Gastric Bypass in Morbid Obesity

Postoperative Changes in Metabolic, Inflammatory and Gut Regulatory Peptides

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Abstract

This thesis examines the effect of surgical weight loss on gut and adipose tissue peptides involved in appetite regulation and energy homeostasis in morbidly obese humans. Roux-en-Y gastric bypass (RYGBP) is the gold standard operation used for effective long-term weight loss and improved health. The exact mechanisms for this outcome are under investigation.

We measured ghrelin, a recently discovered hunger hormone, insulin, adiponectin and leptin along with anthropometry measures in 66 morbidly obese patients prior to and 6 and 12 months after RYGBP. Impressive weight loss occurred postoperatively as did alterations in the peptides. Consistent correlations were found between weight, leptin, ghrelin and insulin. The main findings were low ghrelin concentrations in obesity and an increase after RYGBP.

We explored inflammatory proteins C-reactive protein (CRP), serum amyloid A and interleukin-6 before and during massive weight loss 6 and 12 months after RYGBP in morbidly obese subjects. The studied proteins declined after surgery and a correlation between CRP and homeostatic model of assessment for insulin resistance, independent of BMI, strongly linked insulin resistance and inflammation. CRP declined most in insulin-sensitive subjects.

We examined the excluded stomach mucosa and vagus nerve by measuring gastrin, pepsinogen I (PGI), pancreatic polypeptide (PP) and ghrelin levels during week 1 and year after RYGBP. Ghrelin levels rose with weight loss but declined 24-hours after surgery, like PP, indicating transient vagal nerve damage. Low levels of gastrin and PGI suggest a resting mucosa.

We evaluated gut peptides: peptide YY (PYY), glucagon like peptide-1 (GLP-1), pro-neurotensin (pro-NT) and PP, in lean (young and middle-aged), obese and postoperative RYGBP subjects pre- and postprandially. RYGBP subjects had exaggerated levels of PYY and GLP-1 postprandially and higher basal proNT levels, implying a ‘satiety peptide tone’ that may contribute to the maintenance of weight loss.

In summary, RYGBP results in marked weight loss and alterations in gut and adipose tissue peptides involved in appetite regulation and energy homeostasis. These postoperative peptide changes may contribute to impressive weight loss observed after RYGBP.

Keywords: gastric bypass, morbid obesity, ghrelin, C-reactive protein, pancreatic polypeptide, peptide YY, glucagon-like peptide-1

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Education is the most powerful weapon which you can use to change the world.

Nelson Mandela

To my families and friends
Papers


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<tr>
<td>BPD</td>
<td>Biliopancreatic diversion</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>ECL</td>
<td>Enteroendocrine cells</td>
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<td>ELISA</td>
<td>Immunosorbent assay</td>
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<td>GB</td>
<td>Gastric banding</td>
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<td>IL-6</td>
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<td>HOMA</td>
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<td>PP</td>
<td>Pancreatic polypeptide</td>
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<td>PGI</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>RYGBP</td>
<td>Roux-en-Y gastric bypass</td>
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<td>SAA</td>
<td>Serum amyloid A</td>
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<td>VBG</td>
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Introduction

Obesity epidemic and morbid obesity

Obesity has reached global epidemic proportions and is a condition presently affecting millions of people worldwide. Over the past two decades, the obesity rate has risen steadily and with no barriers present, both developed and developing countries are affected as are all societal strata. No age group is spared, as rates of childhood obesity are rising in many countries.

Obesity is a hypercaloric state associated with a higher energy intake than output and the higher the degree of obesity, the higher the risk of morbidity and mortality compared with lean subjects (1). Caucasians with a body mass index (BMI) greater than 25 kg/m\(^2\) are classified overweight. A BMI >30 is obese, >40 is morbidly obese, >50 is super-obese. A BMI <20 is underweight and is a condition itself associated with increased morbidity and mortality (1). More accurate measures of body fat are waist-hip ratio, waist circumference and sagittal diameter. BMI ranges are slightly different for ethnic groups such as lower ranges for Asian people than for Caucasians.

