Aspects on the functional characteristics of the Roux-limb after Gastric Bypass surgery

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ABSTRACT

Background: Bariatric surgery, and particularly the Roux-en-Y gastric bypass (RYGBP) procedure, has highlighted the central role of the gastrointestinal tract in the regulation of body weight and metabolism. The Roux-limb is the part of the small intestine that has become the food recipient after RYGBP and has generally been very little studied.
Aims: The thesis explores morphological and functional features of the Roux-limb in relation to the clinical effects of gastric bypass surgery. The clinical presence of severe abdominal pain after RYGBP and use of opioid analgesics in a subset of patients also requested elucidation of the Roux-limb as a potential site for problems related to disturbed motor function.

Methods and results:
Paper I compares the mucosa of the preoperative jejunum with the same region in the Roux-limb 6-8 months post-surgery. In the Roux limb the mucosal villi were flattened with an upregulated epithelial replication rate. No histological signs of inflammation were present, but there was increased expression of proinflammatory markers.
Paper II. Mechano-sensory properties of the Roux limb in relation to food intake were examined 6 weeks and 1 year after RYGBP. In general, the preferred meal size decreased after RYGBP and subjects with a low perception threshold in the Roux limb preferred larger meals. Furthermore, the intra-Roux limb pressure correlated negatively to preferred meal size.
Paper III. The motor activity of the gastric pouch and the proximal Roux limb during fasting and food intake was examined by use of high resolution manometry in patients after un-complicated RYGBP. A fasting motility

pattern with migrating motility complexes (MMC) starting in the proximal Roux limb was characterised. Food ingestion was associated with a modest intraluminal pressure increase of similar magnitude in the gastric pouch and the Roux limb.
Paper IV. In a retrospective analysis of 18 patients with chronic abdominal pain after RYGBP referred to high resolution manometry it was found that only 5/17 were evaluated as normal manometries, but there was no clear association to experienced symptoms. In this cohort, 13 out of the 18 patients had longstanding opioid medication suggesting a potential element of narcotic bowel syndrome. Also, to elucidate opioid influence on Roux limb motility asymptomatic RYGBP patients was administered morphine intravenously during manometry. The MMC-pattern did not change but the muscular tone increased during morphine infusion.

Conclusions
Compared to the original jejunum, the phenotype of the Roux-limb mucosa is modestly altered from a digestive-absorptive character into an appearance more suited for food reception⁄transportation and tissue defence. The preferred meal size decreased radically after RYGBP and a close relation to mechano-sensory properties suggests that the Roux limb can be a determinant for food intake. During fasting the Roux-limb exhibited all phases of regular migrating motility complexes (MMC) with an onset close to the GEA. During food intake intraluminal pressure increased transiently without any significant difference between the gastric pouch and Roux-limb indicating that these two segments act as a common cavity. A majority of examined RYGBP-patients with meal associated chronic pain and nausea exhibited a disturbed Roux-limb motility and most of these patients had been prescribed opioid analgesics. In uncomplicated opiate-naïve RYGBP-patients, acute morphine administration influenced the Roux-limb musculature in a fashion that potentially initiates or aggravates dysmotility.
This thesis project demonstrates that biomechanical properties and muscular activity of the Roux-limb can be clinically important, and particularly so during dysfunctional conditions.

Keywords: obesity, bariatric surgery, meal size, morphology, intestinal motility, high resolution manometry, abdominal pain, opioid analgesics

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SAMMANFATTNING PÅ SVENSKA

Antalet operationer för sjuklig övervikt (obesitas) har ökat dramatiskt de senaste tio åren och den vanligaste operationstypen är Roux-en-Y gastric bypass. Vid gastric bypass delas en ficka av från magsäcken i anslutning till matstrupen. Därefter sys tunntarmen fast direkt till den fickan så att maten passerar förbi huvuddelen av magsäcken och tolvfingertarmen. Den del av tunntarmen som nu är ansluten till magsäckfickan kallas *Roux-slyngan* och har, efter operationen, fått ta över magsäckens roll som mottagare av maten. Operationen ger en kraftig och kvarstående viktminskning vilket har satt fokus på magtarmkanalens centrala roll i regleringen av ämnesomsättning och kroppsvikt. Ursprungligen ansågs gastric bypass verka genom en kombination av ett minskat födointag på grund av passagemotstånd i den lilla magsäcksfickan, och minskat upptag av näring eftersom en kortare del av tarmen exponerades för maten. Modern forskning har dock visat att orsakerna till viktnedgång efter gastric bypass snarare är en förändrad reglering av hunger- och mättnadssignalerande hormoner, men också förändringar av tarmens bakterieinnehåll och omsättning av gallan. Även magsäcksfickans tömningshastighet och tiden det tar för maten att nå tarmens nedre delar påverkar matintaget. Tarmrörligheten hos Roux-slyngan har tidigare studerats i efterförloppet till magsäcksoperationer för magsår eller cancer, men väldigt lite är känt om muskelaktiviteten i detta segment (d.v.s. matstrupe-magsäcksficka-tarm) efter viktreducerande gastric bypass.

I den kliniska vardagen har vi blivit alltmer medvetna om problem med långvarig buksmärta efter överviktskirurgi. Ett antal av de överviktsopererade patienterna uppvisar också en långvarig användning av morfinliknande läkemedel som, trots att de initialt är smärtstillande, på sikt kan leda till en ond cirkel med ökad buksmärta. Morfinliknande läkemedel är kända för att påverka mag-tarmkanalens rörlighet, men effekterna i Roux-slyngan efter gastric bypass har inte studerats.

De övergripande målen med den här avhandlingen är att utforska mekaniska egenskaper och rörelsemönster i Roux-slyngan som kan påverka matintaget, samt att belysa hur tarmrörelsemönstret och morfinliknande läkemedel kan bidra till uppkomst av buksmärta efter gastric bypass.

Arbete I. **Syftet** med första delarbetet var att undersöka slemhinnan i den översta delen av Roux-slyngan genom att jämföra provbitar av tarmslemhinnan tagna under operationen med provbitar tagna via gastroskopi

6-8 veckor efter operationen. **Resultat**: Vid mikroskopiundersökning syns att slemhinnans utseende har förändrats efter operation med en minskad veckbildning och planare yta, men också en ökad celltillväxt. Det syns inga direkta tecken på pågående inflammation men en ökad förekomst av proteiner som kan aktivera inflammation. **Slutsatsen** är att tarmslemhinnan, efter operationen, ändrar karaktär och får ett utseende lämpat för att ta emot och transportera föda och för att skydda sig mot nötning och skadliga ämnen.

Arbete II. **Syftet** med andra delarbetet var att studera Roux-slyngans uttöjningsförmåga i förhållande till matintag genom att jämföra undersökningar gjorda före operationen, 6 veckor och 1 år efter operationen. Vid undersökningen jämfördes hur mycket mat en försöksperson kunde äta med hur tarmen reagerade på uttänjning genom uppblåsning av en ballong. **Resultatet** visade att mängden mat försökspersonen ville äta minskade drastiskt efter operationen. Vid en jämförelse av matmängd i förhållande till ballonguttänjning visade det sig: 1; att personer som tidigt kände av ballongen kunde äta större mängd mat, 2; personer där tarmen svarade med en sammandragning vid ballonguppblåsning kunde äta mindre mängd mat. **Slutsats:** Resultaten tyder på att Roux-slyngan är en viktig faktor för reglering av måltidsstorlek efter gastric bypass.

Arbete III. Syftet med tredje delarbetet var att undersöka tarmrörelser i Roux-slyngan under fasta och efter matintag. Tarmrörelser undersöktes med tryckmätning inne i tarmen (högupplöst manometri). **Resultaten** visar hur normala tryckförhållanden ser ut i området matstrupe-magsäcksficka-övre Roux-slynga. Undersökningen visade också att det under matintag sker en förhållandevis liten tryckökning, och i samma storleksordning, i magsäcksfickan och Roux-slyngan. **Slutsats:** Resultaten tyder på att magsäcksfickan och Roux-slyngan utgör ett gemensamt hålrum. Matintag ger en måttlig tryckökning men det gick inte att fastställa om detta påverkar viktnedgången efter operationen.

Arbete IV. Syftet med fjärde delarbetet var att undersöka om det finns typiska, avvikande, tarmrörelsemönster i Roux-slyngan hos personer som har långvariga måltidsutlösta buksmärtor efter gastric bypass. Vidare undersöktes hur tarmrörelserna i Roux-slyngan påverkas av morfin hos opererade patienter som inte har buksmärtor efter måltid. Undersökningarna bestod av två delar: 1; en genomgång av journaler och tidigare genomförda tryckundersökningar från patienter med långdragna buksmärtor, 2; tryckundersökningar på försökspersoner som fick morfindropp i blodet jämförda med tidigare undersökningar utan morfindropp. **Resultatet** visade att av patienter med långdragna buksmärtor använde 13 av sammanlagt 18

morfinliknande läkemedel och bara 5 av 17 bedömdes ha normala tryckundersökningar. Den andra del-undersökningen visade att trycket i Roux-slyngan ökade under morfindropp. **Slutsats:** Morfin påverkar Roux-slyngans tarmrörelser. Avvikande tarmrörelser kan spela en roll vid långdragna buksmärtor efter gastric bypass, särskilt efter långvarigt användande av morfinliknande läkemedel.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Spak E, **Björklund P**. Helander HF, Vieth M, Olbers T, Casselbrant A, Lönroth H, Fändriks L. Changes in the mucosa of the Roux-limb after gastric bypass surgery. Histopathology 2010, 57, 680–688.

II. **Björklund P**, Laurenius A, Een E, Olbers T, Lönroth H, Fändriks L. Is the Roux Limb a Determinant for Meal Size After Gastric Bypass Surgery? Obes Surg (2010) 20:1408–1414.

III. **Björklund P**, Lönroth H, Fändriks L. Manometry of the Upper Gut Following Roux-en-Y Gastric Bypass Indicates That the Gastric Pouch and Roux Limb Act as a Common Cavity. Obes Surg. 2015 Oct; 25(10):1833-41.

IV. **Björklund P**, Maleckas A, Lönroth H, Björnfot N, Thörn SE, Fändriks L. Roux-limb motility in gastric bypass patients with postprandial nausea and abdominal pain – is there an association to prescribed opioids? In manuscript 2017

.

