vid Crohns sjukdom (CD) och ulcerös colit (UC) genom att, 1) utvärdera mag-tarmkanalens motilitet med trådlös motili-tetskapsel (WMC) och videokapsel endoskopi (VCE), 2) studera I-FABP som biomarkör vid CD avseende distribution i mag-tarmkanalen, samband med sjukdomsktivitet, parallellitet med TNFα och Harvey-Bradshaw index (HBI) under anti-TNFα-behandling, 3) utvärdera intestinal permeabilitet vid IBD, 4) studera användning av WMC för att registrera motilitet vid måltids-svar och hormonella mönster.

WMC registrerade pH, tryck och temperatur längs hela mag-tarmkanalen. Referensvärden för ventrikeltömningstid och transittider för tunntarm, colon

och totala mag-tarmkanalen etablerades. Dessa värden har använts för utvärdering av IBD. Mjukvaruberäknade värden var lika visuellt avlästa värden. Mätvärden för VCE under fasta visade snabb transit, medan WMC hade längre ventrikeltömningstid och transittid för tunntarm. Intra-individuell variation måste bedömas särskilt vid kliniska undersökningar.

I-FABP lokaliserades till epitelet i tunntarmen, i mindre omfattning i ventrikel och colon. Cirkulerande I-FABP var förhöjt vid aktiv CD, som TNFα. Plasmanivåer av I-FABP följde parallellt TNFα och HBI under behandling och uppföljning. I-FABP kan användas som selektiv prognostisk markör för CD i jejunum och ileum vid monitorering av CD patienter.

Ökad tunntarmspermeabilitet sågs både vid CD och UC med hyperpermeabilitet för laktulos, medan sukralos, som ersättning för laktulos, kunde mäta permeabilitet i både tunntarm och colon. Hyperpermeabiliteten medför ingen generell malabsorption eftersom absorptionen av riboflavin var normal, och beror av specifika transportmekanismer i tunntarmen.

Med hjälp av WMC kunde endast begränsade avvikelser i motiliteten vid IBD registreras, även för pH. Hos enskilda individer kunde tydliga avvikelser ses. Dessutom noterades generellt förhöjda gastrointestinala och metabola peptidhormoner samtidigt med ett bevarat måltidssvar. Samband mellan hormonnivåer och motilitetsavvikelser saknades, men sannolikt har hormonnivåerna betydelse för hunger och mättnad.

Den sammantagna bilden visar användbarheten av WMC och permeabilitetstest för att bedöma patofysiologiska funktioner i mag-tarmkanalen. I-FABP kan spåra sjukdomsaktivitet i tunntarmen, medan permeabilitetstest ger information selektivt om sjukdom i tunntarm och colon. Användning av WMC tillsammans med mönster av hormonfrisättning kan ge information om motilitetsstörningar och sannolikt förändrad aptitreglering.

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