Presently, the prevalence of overweight and obesity is escalating, especially in the US, UK, Australia and Mexico, the nations leading the epidemic. The prevalence of obesity in Europe in adults aged 40-60 years is 15-44 % for women and 10-18 % for men (2). In Sweden over the past twenty years, the obesity rate rise from 5 to 10 %, with the largest increase in persons aged 25-44 years (3). In Sweden in 2002-2003, 41 % of people were overweight and 11 % obese, up from 31 % and 5 % in 1980-1981, respectively (4).

After smoking, obesity is the second most preventable cause of death and is a major modifiable risk factor for cardiovascular disease. The list of comorbidities associated strongly with obesity is extensive and includes sleep apnoea, dyslipidemia, cancer, arthritis, depression, social isolation, infertility and anxiety. The mortality associated with obesity is astounding, with a ten-fold increased risk of developing Type 2 diabetes (5) and three times greater risk of developing coronary artery disease (6,7). The death rate from all cancers in 900,000 American adults who were obese or larger was 52 % and
62 % higher for men and women, respectively, compared with lean individuals (1). Obese people have shorter lives and may die 5-20 years earlier (8).

**Conventional weight loss**

Obese people are typically treated initially with a combination of calorie restricted diets and exercise, although attempts are largely unsuccessful and the weight lost, between 5-10 %, is rarely sustained (9). However, small improvements in weight provide a benefit by decreasing the risk of cardiovascular disease through reducing blood pressure (10), serum lipids and glucose and increasing HDL cholesterol. Furthermore, for each kilogram lost, a 0.05 mmol/L decrease in total cholesterol occurs (11).

More specifically, various exercise regimens were associated with 4 kgs weight reduction over 1-2 years (12-14). Combined diet and exercise programs lasting 1 year achieve weight loss in the range 2-10 kgs (15-17). A low calorie diet is essential to lose weight, with regular exercise assisting in weight maintenance in the long-term. Behavioural therapies for obesity were introduced in the 1970s to help overcome barriers to compliance with diet therapy or physical activity and thus improve long-term weight loss and adherence to treatment. A five-year randomised controlled trial of health promotion in general practice for patients at high cardiovascular risk found that the benefits reported at 2 years, including increased daily physical exercise, eating a healthier diet and minimal weight loss had disappeared at the study’s completion. The authors advised that prolonged provision of health promotion is desirable (18). Programs incorporating cognitive therapy can induce modest weight loss over 1-3 years as well as improving emotional well-being, increasing fitness, improving dietary quality and reducing cardiovascular risk factors (19). First-line management of obese states should be combination therapy, with regular and long-term contact before any medical or surgical treatment is considered. Morbidly obese people should be managed with lifestyle, medical and or surgical interventions.

**Medical weight loss**

Medical therapy has been employed as one method of treatment for obesity since the 1960s. Currently available medical therapies differ between countries, with Europe introducing the third agent prior to the USA and Oceania. Rimonabant is the first selective cannabinoid type 1 receptor antagonist and it has been shown to aid in significant weight loss (6 kg) and improvements in waist circumference, insulin sensitivity and lipid profile at two years compared with placebo. Adverse effects were uncommon and similar to type and number in placebo group (20).
The two older medicines include orlistat and sibutramine. Orlistat acts by decreasing dietary fat absorption by ~30% through its inhibition of the pancreatic lipase enzyme. It is minimally (<1%) absorbed from the gastrointestinal tract so has no effect on systemic lipases. Sibutramine is an inhibitor of noradrenaline, and serotonin reuptake and so acts centrally to decrease appetite. Both can aid in weight loss of 8-10% over two years but only if medication is taken strictly (21). Weight reduction following medicine compliance is slightly better than for dietary change alone but efficacy is difficult to assess due to compliance issues partly because of side effects (9). Therefore, the rise in obesity, along with unsatisfactory weight loss results, has led to the escalation in obesity surgery.