CONTENT

ABBREVIATIONS

AT1	Angiotensin II type 1
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CCK	cholecystokinin
CNS	Central nervous system
FGF19	Fibroblast Growth Factor 19
GEA	Gastro Entero Anastomosis
GI	Gastrointestinal
GIP	Glucose dependent Insulinotropic Polypeptide
GLP-1	Glucagon-like peptide 1
HAC	High Activity Complex
HPZ	High-Pressure Zone
HRM	High Resolution Manometry
IFSO	International Federation for the Surgery of Obesity and Metabolic Disorders
iNOS	Inducible Nitric oxide synthases
LES	Lower oesophageal sphincter
MMC	Migrating Motor Complex
MPO	Myeloperoxidase
NADPH	Nicotinamide adenine dinucleotide phosphate

NBS	Narcotic Bowel Syndrome
NSAID	Non Steroid Anti Inflammatory Drugs
PIP	Pressure Inversion Point
PYY	Peptide YY
RSS	Roux Stasis Syndrom
RYGBP	Roux-en-Y Gastric Bypass
SOS	Swedish Obese Subjects (study)
T2DM	Type 2 Diabetes Mellitus
TMPD	Transmucosal Potential Difference
VBG	Vertical Banded Gastroplasty
VSG	Vertical Sleeve Gastrectomy
WHO	World Health Organisation

1 INTRODUCTION

1.1 Obesity

Obesity is a rapidly growing problem to public health worldwide. The WHO reports that, in 2014, 39% of adults aged 18 years and over were overweight and 13% were obese. The prevalence of obesity has more than doubled since 1980 and if the incidence continues to rise at this rate, almost half of the world's adult population will be overweight or obese by 2030 (1, 2). In Sweden 2016, 51% of the population between 16 and 84yrs were overweight or obese and the trend is increasing (3). Most of the world's population live in countries where overweight and obesity associated diseases kill more people than underweight (1, 2). Obesity thus also implies a substantial economic burden, both in developed and developing countries (2).

Commonly the term "Body Mass Index (BMI) is used for classifying obesity and is calculated as body weight (kg) / the square of height (m^2). In adults a "normal" BMI is 18.5 to 25 kg m^{-2}; overweight is BMI 25 to 30, while obesity is defined as BMI over 30 kg m^{-2}. Severe obesity is defined as BMI over 40 kg m^{-2} (4). In a person that is 1.75 m tall, a BMI of 30 kg m^{-2} is equivalent to a weight of 92 kg and a BMI of 40 kg m^{-2} corresponds to 122 kg.

Table 1. Classification of obesity, Adapted from WHO (1)

Classification		BMI (kg/m²)
Normal range		18.50 - 24.99
Overweight		≥25.00
	Pre-obese	25.00 - 29.99
Obese		≥30.00
	Obese class I	30.00 - 34.99
	Obese class II	35.00 - 39.99
	Obese class III	≥40.00

During the development of mankind it has been an evolutionary asset to be able to store energy. The individuals that survived periods of starvation were the ones who reproduced. This led to a natural selection of bodily control mechanisms for preserving energy and to promote eating. These fundamental features explain why trying to lose weight by modulating energy balance by, for example, diet or exercise have a limited effect since the regulatory systems strive to compensate by increasing appetite, decreasing satiety and economize energy expenditure. Hunger and satiety, as well as energy expenditure and preservation is controlled by hypothalamic pathways and humoral signaling. Research during the last decade has explored the neuroendocrine systems involved in appetite control and metabolic rate where most attention has been given to gastrointestinal peptide hormones (5-7).

Apart from being a social stigma in many cultures obesity is associated with an increased risk of several medical conditions and also with a shortened lifespan. Typically obesity is associated with type 2 diabetes, hypertension, dyslipidaemia and cardiovascular disease, but also brings an increased risk for sleep apnea, musculoskeletal disorders such as osteoarthritis, certain forms of cancer and impaired fertility. Obesity is also associated with an increased prevalence of mood, anxiety, and other psychiatric disorders (4, 8, 9).

The prime goal for public health care systems is to prevent obesity by lifestyle changes aiming to reduce calorie intake and to increase calorie expenditure i.e. by increased physical activity. Unfortunately these measures only have a moderate effect once a manifest obesity is established and today obesity surgery is regarded as the most effective treatment for achieving sustained weight loss (4). Obesity surgery is also called bariatric surgery and can be defined as a surgical intervention in the gastrointestinal tract with a weight reducing purpose. One very common procedure is the Roux-en-Y Gastric Bypass (RYGBP) and the Swedish Obese Subject (SOS) trial reports a sustained weight loss following RYGBP of 27 ±12% after 15 years. This effect was compared to non-surgical obesity treatment that was principally without effect. Short time studies have reported weight loss after RYGBP of 25-28% with follow up time between 12 and 60 months (10). Several studies and reviews have reported concomitant improvements in obesity related comorbidities like type 2 diabetes mellitus, hypertension, metabolic syndrome, sleep apnea and to the end, overall mortality (4, 8-12). The indication for surgery as treatment for obesity follows, in most countries, the NIH consensus statement in 1991 that recommended bariatric surgery for patients with severe obesity who had a BMI of ≥ 35 kg/m^2 with at least one comorbid condition (such as T2DM, hypertension and obstructive sleep apnea) or a BMI of ≥40 kg/m^2 (13). A joint statement by several international diabetes organizations stated in 2016 that metabolic surgery (i.e. obesity surgery procedures with the intent to treat type 2 diabetes) should be recommended to treat Type 2 Diabetes in patients with class II & III obesity, and considered in patients with class I obesity with poor glycemic control (14). This statement has however not yet been accepted by Swedish diabetologists.

1.2 Obesity surgery

The limited weight-loss effect of conventional therapies has led the medical profession to search for other remedies against obesity. In the 1950s the first surgical procedures to induce weight loss was introduced in the form of intestinal shunting operations aiming to induce malabsorption. Another principal concept apart from the malabsorption is restriction to food intake. One dramatic historic example of the latter was to clamp the patient´s teeth together, thereby disabling them from eating. A somewhat more sophisticated way to restrict food intake has been to place a rigid band around the stomach, with the idea of inducing early satiety when the upper part was filled. Based on the observation of weight loss among patients that underwent partial stomach removal for ulcer or malignant diseases, the gastric bypass procedure was developed by Dr E Mason during the 1960ies (15). The gastric bypass has since then been developed further into its current form, the Roux-en-Y gastric bypass (RYGBP).

The initial idea of the Gastric Bypass was that it combined the two principals of restriction and malabsorption. The procedure is performed by creating a small (15-30cm^3) gastric pouch distal to the lower oesophageal sphincter and cardia that would limit food-intake and accomplish a restriction. The small intestine is then divided ca 50 cm distal to the ligament of Treitz and the distal end anastomosed to the gastric pouch while the gastric remnant with duodenum is re-connected to the intestine approximately 120 cm further distally (Fig 1). By disallowing the bile, pancreatic enzymes and gastric secretions to exert effect in the upper 120 cm of the small intestine, a functional malabsorption was thought to be created. This construction constitutes the Roux-en-Y Gastric Bypass of today.

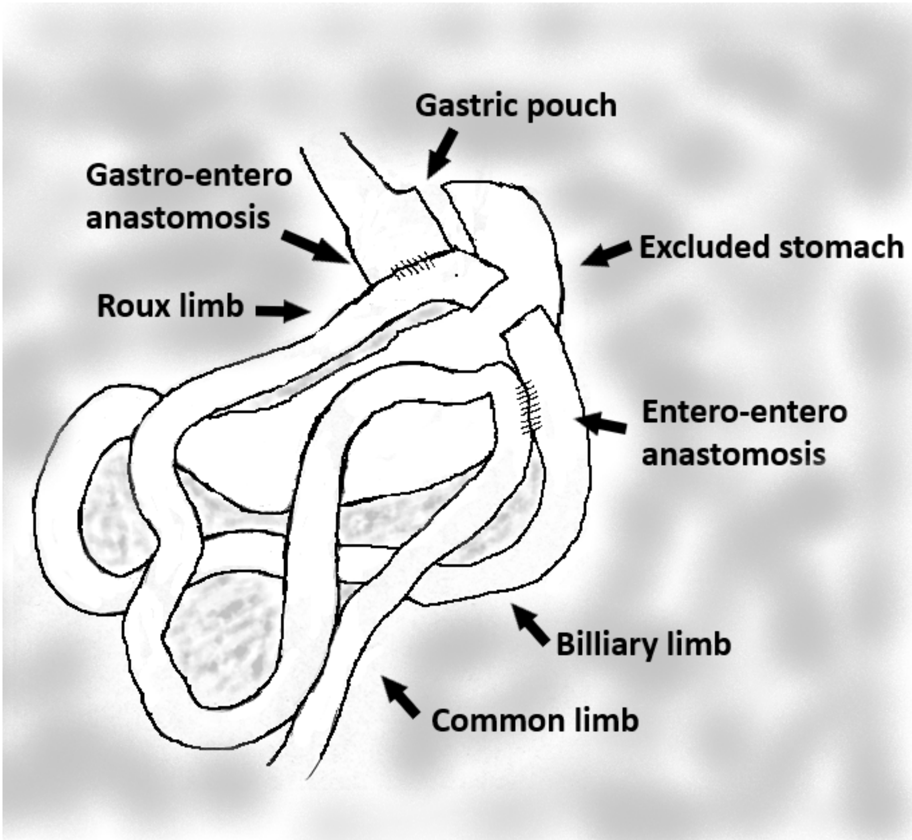

Figure 1. Anatomical features of the Roux-en-Y gastric bypass. Note that the anatomical proportions are erroneous.

In the mid-1990s the development continued with an adaptation of the procedure to laparoscopic technique. This brought the advantages of reducing the risk of wound infections and incisional hernias, as well as reducing time for postoperative recovery, all which greatly increased the popularity of the operation. Several other surgical procedures to treat obesity has been developed over the years, and the most recent major contribution is the vertical sleeve gastrectomy (VSG) in which the stomach is divided length-wise and the outer curvature is removed, leaving only a narrow tube. The VSG was initially a part of the more extensive biliopancreatic diversion with duodenal switch, but was introduced as a single procedure in 2005(16).

RYGBP has long been the most common bariatric procedure, and still is worldwide, even though it is now surpassed by VSG in the United States (10, 17). The IFSO Worldwide Survey of the year 2014 that 579,517 bariatric/metabolic surgical procedures were performed worldwide and of these 39.6% was Roux-en-Y Gastric Bypass (18). In Sweden the number of operations for obesity increased from ~800/year prior to 2004 to a peak level of ~8500 in 2011. Since then there has been a decrease to ~5500 during year 2016, of which 64% were RYGBP and the majority of the rest were VSG (19).

1.3 RYGBP; The mechanisms of action – a very brief note on today's view

Further studies on the Gastric Bypass have made it apparent that the malabsorption and restriction were only parts of the mechanisms behind the weight loss and improvement of comorbidities. The main driver for weight loss is rather an altered eating behaviour that reduces energy intake (20, 21). Two connected principles have emerged as potential working mechanisms of gastric bypass; the foregut theory and the hindgut theory. The foregut theory teaches that by bypassing the stomach and the duodenum these organs are deprived of the exposure to food and nutrients thereby altering the secretion of gut-derived hormones originating from this area, e.g. the liberation of GIP from the duodenal mucosa. The hind gut theory, on the other hand, is based on the fact that the parts of the intestine that originally are situated more distally, i.e. in the distal the jejunum and ileum, are now exposed to nutrients earlier during food intake resulting in a faster humoral response. Changes in the concentrations of gut-derived hormones, including peptide YY (PYY), glucagon-like peptide 1 (GLP-1), ghrelin, glucose dependent insulinotropic polypeptide (GIP), oxyntomodulin and cholecystokinin (CCK) have been reported to occur after RYGBP (6, 20-23). PYY and GLP-1 are secreted from L-cells in the ileum and colon and acts as satiety promoting hormones, while hunger is stimulated by ghrelin secreted from P/D1 cells in the gastric fundus. GLP-1 is also active in the glycaemic control. It has been shown that the meal induced release of PYY and GLP-1 increases after RYGBP, from subnormal levels in obese subject, to more normal levels which also reduces food intake (24). It has been proposed that modulating these humoral systems by pharmacological intervention may be a future way to treat obesity without surgery (25).

In addition to regulating food (energy) intake, data have emerged demonstrating increased energy expenditure in RYGBP patients. Interestingly, it appears not to be the basal metabolic rate (BMR) that becomes upregulated, rather the thermogenesis associated to meal intake (26, 27). The mechanism is not completely known, but according to experimental animal data it can be explained as a reprogrammed mucosal metabolism in the Roux limb (28). Two other recently investigated aspects on the working mechanism of RYGBP are alterations of circulating bile acids and the intestinal microbiota. It is hypothesized that bile acids regulate glucose metabolism through the TGR5 receptor expressed on L-cells, causing release of GLP-1, and also induce synthesis and secretion of fibroblast growth factor 19 (FGF19) which might improve insulin sensitivity, thus improving glycaemic control (29). Studies have shown that transplanting faeces from RYGB-treated to germ-free mice resulted in significantly greater weight loss compared with mice receiving faeces from sham-surgery treated mice (21). Also, germ-free mice transplanted with faecal microbiota from obesity operated human patients accumulated less fat than mice transplanted with microbiota from obese patients (30). The development of knowledge in these areas is evolving precipitously, but few results have yet shown clinical relevance.

1.4 The Roux-limb – new terms and conditions

The science is well established around the bypassed stomach and duodenum, as well as the rapid delivery of nutrients to the distal intestine, but up to day very little interest has been put to the novel prime food recipient, the Roux-limb. By repositioning the small intestine to a position principally following on the oesophagus its internal environmental is radically changed. Prior to RYGBP surgery the role of the stomachs was to store ingested food, grind it to smaller parts, disinfect it from bacteria with gastric acid, initiate degradation, and then to deliver it into the duodenum and small intestine in a regulated manner. After gastric bypass surgery those tasks have to be taken over by the Roux-limb implying that several morphological and functional features become challenged.

1.4.1 The mucosa

Naturally the mucosa of the stomach is well specialized for the purpose of withstanding chemical and mechanical wear and tear but, intuitively, this should not be the case for the small intestine. It can be hypothesized that the new demands on the mucosa of the small intestine, now situated as recipient of undigested food and exposed to bacteria, would render a change in its basic properties with a reactive inflammation in response to mechanical and chemical stress. This inflammation could, in turn, influence the signalling of hunger and satiety. **So how is the mucosa of the small intestine adapted to its new anatomical position?**

1.4.2 Wall distension

Normally the human stomach has a very variable volume and is able to receive 1-1.5 litres of ingested food within a short period of time, an ability that the small intestine lacks. A part of the satiety sensation in a non-operated person is achieved by stretch receptors in the wall when the stomach is full. Returning to the theory of restriction, the mechano-sensory properties of the gastric pouch and proximal Roux-limb becomes of interest. The clinical impression is that the patients eat less after surgery and it can be speculated that the sensitivity for distension of the Roux-limb affect satiety and the quantity of food intake. **So how does the Roux-limb react to distension, and does it matter for meal size?**

1.4.3 Luminal transport and motility patterns

The anastomosis between the gastric pouch and the Roux-limb, in our hands, is created by a dorsal 45mm long linear staple after which the anterior defect is over sewn with a running suture. It is debated to what extent the width of the anastomosis contributes to a restriction or not. It has been shown that the width of the anastomosis, when constructed by circular staplers of different diameter, does not affect weight loss after surgery (31), on the other hand it is disputed whether reinforcing the anastomoses with a band is effective against weight regain or not (32, 33). If the anastomosis causes a transport restriction, then it is reasonable to expect an increasing intraluminal pressure in the proximally located gastric pouch during food intake. However, on the assumption that the pouch and Roux-limb instead acts as a common cavity, and that this cavity is not able to expand to any greater extent during food intake, the ability to transport intraluminal contents in a distal direction should be important for the ability to continue ingesting food.

Transport of the luminal contents is dependent on the activity of the intestinal wall musculature. This motor activity has two strict patterns that occur in the fasting and the fed state, respectively. The intestinal motility of the fed state is characterized by phasic contractions that provide mixing and slow distal propulsion, where the rate of propulsion decreases and mixing increases distally. In the fasting state the motor activity is characterized by Migrating Motor Complexes (MMC) divided into three phases; Phase I with no contractile activity, Phase II with intermittent, random, contractions, and Phase III is a migrating high activity complex (HAC) of coordinated contractions with a purpose of cleaning the lumen from debris. The cyclic recurrence of the Phase III is approximately 85 -110 min. Two thirds of the Phase III HAC originate from the stomach but can also be initiated in the proximal small intestine. The propagation velocity of the HAC is 5-10 cm/min in the duodenum and decreases more distally (34-36). The intestinal muscular activity is neurally controlled mainly by enteric nerves in the myenteric plexus, being situated between the longitudinal and circular muscle layers. An intrinsic electrical activity is generated by the intestinal cells of Cajal so the intestine can operate independently of the central nervous system to maintain its motor function. However, the intestinal motor activity can be influenced by parasympathetic signals via the vagal nerves and sympathetic signals via the splanchnic nerves (37). Following gastrectomy it can be assumed that the vagal innervation to the small intestine is disturbed, especially after resections for cancer were the lymph nodes surrounding the stomach is removed. When performing a laparoscopic gastric bypass, the dissection for creating the gastric pouch is very close to the wall of the stomach and the impact on the innervation should be limited. It can thus be assumed that the motor behaviour of the Roux-limb after a gastric bypass is different from that of a Roux-limb after gastrectomy. **So what is the normal motor pattern for the Roux-limb, and is a pressure gradient between the gastric pouch and the Roux-limb developing during food intake?**

1.4.4 Abdominal pain and opioids

The Roux-en-y construction was originally developed to ameliorate the problem of bile-reflux after partial gastrostomies for gastric cancer or ulcer disease. A disadvantage of this method is that it sometimes results in a series of symptoms called the Roux stasis syndrome (RSS) which presents with pain, nausea and vomiting that is worsened by eating (38), and the motility of the Roux-limb has, this far, essentially been studied in this context.

The Roux stasis syndrome with nausea and postprandial abdominal pain carries much resemblance to symptoms that is encountered by patients with problems after gastric bypass surgery. Studies has shown that 54% of RYGBP operated patients had symptoms of abdominal pain and that 34% had been in contact with the healthcare system because of such symptoms, and that chronic abdominal pain is reported in 34% (39, 40). It has also been reported that the use of prescribed opioid analgesics is increased after gastric bypass and it is well known that this class of drugs affect the motor behaviour of the small intestine (41, 42). Opioids induce an increased tone in the circular muscle layer and enhanced rhythmic contractions in the small intestine resulting in increased segmental contraction and decreased propulsive peristalsis (43). Chronic opioid use is an escalating problem following surgery in general (44, 45). It has actually been reported that 4% of patients that were not chronic opioid user became chronic opioid users after bariatric surgery and opioid analgesics are increasingly prescribed 7 years after surgery (46, 47). One side effect of longstanding opioid medication can be the Narcotic Bowel Syndrome (NBS), which is characterized by chronic or intermittent colicky abdominal pain that worsens when the narcotic effect wears down and where tachyphylaxis leads to need for increasing doses (48). The increased presence of gastrointestinal symptoms after gastric bypass and the similarity to the RSS and NBS raises the question on the role of Roux limb motility in abdominal pain and also if opioid analgesics may have effect on Roux limb motility. **So is there a connection between disturbed Roux-limb motility and abdominal pain after gastric bypass surgery, and is the Roux-limb motility affected by opioid analgesics?**

2 AIM

2.1 Overall goal of the thesis project

This thesis project was initiated to elucidate morphological and functional features of the Roux-limb in relation to the clinical effects of gastric bypass surgery. The clinical presence of severe abdominal pain after RYGBP also requested elucidation of the Roux-limb as a potential site for problems in affected patients.

2.2 Specific aims

1st aim: to examine the Roux-limb mucosa after gastric bypass surgery, focusing on basic morphology and inflammation

2nd aim: to explore the mechano-sensory and biomechanical properties of the Roux-limb in relation to preferred meal size

3^{d} aim: to elucidate motor activity and pressure relationships along the gastro-Roux-limb during fasting and after food intake

4th aim: to search for potential relationships between chronic abdominal pain, Roux-limb motor activity and intake of opioid analgesics.

3 PATIENTS AND METHODS

The present thesis project examines various aspects of weight-reducing surgery and related pathophysiology. All studies involved the need for patient compliance and interaction which made the use of animal models inappropriate. The studies involve 65 RYGBP and 3 VBG patients distributed in the following manner:

Table 2. Study populations involved in the thesis.

Paper	Study popu-lation		Number (female)	Mean BMI (kg/m²) at investigation	Mean BMI (kg/m²) at surgery	Median Age (years)	Catheter type	Investigations
I	1	All Investigations	8(4)	30.1	40.3	44	N.a.	Histology / Morphometry / Western blot
II	2	Ballon Inflation	10(8)	40.0 6w/ 31.2 1yr	46.3	43	Fluid perfused + balloon	Ballon Inflation / Test meal
III	3	RYGBP fasting	9(5)	27.7	40.5	48	Fluid perfused	Motor Activity in Fasting / Baseline Pressure
	4	RYGBP meal test	10(2)	29.5	41.2	51	Fluid perfused	Baseline Pressure / Effect of Food Intake
	5	VBG meal test	3(2)	30.7	?	45	Fluid perfused	Effect of Food Intake
IV	6	Retrospective review	18(17)	?	?	42	Fluid perfused / Solid state	Clinical assesment / Motor Activity in Fasting / Effect of Food Intake
	7	Time controls (from 3+4 with 2+ HAC)	11 (3)	25	41	48	Fluid perfused	HAC pressure comparison
	8	Morphine intervention	10 (9)	28	42	44	Solid state catheters	HAC pressure comparison

3.1 Ethical considerations

According to some of the study protocols the examined subject needed to ingest food in a controlled manner and evaluate variables as discomfort, pain, hunger and satiety. Gastrointestinal intubation procedures like endoscopy and luminal manometry can be stressful for the examined subject and, in turn, jeopardize the scientific value of the assessments. It was, therefore, important to try to minimize the instrumentation-dependent discomfort caused by e.g.

nasal intubation. Furthermore, the investigator had to be responsive to signs of intolerable conditions and if necessary to abort the investigation. In paper I the peroperative mucosal sampling was considered as not bringing any additional surgical risk or discomfort for the patient since it did not alter the final surgical result. Postoperative mucosal biopsy takings by endoscopy were done by a skilled endoscopist to minimize both the procedural risk and the discomfort for the study persons.