**Bariatric surgery and Roux-en-Y gastric bypass**

Bariatric surgery comes from the Greek word *Baros* for fat. It was first used in Scandinavia in the 1950s but procedures have been re-developed and are performed today at expanding rates primarily for those who are morbidly obese or have BMI>35 with co-morbidities. Criteria for surgical treatment include failure of other methods, knowledge of procedures and risks, motivation, support and commitment to life-long follow-up. Surgical treatment is essential for patients with double the risk of death compared with lean people (6) and is now the preferred method for clinically obese patients to lose weight and reduce their risk of developing diabetes and cardiovascular disease (7).

Obesity surgery options are two: restriction or bypass operations. Restriction operations are of two main types: gastric banding (GB) and vertical banded gastroplasty (VBG). Bypass operations include Roux-en-Y gastric bypass (RYGBP) and biliopancreatic diversion (BPD). GB involves a silicon band, which may be adjustable, securely fastened around the cardiac area of the stomach to result in earlier satiety due to early gastric distension. Nonadjustable operations, including VBG produces weight loss of approximately 41 kgs (range 45-63) over 1-2 years and 25 kgs (range 17-39) over 3-8 years, while adjustable bands aid in a loss of 31 kgs over 1-2 years and 34 kgs during 3-4 years (19). Re-operation rates vary between 2-41% (22-24) mainly due to band migration as well as oesophageal and gastric pouch dilation (25). VBG was developed to leave a small 30 ml vertical pouch, kept in position by staples. Even though more successful with 40-60% weight loss (26), this method has re-operation rates of 20-40% due to poor weight loss maintenance, staple line breakdown (27), stomal outlet stenosis and severe gastrooesophageal reflux (28). BPD is not as commonplace despite weight losses of about 53 kgs (range 42-62) over 1-2 years and 54 kgs (range 37-84)
over 3-9 years (19). Postoperative problems include protein and caloric malnutrition, diarrhoea, gas and kidney stones (29).

RYGBP is now the ‘gold standard’ for surgically induced weight loss (30-32) (Fig 1). The prevalence in the USA and Canada is 70 % compared with 5 % for GB, 7 % for VBG and 12 % for BPD. It typically results in weight loss of 46 kgs (range 35-53) over 1-2 years and 42 kgs (range 29-62) after 3-15 years (19) and has therefore superseded the formerly described bariatric procedures. Hickey et al. claimed excess weight loss was 55 % at 10 years and 49 % at 14 years (33). The success of RYGBP is attributable to reduced adipose tissue mass [indicated by decrease in subcutaneous fat-cell weight and fat-cell number (34)], food intake (35), hastened early satiety (36), transient nutrient malabsorption, ‘dumping’ syndrome and the exaggerated hormonal responses involved in appetite regulation and energy homeostasis. The newly achieved state of negative energy balance lessens morbidity (37) and decreases mortality by 25 % with respect to an obese control group (38). More specifically, mortality was reduced by 40 % in a large cohort at 7 years, especially from diabetes (92 %), heart disease (56 %) and cancer (60 %) (39). Furthermore, patients experience a dramatically improved quality of life (40), psychosocial functioning (41-42), self-image, happiness, social interaction and employment opportunities, all of which were increased 3 years after surgery (43).

Obesity surgery is cost-effective in morbidly obese patients after 2 years (19). The quality of life (QOL) before and postoperatively are opposite, with a poor QOL prior to surgery and a major improvement after massive weight loss, irrespective of the procedure (44-46). The postoperative mortality rate of 0.22 per cent in the Swedish Obese Subjects study demonstrates the high standard of safety that has been achieved in the past decade (47). The prevalence of the dumping syndrome is 10-15 % (48). This is a postprandial condition characterised by sweating, tachycardia, nausea and vomiting. It is due to rapid emptying of partially digested foods, with mechanical distention, and altered secretion of intestinal hormones and may result in systemic volume contraction, adrenergic stimulation and late hypoglycemia (48).
Figure 1. Diagram of the gastrointestinal tract (A) before and after RYGBP (B). Major sites of micronutrient absorption and gastrointestinal hormone production are shown. In RYGBP, the stomach becomes a small pouch, excluding much of the ghrelin-secreting regions. The pouch is anastomosed with the proximal jejunum. The duodenum and jejunum are attached to the distal jejunum. (Modified from Shah et al. JCEM 2006; 91:4223)