All studies were performed according to the principles for experimentation with human beings as defined in the Declaration of Helsinki. The study protocols were all approved by the Human Research Ethical Committee at University of Gothenburg (paper I) and the Regional Ethical Review Board in Gothenburg (II-IV). All subjects were informed about the experimental procedures and signed an informed consent form.

3.2 Tissue sampling during and after RYGBP surgery (I)

Paper I contains data from mucosal tissue sampled from patients undergoing RYGBP and 6 to 8 months after surgery. During surgery a full thickness sample was obtained by discharging an extra linear stapler some centimetres from the division of the small intestine being part of the standard operation. Small mucosal biopsies were dissected and used for morphological and biochemical analyses. From the same individuals new mucosal biopsies were taken 6-8 months postoperatively by use of upper gastrointestinal (GI) endoscopy~ 8cm distal to the gastro-entero anastomosis. Thus, the samples were taken from two locations that, before operation, were only~ 20cm apart and therefore considered to represent the same anatomical segment, i.e. the jejunum prior to surgery and the Roux-limb after surgery (Fig 2)

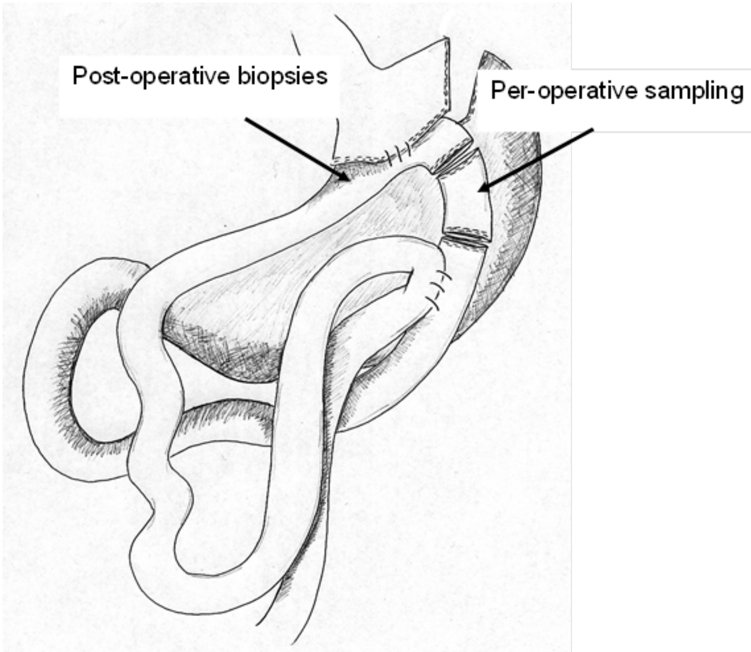

Figure 2. Sites of peroperative sampling and postoperative biopsies.

3.3 Intra-Roux balloon distension and meal testing (II)

In paper II a multi-lumen PVC catheter (modified Zinectics tube, Synmed AB, Stockholm, Sweden) was inserted nasogastrically and placed with its tip in the descending part of the Roux limb (Fig. 3). The catheter included eight separate channels with corresponding side holes positioned 20 mm apart with the most distal side hole 5 cm from the distal end of the tube. A latex balloon with a length of 35 mm was positioned at the distal end of the catheter and was connected to the central channel that in turn was used for in- and exsufflation of air and recording of intraballoon pressure. The position of the balloon in the Roux limb was standardized to be 8–10 cm below the gastro-enteroanastomosis by utilizing measurements of transmucosal potential difference (TMPD) (see below). On another study day, the study subject ingested unrestricted amounts of a standardized meal, comprised of Swedish hash, i.e. a mixture of meat, potatoes and onions, and the weight of the meal was recorded. The study days were separated to not disturb the preferred meal size by the discomfort of the catheter, but since the days was separated by not more than a week, we assumed that the results are corresponding.

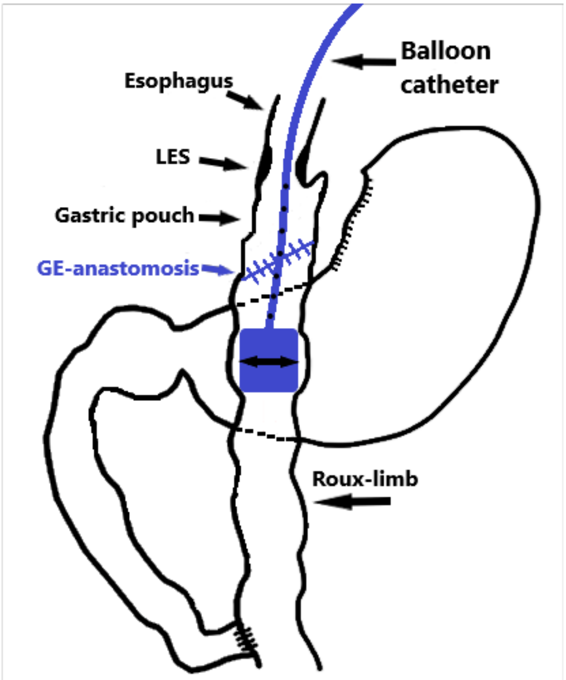

Figure 3. Principal sketch of balloon catheter placement.

3.4 High resolution manometry (III & IV)

In papers III and IV the catheter was inserted trans-nasally and fixed at one nostril and so positioned that it straddled the oesophago-gastric junction, leaving three to five measurement points in the distal oesophagus including the lower oesophageal sphincter (LES) and the rest in the gastric pouch and the proximal Roux limb. Attention should be put on the fact that, at the gastro-entero anastomosis, the jejunum is connected to the posterior wall of the gastric pouch linear staple. By this the anastomosis becomes obliquely oriented in relation to the cranial–caudal axis with the posterior wall of the pouch consisting of jejunum whereas the anterior wall is derived from the stomach in an overlapping manner (Fig 4).

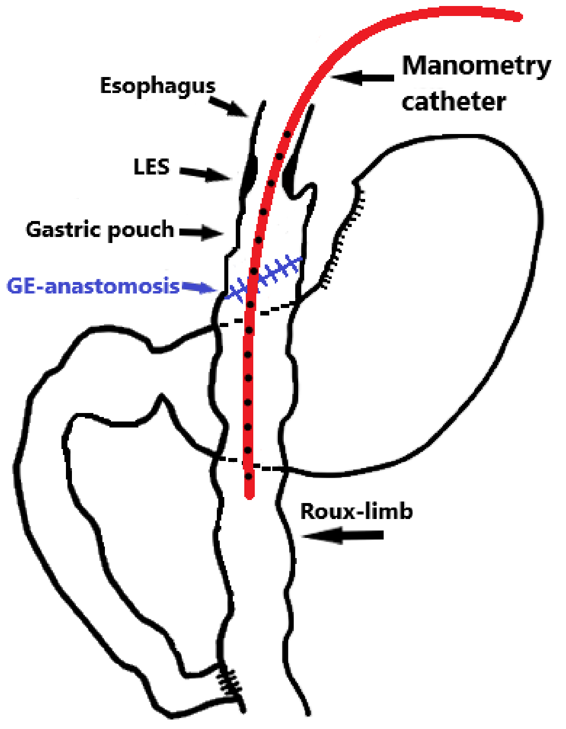

Figure 4. Principal sketch of manometry catheter placement; note the oblique orientation of the GEA.

3.4.1 Catheter placement

A correct catheter placement can be achieved by different methods. Fluoroscopy, and the use of radiopaque markers on the catheter, is easy to use but with the drawback of two dimensional imaging. It also entails radiation safety precautions. When performing pressure measurements, the use of 'manometric landmarks' is a useful method when orienting in close vicinity of the LES but require a long segment of measurement points along the catheter when investigating areas further distally. We used the high-pressure zone indicating the LES, and the pressure inversion point (PIP) following forced inspiration indicating the border between the thorax (negative pressure) and the abdomen (positive pressure) and vice versa at expiration. When using fluid perfused side-hole catheters, each pressure recording line is electrically isolated from each other which enable it to be used as an electrode for electrical potential recording related to a corresponding subcutaneous electrode. Transmucosal potential difference (TMPD) during baseline fasting conditions is generally lumen-negative with typical value of 10 to 15 mV in the oesophagus, 20 to 50 mV in the gastric lumen, and 1 to 5 mV in the small intestine, which can be used for orientation.

3.4.2 Catheter types

We have used two principle types of set-up for high resolution manometry (HRM), either fluid perfused side hole catheters or solid-state Manoscan-catheters, each with strengths and drawbacks. The fluid perfused catheter technique was used in paper III, whilst in paper IV also solid state catheters were used. Both techniques have similar recording characteristics with pressure sensing at 1 cm intervals, but the latter one covered a longer segment (35 cm) than the former technique (21 cm). For reasons given below both techniques exhibit weaknesses regarding the assessment of absolute pressure and the manometry recordings were, therefore, used for relative pressure and pattern analysis.

Fluid perfused catheter

We used a multi-lumen silastic catheter, including 22 separate channels with corresponding side holes positioned 10 mm apart, thus covering 21-cm length (customized Ch12 gastrointestinal manometric catheter, CE4-1024, Dentsleeve International LTD, Mississauga, Ontario, Canada). Each side hole was connected to a pressure transducer that was separately fed with a low flow of 3mL/h of 150mM NaCl. The use of isotonic saline instead of water allowed assessment of TMPD. A drawback with the fluid perfused technique is that pressure measurements demand externally placed transducers requiring meticulous preparations to avoid air bubbles in the channels that can interfere with the measurements (air constitutes electrical blocks and damps pressure oscillations due to its high compressibility). It also covers a rather short part of the Roux-limb, it is sensitive to body movements, and there is an increased hydrostatic pressure along the manometric catheter secondary to the semi-recumbent body position, which has to be compensated for. The advantage is that pressure readings are consistent over time and that TMPD readings can be used for positioning.

Solid state catheters

We used the solid-state catheter assembly Manoscan from Given Scientific Instruments Inc. It has 36 circumferential pressure sensors spaced at 1-cm intervals, thus covering 35 cm. This technique has the drawback that the pressure sensors are sensitive to clogging and thermal errors during ingestion of heated food. The method is also flawed by inborne errors of “pressure drift” due to temperature changes when introducing the catheter into the body, and also due to “average pressure exposure” during measurement. The former “thermal drift” can, to some extent, be compensated for by a correction algorithm. The latter is highly variable across sensors and is not possible to be corrected for via standard operating instructions (49-51). The errors from pressure drift, and the fact that these are not completely studied and understood, seems to become an increasing problem in prolonged measurements (> ≈1h) and makes it necessary to be cautious when interpreting absolute pressure data. Naturally, data on contraction frequencies, time intervals, etc. are not affected by those errors. The advantage of the solid state catheter is the ease of use in the study situation and that it covers a relative longer distance.

3.5 Statistics

In papers I and II all study persons acted as their own controls and the observations were thus considered dependent. Wilcoxon’s test was used for paired comparisons. In Paper II, Friedmans test was used to compare data over time and Pearson’s product moment correlational analysis was used to evaluate correlation between meal size and distension.

In paper III Shapiro-Wilks test was used to test for normal distribution. Since that could not be confirmed, the non-parametric Spearmans correlation was used in the analyses of meal associated data and Kruskal-Wallis test was used comparing pressure data.

In paper IV two-tailed Students t-test was used when comparing mean values within or between groups.

In all studies a p-value of < 0.05 were considered significant. The statistical software used in the thesis was SPSS (SPSS, Chicago, IL, USA) or Prism (GraphPad Software, La Jolla California USA) of the latest version available at the time.

4 RESULTS AND COMMENTS

4.1 Does the small intestinal mucosal appearance of the Roux-limb change after gastric bypass surgery? (I)

The gastric pouch of the RYGBP construction is most likely neglectable as a reservoir considering its small volume. The chewed and swallowed food will instead be directly delivered into the Roux-limb. As the primary recipient of undigested food the Roux limb mucosa is subjected to a markedly altered intraluminal environment compared to its original position further down along the digestive tube. Thus, after surgery this intestinal mucosa will be exposed to an augmented microbial, chemical as well as mechanical load. Hypothetically the mucosa should react with an inflammatory reaction and/or adapt its morphological appearance to the novel intraluminal milieu. To elucidate this assumption, mucosal samples from the part of the naïve jejunum that was to become the Roux limb were gathered peroperatively in eight patients. These mucosal biopsies were compared to endoscopically acquired biopsies from the same intestinal part 6 to 8 months after surgery.

4.1.1 Endoscopic and histologic appearance with no signs of inflammation

Somewhat surprisingly, the endoscopic evaluation of the Roux-limb showed a normal mucosal appearance in all eight patients. Still, on the microscopic level there were several findings speaking in favour of that the mucosa had adapted to the changed luminal environment. For example, the villi-derived amplification of the mucosal surface area was significantly reduced in the Roux-limb indicating a flattening of the villi, i.e. a reduced mucosal surface area, compared to before surgery. Furthermore, there was a significant increase in the proportion of Ki57 labelled cells after surgery indicating an increased mitotic rate. Most of the labelled cells were found close to the bottom of the crypts of Lieberkühn where the epithelial stemcells are situated, thus supporting a hyperproliferative condition. There were no differences detected regarding volume density of the *lamina propria* or of the numerical density of the goblet cells (Fig. 5). Taken together, the signs of a reduced villous geometry and increased mitotic rate suggest a markedly increased cell turnover possibly indicating a protective behaviour against luminal aggressors. However, signs of active inflammation, defined as the presence of neutrophils, macrophages and intramucosal oedema, were neither detected at the time of surgery, nor postoperatively. The number of mucosal lymphocytes was significantly lower after surgery, as was the number of intraepithelial lymphocytes. No significant differences were detected in the amount of plasma cells or eosinophils in the mucosa. In other words, despite the above-proposed hyperproliferative state there were no signs of "histological inflammation" in the Roux limb mucosa.

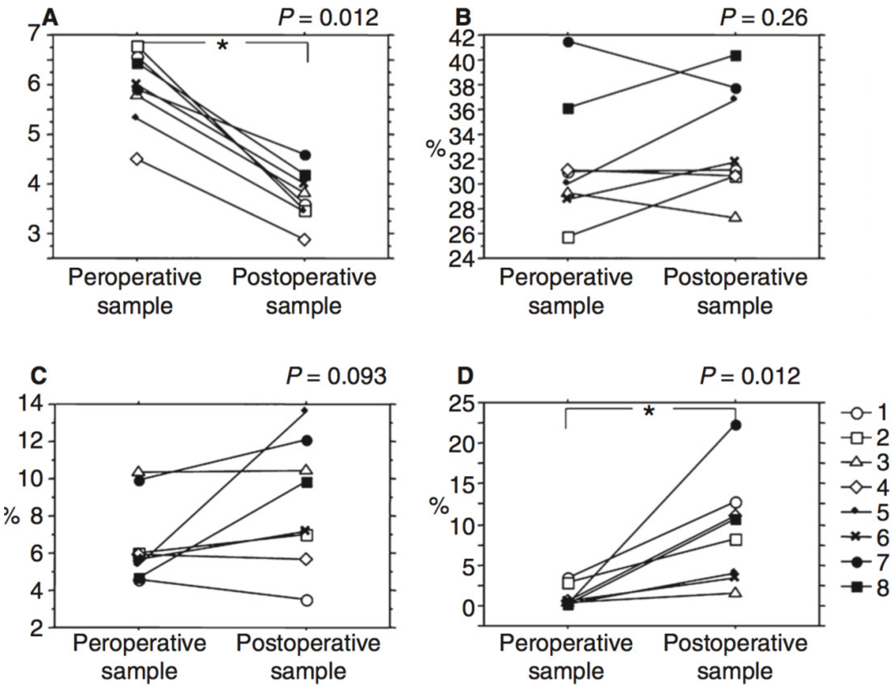

Figure 5. Morphometric evaluation of per and postoperative samples, all patients (numbered 1 to 8) act as their own controls and are displayed as individual observations (denotes a p-value of ≤0.05).*
Panel A: Villi derived area amplification factor.
Panel B: Lamina propria density given as % of the mucosa
Panel C: Goblet cell count given as % of the epithelium
Panel D: Ki67 staining signifying mitosis frequency given as % of counted cells

4.1.2 Protein expression analyses indicated a proinflammatory condition

The formation of the reactive cytotoxic radical peroxynitrite constitutes an important part of the host defence system and increased expressions of oxy- and nitroradical forming enzymes are commonly used as indicators of tissue reactions of infection or mechanical trauma (52). Western blot was used for semi-quantitative immunoblotting of the oxyradical-promoting enzymes MPO and NADPH, as well as markers of the peroxynitrite formation; nitrotyrosine and iNOS. A significant increase of NADPH oxidase protein expression was detected, as was a significant increase of MPO protein expression, but no significant differences in per- to postsurgical expression of iNOS or nitrotyrosine. The absence of nitrotyrosine speaks against inflammatory reactions, thus supporting the histological analysis. The somewhat increased capacity of oxy-radical formation may indicate a pro-inflammatory state and is supported by the increased expression of the pro-inflammatory Angiotensin II type 1 (AT1) receptor.

4.1.3 Conclusion

The appearance of the Roux-limb mucosa had undergone a moderate change at 6–8 months after RYGBP surgery, as depicted by a flattened villous surface area and increased cell proliferation. The increased epithelial cell turnover indicates that the mucosal integrity is challenged by the altered intraluminal conditions. Still no histological signs of inflammation were present, but increased expression of pro-inflammatory proteins, NADPH oxidase, MPO and AT1-receptor was found. These results indicate that the phenotype of the jejunal mucosa changes from a digestive-absorptive character into an appearance more suited for food reception / transportation and tissue defence when it becomes positioned as the Roux-limb.

4.2 Is the Roux Limb a Determinant for Meal Size After Gastric Bypass Surgery? (II)

The role of the Roux-limb in achieving a reduced food intake and weight loss following RYGBP has been very little explored. Because the data in paper I showed a reasonably normal appearance of the mucosa of the Roux-limb mucosa it seems improbable that the mucosa itself exerts any major impact on food intake. It was hypothesised that mechano-sensory and the biomechanical properties of the Roux limb wall instead could be of relevance. To investigate this possibility we assessed volume-pressure relationships in response to balloon distension of the Roux-limb and made correlations to the individual´s preferred meal size. Ten study persons were examined on two separate study days; 6 weeks, as well as 1 year after RYGBP surgery.

4.2.1 Preferred Meal Size and Body Mass Index

The clinical impression was that RYGBP-patients reduce the size of each meal but quantitative data were lacking at start of the thesis project. Therefore, all ten study persons were examined regarding preferred meal size before, 6 w and 1 y after RYGBP. Under standardized fasting conditions the subjects were instructed to serve themselves and eat until satisfied. Compared to before surgery nine out of the ten subjects ingested less at 6 weeks (median: −62%), and at 1 year (median: −41%) (Fig.6). All patients reduced their body mass index (BMI) following surgery with a group median of minus 13% at 6w and minus 33% at 1 year.

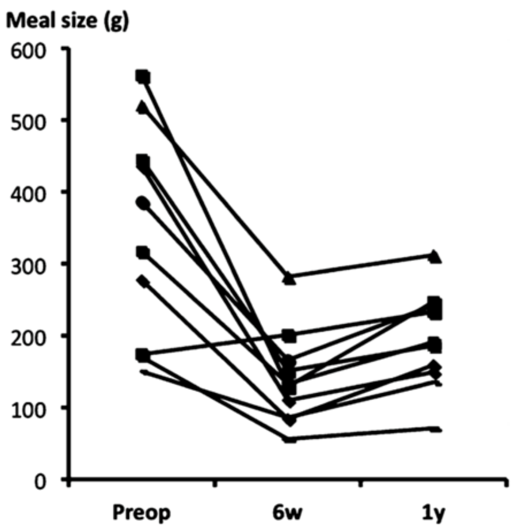

Figure 6. Preferred meal size before as well as after RYGBP.

4.2.2 Roux Limb Distension

The examination was performed after an overnight fast. To standardize experimental conditions, the distensions were conducted during MMC phase II-like conditions, i.e. periods of irregular contractions occurring in the Roux limb. The balloon was repeatedly inflated with air over 2 min with gradually increasing volumes and with complete emptying in between, starting from 20 mL and then consecutively increasing the volume by 7.5 mL to maximal 72.5 mL. At each level of inflation, the subjects reported perception of gastrointestinal sensations and the inflation sequence was interrupted if the induced sensation was not tolerable.

It was difficult for the study participants to differentiate between the non-pain and painful sensations, and sensations varied considerably between individuals. It follows that there was no clear statistical difference between the threshold pressure for induction of non-pain and pain sensations. With regard only to non-pain sensations, there was a statistically significant negative association between meal size and thresholds for sensation following distension both at 6 weeks (Fig. 7) and at 1 year.