Gastric peptides: ghrelin, gastrin and pepsinogen I (PGI)

**Ghrelin**, an endogenous orexigen, is the only hormone that stimulates appetite to date. Ghrelin was initially isolated from rat gastric mucosa once it was recognised as the first endogenous ligand for the growth hormone secretagogue receptor (GHS-R) and the most potent stimulator of growth hormone secretion. Ghrelin and its receptor have been found in the entire human intestine as well in hypothalamus (49), pancreas (50), kidneys (51) and pituitary gland (52) and testes. Ghrelin initiates feeding if given centrally or peripherally through its ability to stimulate appetite and food intake in animals and humans (53), as well as chronic administration causing an augmentation in body weight (54). Ghrelin acts on neuropeptide Y (NPY) and Agouti gene-related protein (AGRP) neurons in the arcuate nucleus of the hypo-
thalamus (55). Ghrelin rises preprandially and drops postprandially within one hour of eating to instigate feeding (56-58). In addition, it affects energy partitioning, by utilising glucose and sparing adipose tissue, as evidenced by an increase in respiratory quotient (59). It also increases gastric acid secretion and motility. Active ghrelin has an octanoic acid group on the third residue, essential for binding to the GHS-R (60-62) (Fig.2).

The control of ghrelin secretion is via the vagus nerve and endocrine paths. Ghrelin receptors are present on the vagus nerve and are axonally transported to the periphery (63-64). Vagotomy results in less food intake but meal oscillations in ghrelin levels still occurs in rats (65). In humans, truncal vagotomy increases ghrelin levels indicating an inhibitory tone on its secretion (66-67). Furthermore, exogenous ghrelin did not affect appetite and intake (68).

Ghrelin is suppressed in states of positive energy balance such as obesity (57, 69) and this may be a counter-regulatory mechanism to limit the development of further adiposity, such as that which occurred in rats consuming a high calorie diet (70). Interestingly, ghrelin knockout mice exhibit normal food intake and weight (71). Ghrelin is strongly correlated with BMI (57, 72). It is upregulated in negative energy balance situations including low calorie diets, chronic exercise, cachexia and anorexia nervosa.

In terms of dietary macronutrients, ghrelin is decreased by all but mostly by carbohydrate (CHO), followed by protein and fat. A high protein meal caused lower ghrelin levels than at baseline, as well as the highest postprandial insulin level (73). Furthermore, frequent feeding is linked with high insulin and low ghrelin levels, whilst fasting and two meals (64 % CHO, 23 % fat, 13 % protein) in 8 hours showed an inverse relationship (74). The mechanisms by which feeding suppresses ghrelin (75-76) are not elucidated.

There are conflicting reports regarding an effect of insulin on ghrelin regulation. Parenteral administration of insulin and glucose did not suppress serum ghrelin, suggesting that the effect of food intake or oral glucose on serum ghrelin is unlikely mediated by changes of plasma insulin or glucose (77). An insulin infusion though into humans via an euglycemic clamp decreased plasma ghrelin until it returned to basal levels after infusion withdrawal (78).
Figure 2. Molecular structure of the hunger hormone ghrelin. Ghrelin has a unique post-translational modification on its serine residue, third from the N-terminal end. An octanoic acid group is present and necessary for its biological activity.