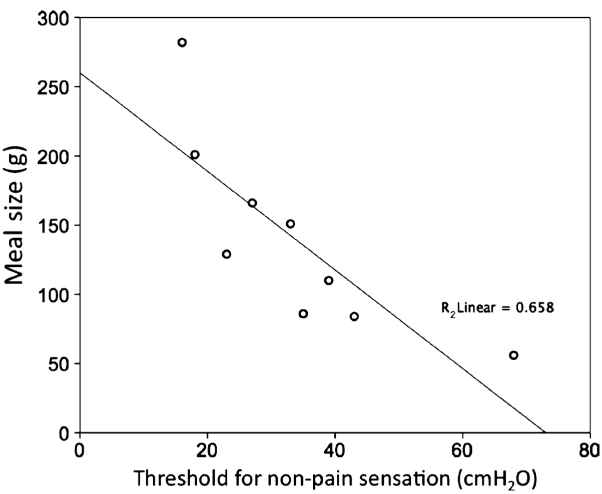

Figure 7. The recorded thresholds for induction of non-pain sensations in response to distension of the Roux limb plotted against preferred meal size. Data collected 6 weeks after RYGBP, n=10. Pearson Correlation -0.81)

At 6 weeks after surgery, the preferred meal size correlated closely to baseline intraluminal pressure (Fig 8a) as well as to the highest intraluminal pressure recorded for each individual (Fig 8b). A similar, but not statistically significant, picture was obtained at 1 year postoperatively.

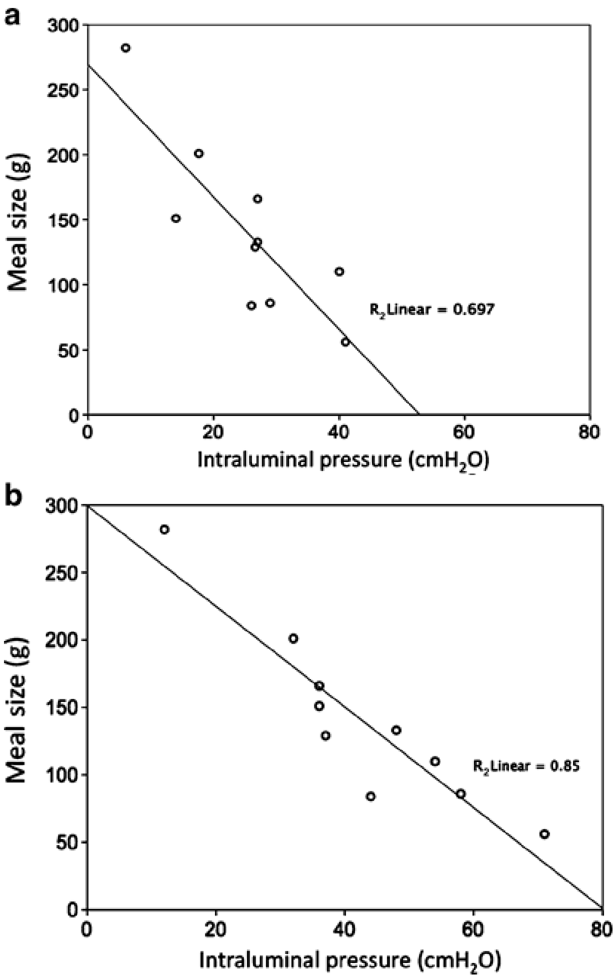

Figure 8. Baseline intraluminal pressures at a balloon volume of 20 mL (panel a) and at the highest recorded pressure in each individual (b) plotted against preferred meal size. Data collected 6 weeks after RYGBP, n=10. (Pearson Correlation panel a -0.83, panel b -0.92)

4.2.3 Conclusion

The size of voluntary food intake decreased radically in 9 out of 10 patients after RYGBP. Subjects with a low perception threshold for distension in the Roux limb preferred a large meal and *vice versa*. There was also a negative correlation between the size of meals and the intraluminal pressure that developed during balloon-induced distension; i.e. subjects with high luminal pressure in response to balloon inflation preferred smaller meals. Because of the small size of the investigated population it was not possible to conclude to what extent this influenced BMI, but the results suggest that the mechano-sensory properties of the Roux limb is an determinant for the regulation of food intake after RYGBP.

4.3 Motor activity of the Upper Gut Following Roux-en-Y Gastric Bypass (III)

The relationships between distension induced intraluminal pressure and preferred meal size in paper II are intriguing. For example, the individuals that developed a large pressure increase upon a standardized luminal volume increase were also the ones that preferred small meal sizes. It is, therefore, reasonable to assume that the Roux limb can exert a flow resistance that restricts the ingestive ability of the subject. However, the receptive behaviour of a hollow organ like the Roux limb is only partly dependent on mechanical compliance (Δvolume per Δpressure), also the clearance of the lumen is of importance to allow continued filling (i.e. ingestion). Both compliance and clearance are dependent on the biomechanical properties of the Roux-limb wall and can change depending on the state of the wall motor activity. The motility patterns of Roux-limb constructions following resective surgery have been previously published, but very little is known about Roux-limbs after the RYGBP procedure. Therefore, a study was designed to explore the oesophago-gastro-Roux motor activity during fasting and during food intake in weight-stable patients at least 2 years after uncomplicated laparoscopic RYGBP. High resolution manometry was used according to two different protocols with nine and ten investigated subjects, respectively.

4.3.1 The pressure profile along the Oesophago-Gastro-Roux Segment

By pooling baseline pressure data from twelve subjects in the two protocols, the oesophago-gastro-Roux pressure profile was calculated. Baseline conditions were defined as periods without significant motor activity (thus excluding, for example, oesophageal primary peristalsis and Roux limb phase III motor activity). By correlating PIP (indicating the border between the thorax and the abdomen) to the high-pressure zone (HPZ) (indicating the position of LES) it was concluded that LES was positioned at, or immediately below, the PIP in all cases. The pressure curve was also correlated to the TMPD that corresponded well to expected values for each type of mucosa.

The individual pressure profiles were adjusted to the highest individual pressure value in the HPZ, and the merged pressure profiles showed a difference between the HPZ (max) and the distal gastric pouch/gastro-entero anastomosis (GEA) of median 36 cmH_2O. It was noted that there was no difference in pressure between the GEA and the Roux limb (Fig. 9).

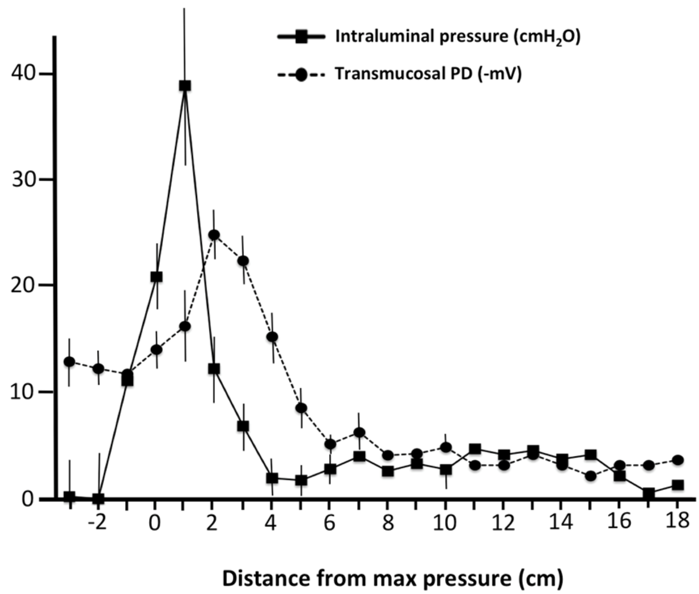

Figure 9. Intraluminal pressure and transmucosal potential difference (PD) along the manometry catheter during <u>*baseline*</u> *conditions in twelve RYGBP-patients. The values are related to the highest pressure recorded in the high-pressure zone (HPZ) of the oesophagogastric junction in each individual (0 on the x-axis). Note that transmucosal PD values typical of oesophageal mucosa (<15mV) and of gastric mucosa (>20 mV) were recorded immediately before and after the HPZ. TMPDs indicative of small intestinal mucosa (<5mV) were recorded more distally (nb all TMPD values being lumen-negative). Data are plotted as mean values ±SEM (if >1.5 mV), n=12.*

4.3.2 Roux limb Motor Activity in Fasting resembles the normal jejunal motility

Spontaneous pressure activity in the oesophago-gastro-Roux segment was recorded over 2 to 3 h in nine study persons. The pressure in the gastric pouch area remained low during the observation period whereas the Roux-limb exhibited the different phases of the migrating motor complexes (MMC). All phase III complexes started in the most proximal part of the Roux limb and propagated aborally. Median MMC cycle duration, defined as time from end of two consecutive phase III complexes, was 72 min, and the propagating velocity of phase III was 2.7 cm/min.

One pressure channel in the proximal Roux limb was used in analysing the frequency characteristics of the MMC. The contraction frequency of the migrating high activity complex (HAC) was median 12 pressure waves per minute. The late phase II (assessed 15 min before HAC) was characterized by irregular contractions with a median frequency of three pressure waves per minute. Phase I motor activity (immediately after phase III HAC) was present in all subjects and, per definition, characterized by motility quiescence with no contractions.

4.3.3 Gastric pouch and Roux limb pressure increased similarly during food intake

After catheter placement (as above) the completion of a phase III HAC in the Roux limb was awaited in nine study subjects. After the HAC had passed; the study subject was served 300 g of Swedish Hash, and instructed to eat until they felt comfortably full. Eating and the amount of ingested food were measured, after which the manometric recordings were maintained to a median total recording time of 163 min. (example in Fig. 10)

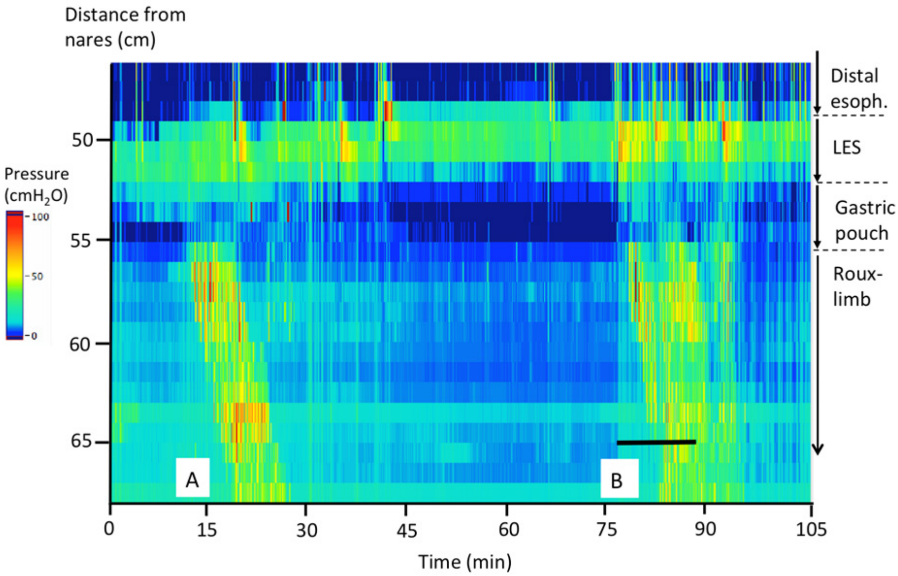

Figure 10. High resolution manometry of the oesophago-gastro-Roux area (with distance from nares on the left y-axis and corresponding anatomy at the right) in one RYGBP patient. Pressure is encoded in color (see scale at left). Note the appearance of a MMC phase III like motility complex starting in the Roux-limb some 15 min after onset of recording (A). At time 75 min the patient ingests food until satiated (13 min, marked with a bar at B). Note the moderate pressure increase in the gastric pouch during and after food intake speaking against outlet obstruction at the gastroentero-anastomosis.

Pressure data were integrated into 2-min intervals and related to the highest value in the HPZ of LES and then merged to allow group analysis. Food intake caused a moderate and gradual pressure increase in the gastric pouch as well as in the Roux segment. In most subjects, the gastro-Roux pressure was highest after 8 min and tended then to decrease despite continued food ingestion (Fig. 11). When comparing pressure change from baseline to when the individual stopped eating, as well as the pressure progression during the ingestion period, there was no difference between the gastric pouch and the more distal parts of the Roux-limb. In other words, these two segments acted as a common cavity indicating strongly that there was no transport restriction in the GEA.

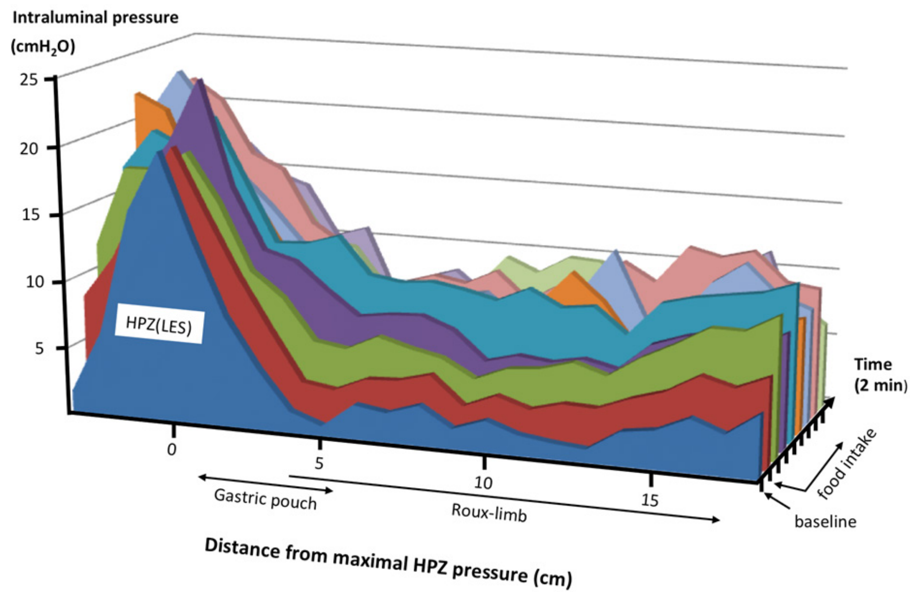

Figure 11. Mean intraluminal pressure (y-axis) in 2 min periods (z-axis) in 10 patients with RYGBP before (baseline) and during voluntary food intake. Pressure is plotted along the oesophago-gastro-Roux catheter in relation to the highest pressure recorded in the HPZ (high pressure zone) at the level of the LES (lower oesophageal sphincter) during baseline conditions. Estimated anatomical landmarks are indicated. Note a general upward transposition of the gastro-Roux pressure curve during food intake.

4.3.4 No support for that the meal-induced Roux-pressure determines meal size and weight loss

There were no correlations between pressure changes and meal size, meal duration or percentage total body weight loss. The most obvious reason was that the study was not powered for these analyses.

4.3.5 Conclusion

When examining the motor pattern in the fasting state, the Roux-limb exhibited regular MMC activity including phases I, II and III as in a normal small intestine, with onset immediately distal to the gastro-entero anastomosis. The absence of a difference in intraluminal pressures of the gastric pouch and the Roux-limb during food intake indicates that these segments act as a common cavity. The study could not give support to that the meal-associated increased intraluminal pressure influences meal size or weight loss.

4.4 Roux-limb dysmotility and postprandial nausea and abdominal pain (IV)

RYGBP is in most cases successful with a sustained weight loss and improved metabolic state. However, a subset of patients that have undergone RYGBP suffers from abdominal pain and/or nausea after meals at a level that demands contact with the health care system. Also after excluding obvious surgical reasons, including expected procedure-dependent dumping, there are a substantial number of RYGBP-patients with postprandial chronic pain of unknown origin. The enigmatic association between meal intake and abdominal pain had led to that a number of our patients were referred to upper gut manometry as part of a clinical evaluation. It was considered of interest to review these clinical investigations to see if there was a connection between Roux-limb motor patterns and postprandial chronic abdominal pain.

4.4.1 Dysmotiliy, revisional surgery and opioid use are common in gastric bypass patients with postprandial abdominal pain

Disturbed Roux-limb motor patterns were frequently noted in the group of RYGBP patients with postprandial symptoms. Of the 17 assessable investigations only 5 were evaluated as normal manometries, thus comparable to those described for the uncomplicated patients assessed in paper III. There were signs of distal pacemaker activity in 4/17 study persons and 4/17 had distinct retrograde propulsion. Despite this, only one patient out of the 17 had symptoms isochronous to pathological manometry findings. Furthermore, no distinct manometric signs of congestion in the gastric pouch or Roux-limb after food intake were noted. This speaks against the Roux Stasis Syndrome that previously has been described to occur after resective surgery (38). Interestingly, 17 out of the 18 symptomatic patients were women suggesting an overrepresentation of female gender, although this may be a result due to selection bias. Furthermore, in this cohort of RYGBP patients the median number of re-operations due to abdominal symptoms was 5 (range 0 to 11) highlighting that revisional surgery may have a low efficacy. Also another phenomenon that caught our interest was that a substantial number of patients, 12 out of the 18, used prescribed opioid-based analgesics.

The high prevalence of prescribed opioid analgesics in this group of patients is alarming as long term intake of morphine-derivatives for analgesic purposes may lead to Opioid Induced Bowel Dysfunction with constipation, nausea and vomiting, as well as the Narcotic Bowel Syndrome (NBS). Grunkemeier et al. characterize NBS as chronic or intermittent colicky abdominal pain that worsens when the narcotic effect wears down and where tachyphylaxis leads to need for increasing doses (48). NBS could thus explain both the high prevalence of prescribed opioids and the resemblance in symptomatology in our cohort of RYGBP patients with severe chronic abdominal pain. Moreover, this suspicion led us to hypothesize that the opioids could be both the trigger and driver of NBS by inducing Roux-limb dysmotility.

4.4.2 Can administration of an opioid trigger Roux-limb dysmotility and pain?

In 1982 Sarna et al. showed that the migrating motor complex (MMC) could be influenced by morphine (42). Later Levis demonstrated that intravenous infusion of morphine induced duodenal phase III-like activity (53). To test if Roux-limb motor activity is sensitive to circulating opiates we performed oesophago-gastro-Roux manometry in asymptomatic and opiate-naïve gastric bypass patients (n=9), and compared the results to an untreated control group (n=11). As motor activity is extremely difficult to standardize we concentrated the analysis on the phase III-like HAC of the fasting motility pattern that allowed quantification.

No significant differences between the groups were found regarding duration, contraction frequency or propagation velocity of the HAC's. *Mean Roux-limb pressure*, however, was significantly higher (+7mmHg) during HAC in presence of morphine than in a preceding baseline HAC. This effect was not seen in the untreated time control group. The higher intraluminal pressure during morphine administration indicates that the Roux-limb compliance is reduced and supports the hypothesis that pain-killing opioids actually can contribute to induction of pain. Theoretically, a "pre-tension" in the wall musculature facilitates activation of tension-sensitive pain receptors in response to a luminal volume expansion after food intake, thereby increasing the probability for eliciting abdominal pain sensations that in the long term can develop into NBS

4.4.3 Conclusion

A majority of the RYGBP-patients with chronic pain and nausea exhibits a disturbed Roux-limb fasting motility. In opiate-naïve RYGBP-patients, acute morphine intravenously increased the muscular tone of the Roux-limb. This can be a triggering signal to a viscous circle ending in NBS in susceptible RYGBP-patients.

5 CONCLUSIONS

1. The results indicate that compared to the original jejunum, the phenotype of the Roux-limb mucosa is modestly altered from a digestive-absorptive character into an appearance more suited for food reception ⁄ transportation and tissue defence.

2. The preferred meal size decreased radically after RYGBP and a close relation to mechano-sensory properties suggests that the Roux limb can be a determinant for food intake

3. During fasting the Roux-limb exhibited all phases of migrating motility complexes (MMC) with an onset close to the GEA. During food intake intraluminal pressure increased transiently without any significant difference between the gastric pouch and Roux-limb indicating that these two segments act as a common cavity.

4. A majority of examined RYGBP-patients with meal associated chronic pain and nausea exhibited a disturbed Roux-limb motility and most of these patients had been prescribed opioid analgesics.

5. In uncomplicated opiate-naïve RYGBP-patients, acute morphine administration influenced the Roux-limb musculature in a fashion that potentially initiates or aggravates dysmotility.

6 GENERAL DISCUSSION

The present thesis focuses on functional characteristics of the Roux-limb, which in the context of Gastric Bypass surgery had not been studied to any greater extent. The intellectual process started with the question "why do patients lose weight after Gastric Bypass surgery". Today the discussion focuses on "metabolic surgery" and particularly the positive effects on diabetes. The observant reader notices that the first two papers (I & II) were published in 2010, and when the project started a few years earlier the question regarding weight loss was very valid. In those days, around 2004, the research on gastrointestinal hormones was still in the bud and there was an ongoing debate on whether the stoma size of the gastro-entero anastomosis, by restriction, contributed to the weight loss. It was even proposed that the restrictive feature of RYGBP should be reinforced by applying a band around the GEA (54). One of the initial aims of the thesis project was actually to refute the restrictive part of the Gastric Bypass surgical procedure. In doing this, two questions became evident: "Do the patients eat less after surgery" and "if so, might that be for pure mechanical reasons".

The clinical experience was that the patients ate less after surgery, which was also confirmed in paper II where we found a radical decrease in preferred portion size after surgery. A role for the gastric pouch as a reservoir had principally already been ruled out by the fact that the surgical technique, "the Lönroth procedure", prescribed a minimal gastric pouch (≈20-30 mL) and a large-calibre GEA (theoretically without outflow "restriction") (55, 56). Thus, instead of continuing the debate on the role of the pouch being a reservoir or not, two hypotheses were formulated based on the fact that the Roux-limb was the primary recipient for ingested and undigested food:

1. / the jejunal mucosa in the Roux limb gets inflamed by the new intraluminal milieu in turn starting anti-ingestive signalling,

2. / satiation is induced by bolus distensions of the jejunal segment (i.e. the Roux limb) that follow the swallows of food.