Furthermore, hyperinsulinemia was found to suppress ghrelin levels in hyper- or hypoglycaemic states (79). In patients with Type 1 diabetes, insulin is essential for meal-induced ghrelin suppression and basal insulin is sufficient for the postprandial ghrelin decline whilst the lack of postprandial ghrelin suppression may contribute to the hyperphagia experienced by patients prior to treatment (80). We reported low ghrelin levels prior to commencement of insulin therapy in newly diagnosed Type 1 diabetes patients. The ghrelin level rose as initial hyperglycemia and lipid disturbances resolved with ongoing insulin treatment (81). Exposure of human hepatocytes to ghrelin upregulated phosphorylation of insulin receptor substrate-1 and gluconeogenesis (82). Thus, ghrelin possibly modulates insulin action.

Following dietary weight loss, ghrelin levels augment (72,83). Surgical weight loss and ghrelin levels were first reported by Cummings and colleagues (72) in five post-RYGBP patients. Ghrelin levels were 72 % lower than in weight-matched obese controls, with no meal-related oscillations or diurnal secretory pattern. They hypothesised that the success of RYGBP results from gastric fundus disconnection and secondary hypogrelinaeemia, contributing to less appetite and caloric intake and leading to weight loss. Multiple studies analysing the effect of RYGBP on ghrelin levels have been
published but with conflicting results: hypoghrelinemia, no change or increased ghrelin concentration, indicating that surgical technique may vary and affect the vagus nerve and postoperative ghrelin secretion.

*Gastrin* is a hormone released by G cells in the gastric and duodenal mucosa to stimulate secretion of hydrochloric acid (HCl) from parietal cells. It binds to gastrin receptors on ECL cells which then release histamine and act on adjacent parietal cells. Direct binding of gastrin to the parietal cells is involved in parietal cell maturation and fundal growth. Gastrin also stimulates antral contractions against the pylorus and constricts the pyloric sphincter, slowing gastric emptying. Gastrin is released in response to multiple stimuli including stomach distension, vagal stimulation (mediated by Gastrin Releasing Peptide), amino acids and hypercalcemia. Gastrin is inhibited by hydrochloric acid, somatostatin, secretin, gastric inhibitory peptide, vasoactive inhibitory peptide, glucagon and calcitonin (84).

In a recent rat study, gastric bypass reduced body weight, stomach weight, oxyntic mucosal thickness, serum gastrin and activity of ECL cells. Gastrin given to these rats prevented mucosal atrophy, ECL cell inactivation and attenuated postoperative body weight reduction. Serum ghrelin and gastric ghrelin cells were not affected by surgery or gastrin so authors suggested that hypogastrinemia and impaired ECL cell function is linked with postoperative weight loss (85). Another report on rats showed a decrease in food intake, meal size and gastrin levels postgastrectomy but not after gastric bypass (86). After GB and 45 % loss of excess weight in 24 subjects, no changes in gastrin, ghrelin and PGI at 12 months occurred (87).

*PGI* is the inactive zymogen of the active enzyme pepsin. Chief cells in the gastric body and fundus release PGI in response to HCl secretion from parietal cells. Besides gastrin, the vagus nerve also triggers pepsinogen and HCl secretion upon food ingestion. HCl allows pepsinogen to unfold and cleave itself generating pepsin. Pepsin cleaves the 44 amino acids from pepsinogen to create more pepsin. There are few reports on weight loss and PGI levels. Data is limited regarding mucosal activity in the excluded stomach post-RYGBP. Several studies have measured PGI levels as a reflection of mucosal activity state (88-92).
Intestinal peptides: Peptide YY (PYY), glucagon like peptide-1 (GLP-1), pro-neurotensin (Pro-NT)