As discussed above, according to the findings in paper I the mucosa of the Roux-limb mucosa did not show any manifest inflammation, as consistent with an earlier study by Csendes et al. (57), although some pro-inflammatory signs were present. The appearance with flattened villous structure combined with increased epithelial renewal speaks in favour of a mucosal adaptation to food reception/transportation/protection rather than appetite signalling. Although the latter cannot be completely excluded, more attention was put on the second hypothesis. It was reasonable to assume that the food-receiving capacity of the Roux-limb was restricted due to its mechanical properties. Thus, food intake would be followed by an increased intraluminal pressure, immediately propagated to volume- or stretch-sensitive neural elements in the intestinal wall that in turn report to the CNS. To mimic the Roux-limb's response to a food bolus we exposed the Roux-limb to pressure stimulation by inflating a balloon in the lumen. An obvious question is why we did not use a food bolus and intraluminal manometry. The reason was to standardize the stimulus and avoid accounting for the outflow in distal direction that would make us not to have control over the volume of the bolus. We choose, therefore, to separate the intubation and meal size events. Another advantage of this was that a nasal catheter probably disturbs the study person's willingness to eat, why it would be difficult to achieve a true "*ad libitum*" measure. (Food bolus as stimulus was actually tested later as reported in paper III.) Balloon distension not only allowed standardised local mechanical stimulation, it was also without simultaneous activation of mucosal chemosensitivity. When using this technique we showed that subjects with a low perception threshold to distension of the Roux limb preferred large meal sizes and *vice versa* (II). This finding was a bit counter-intuitive. At first it seems reasonable that an abdominal sensation to intraluminal distension is interpreted as a satiety signal thereby reducing food intake. However as the opposite was the case, one can instead speculate that in parallel to the perceived sensation there is a triggering of peristalsis in distal direction that clears the lumen and makes it ready to receive more food (i.e. a larger meal). This indeed appears to be the case as shown in paper III where food ingestion clearly induces Roux limb propulsive motility. However, in the current thesis project we did not assess clearance of the Roux-limb lumen. At present, therefore, we cannot confirm the above proposed idea that that "big-mealers" have a low-threshold for inducing Roux limb clearance motility.

Another perhaps more expected finding was that the subjects that responded with high intra-balloon pressure to a given volume distension were the ones that chose to eat small meals (II). These results suggest that individuals with low mechanical compliance in the Roux-limb (i.e. a large net pressure increase following a given volume), have the most optimal effect regarding reduction of food intake. This finding supports that RYGBP has a restrictive component and that the restriction is located to the Roux limb. Because of the small size of the investigated population, it was not possible to conclude to what extent this "Roux-limb restriction" influenced BMI.

So, in paper II we proposed that that food intake and intestinal sensing can be determined by the bio-mechanic properties of the Roux limb wall. One important constituent of the GI-wall is smooth musculature that have a functional state that can vary considerably for example in response to luminal nutrient degradation (e.g. fat digestion), or to neuroendocrine factors (e.g. vagal activity, prokinetic hormones), as well as to pharmaceuticals (e.g. NSAIDs and opioids) (35, 58, 59). It follows that the Roux-limb should be ascribed a sort of "dynamic restrictive property". To what extent a potential dynamic restriction of the Roux limb really has a food-intake regulating relevance remains to be investigated. If verified, it opens for exciting possibilities. One is that the Roux-limb mechanical compliance should be possible to regulate by pharmaceutical agents. This may be useful to, for example, counteract or treat weight regain that sometimes occur also after RYGBP.

Even though we, for surgical technical reasons, did not believe in a restriction in the GEA, the question remained; does the GEA exert a flow resistance that creates distension of the gastric pouch during meal intake? Since mechano-receptors for distension are present also in the gastric pouch, it could still be the site for a pressure induced satiation response. Why did not we perform the balloon dilatation in the pouch? The problem with investigating the pouch, in the same manner as we did in the Roux-limb, is that the pouch size varies between individuals making investigational conditions difficult to standardize. Even though we strive to standardize the pouch during the operation the diameter might differ markedly. Also, with our fashioning the GEA by use of a dorsal linear stapler, it follows that the dorsal wall of the pouch, to a varied extent, is comprised of the connected small intestine having different biomechanical wall features than the gastric wall. Instead of balloon measurements, therefore, a more relevant measure is the pressure-profile that is built up in the pouch-to-Roux segment during feeding, which also takes into account the distal outflow. This is done in paper III where we show that there was no intraluminal pressure-gradient between the pouch and the Roux-limb during eating. Thus, the pouch and Roux-limb should be regarded as a common cavity and a role of the GEA as the site for restriction can, at least with regard to the present surgical procedure, be excluded. Still, even if the restriction is distal to the GEA, the pouch will, together with the Roux limb, be subjected to meal induced distensions and can thus contribute in the signalling of satiation.

In paper III we did not see any relation between Roux-limb pressure and meal size, which thus speaks against the findings in paper II. However, the investigational conditions, particularly the presence of a nasointestinal tube, might have influenced the ingestive behaviour of the study participants. Speaking in favour of such an influence are 2 years follow-up data from Laurenius et al 2012(60) showing larger preferred meal size (mean meal ≈250 g) in study subjects without naso-intestinal intubation compared to the instrumented subjects of III (mean meal ≈160 g). Based on this difference and the low number of study subjects it is not surprising that data from III does not support the proposed mechanism.

Today, the research front of has moved away from the restriction/malabsorption paradigm. This thesis project demonstrates that the biomechanical properties and muscular activity of the Roux-limb and gastric pouch may still be clinically important, and particularly so during dysfunctional conditions potentially leading to problems with nausea, pain and eating disorders. Also after excluding obvious surgical reasons, including procedure-dependent dumping symptoms, there are still a substantial number of RYGBP-patients with chronic pain of unknown origin. In the present survey of patients with such symptoms after RYGBP we found that as many as 12 out of the 18 patients had prescribed opioid medication (IV). This observation may be of clinical relevance as opioid based analgesics not seldom are associated with abdominal discomfort and pain after meals. It is well known that opioids can induce a dyscoordinated gastrointestinal motility, for example manifested as an increased contractility tone in the small intestine. In paper IV we confirm that an acute intravenous morphine infusion increases the muscular tone of the Roux-limb in uncomplicated RYGBP-patients. This finding has a direct relation to the above-proposed dynamic restriction of the Roux limb, but not in the context of gut satiety signalling, rather as trigger of discomfort and pain. Thus, as suggested in IV, postoperative administration of opioids for analgesic purposes can by inducing a contracted Roux limb, and the following altered mechanosensory thresholds, trigger meal-induced abdominal pain resulting in iterative demand of the pain-killing drug. In the long term this is a viscous circle that may end up in the narcotic bowel syndrome, NBS.

To substantiate this, so far, hypothetical chain of deleterious events it would be necessary to investigate the basal muscular tonus and the effect of opioid administration in patients with longstanding opioid medication, and preferably also after drug detoxification, to see if the basal tonus was higher in this group, or if the increased tonus only applies to acute administration of opioids. It would also be of interest to compare the sensation thresholds and pressure response to balloon inflation in presence and absence of opioids. Since we found in paper II that subjects with a low perception threshold also responded to inflation with a low pressure increase to volume expansion, it can be speculated whether opioids increase intraluminal pressure due to induced increase muscular tone and thereby changes the perception threshold upwards. On the other hand, as we speculate in paper IV, the elevated muscle tonus might induce a "relative narrowness" with reduced compliance to distension that would facilitate activation of tension-sensitive pain receptors in response to a luminal volume expansion. There might be a difference between "non-pain" and "pain" perception that we were not able to sort out in paper II.

There are several weaknesses in this thesis project and some need to be commented: First; the study populations are small, of which follows the apparent risk for type 2 errors, that is, the risk of not detecting an existing difference. One reason for this is that the investigations demanding oro-pharyngeal intubation (e.g. manometry) are uncomfortable and time-consuming and it is thereby hard to find volunteers, especially those who agree to undergo repeated investigations. The investigations are also demanding large resources from the laboratory. This is also the reason why we re-cycled investigations from paper III in paper IV, albeit with new analyses. Second; as has been discussed above, the problems with fluid perfused technique made us change to solid state catheters which brought new challenges. By this there is a mixture of techniques in the thesis which, from a methodological point of view, is less elegant. The fact that a nasal catheter also irritates the pharynx makes *ad libitum* food intake unreliable. The discomfort of the catheter urged for a limitation of investigation time, which made us unable to use the prolonged protocol needed for a meticulous study of the transition from fasting to fed state.

7 FUTURE PERSPECTIVES

One general message from the present thesis project is that the Roux-limb has a number of features that can help to explain both weight loss and un-wished consequences of RYGBP. Although the data are mainly hypotheses generating, the thesis has brought forward some issues that would be very profitable to further explore both as basic science and in the clinical arena. In our retrospective cohort study (paper IV), an appalling proportion had been re-operated several times without reaching alleviation of their symptoms. The growing number of patients that has undergone RYGBP, and the fact that many of these patients continue to seek medical care for abdominal pain, stresses the importance of a better understanding of the underlying mechanisms behind these symptoms, all with the objective to avoid unnecessary abdominal reoperations which otherwise aggravate the situation.

The presence of abnormal motor patterns in patients with gastrointestinal symptomatology (paper IV) needs to be further addressed, preferably in combination with validated rating instruments for gastrointestinal symptoms, such as the Gastrointestinal Symptom Rating Scale (GSRS), which unfortunately was not done in our retrospective cohort. The variety in motor disturbances also needs to be addressed by a larger number of investigations both in symptomatic and asymptomatic patients. The fact that we did not see any abnormal patterns in the cohort studied in paper III does not exclude the possibility that retrograde contractions and premature onset of distal contractions may also be present in asymptomatic patients.

The drawbacks of catheter manometry, as discussed above, made it difficult to investigate the transition from fasting to feed state, as well as the relations between symptoms and motor behaviour during feeding. They also implies a challenge in bringing these investigations to a clinical setting with a greater number of investigations, both concerning the patient's willingness to undergo the investigations, and the resources required from the health care system. It is therefore of importance to develop methods for further research on Roux limb motility during and after food intake, that does not demand intubation.

8 EPILOGUE

The thesis discusses the functional characteristics of the Roux-limb after Gastric Bypass surgery in the aspects of; the decrease in preferred meal size, the adaptation of the mucosa, the mechano-sensory properties of the in relation to food intake, the normal MMC activity, the intraluminal pressure during fasting and fed state, and the role of the Roux-limb and opioid analgesics in abdominal pain after surgery. The scientific field of obesity, as the number of patients, is growing rapidly and this is only a small contribution, but hopefully, some vague insights lead on into memory.

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APPENDIX

Paper I-IV