**PYY** has 36 amino acids and is a member of the neuropeptide Y (NPY) and pancreatic polypeptide family. It’s secretion from L-cells occurs in greatest concentrations in the terminal ileum, colon and rectum and is stimulated by intraluminal nutrients, mainly protein and fat, as well as neuronal and humoral factors (97). It is released into the circulation, within minutes of eating and in proportion to caloric load (98), as PYY$_{1-36}$ and PYY$_{3-36}$, the latter of which appears post-cleavage of PYY$_{1-36}$ by dipeptidyl peptidase IV (DPP-4) (99,100). Peripheral administration of PYY$_{3-36}$ into rodents results in a dose-dependent reduction in food intake (101-104). Intravenous infusion into humans decreased appetite and food intake for more than 24 hours. Moreover, energy intake by obese subjects during a buffet lunch was reduced by 30 % after intravenous infusion of PYY$_{3-36}$ (105). Chronic administration of PYY$_{3-36}$ inhibited food intake and reduced body weight gain in animals (102,106-107). In knockout mice, daily food intake, body weight, and body fat increased (108). PYY$_{3-36}$ did not decrease food intake in Y2 receptor-deficient mice (102). Moreover, Y2 receptor deletion in the hypothalamus lowered body weight and increased food intake in mice (109). It appears that PYY$_{3-36}$ inhibits food intake via the Y2 receptor. However, central administration into the cerebral ventricles of rodents stimulated food intake (110-112). This orexigenic action of PYY is lower in Y1 and Y5 receptor-deficient mice (113). Therefore these receptors play a vital role in this PYY function.

High-protein diets cause greatest satiation and PYY release in humans, and a reduced caloric intake in mice. In the longer-term, weight gain was reduced and PYY synthesis and secretion augmented (108). Besides macronutrients, bile acid, gastric acid, vasoactive intestinal polypeptide, and cholecystokinin also stimulate PYY release (114-116). More specifically, intraduodenal liquid chyme given to rats induced the release of PYY from the distal intestine before the food content reaches the ileum, suggesting that PYY release occurs via a neural reflex, probably mediated by the vagus nerve (117-119). Infusion into humans caused delayed gastric emptying, slower transit time (120) and inhibition of gastric acid and pepsin secretion (121).

There are divergent results in the literature regarding fasting PYY concentrations in obesity. Lower basal concentrations in obese compared to lean adults and children have been reported (105,122), as have similar concentrations in anorexic, obese and gastric bypass subjects (123-126). Collectively, no consistent evidence supports a relationship between PYY and BMI.
GLP-1 is a 29 amino acid peptide derived from the proglucagon gene and co-localised with PYY in ileal and colonic mucosa (127). Like PYY, it is stimulated by nutrients including fatty acids and dietary fibre and it lowers food intake in rats (128-129) and humans, including lean and overweight persons with Type 2 diabetes (130-133). Levels rise postprandially within minutes pointing to neural and endocrine mediation of its secretion. Vagotomy decreases its fat-stimulated increase (134). Its short half-life results from rapid degradation by the enzyme dipeptidyl peptidase (DPP IV).

One study assessed appetite and found a tendency for reduced hunger and prospective consumption, as well as increased satiety during infusion of GLP-1 in type 2 diabetic patients (135). A meta-analysis indicated that an intravenous GLP-1 infusion reduced energy intake in lean and overweight humans but only explained 20 % of this change. Also gastric emptying was slowed by GLP-1 (136).

GLP-1 receptors are ubiquitous as they are present on many cell types including pancreatic β-cells, hepatocytes, adipocytes, hypothalamus and myocytes. Receptor knockout studies indicate that mice are lean with normal intake, even after months of a high fat diet (137-138). When combined with a leptin gene knockout, mice do not eat more or gain weight compared with ob/ob mice (139). Moreover, transgenic mice producing exendin-4, a GLP-1 agonist with 50 % lizard and mammal homology, eat and grow normally despite months of continuous expression (140).

In obesity, GLP-1 levels are lower (141-142), higher (143) or similar to normal weight individuals (144). Furthermore, the postprandial GLP-1 response is lower in obese subjects (141). Dietary weight loss decreases GLP-1 levels (145-146), in combination with aerobic exercise (147), perhaps resulting from a state of negative energy balance. However, an increase and a postprandial response closer to that in lean subjects was observed in obese subjects after 15 % total weight loss following a 24 week intervention, possibly reflecting more normal GLP-1 secretion with a more normal weight (142). Interestingly, a gastric pacer implanted into morbidly obese subjects lowered weight and basal GLP-1 levels 6 months after its activation (148).

Pro-NT, the most recently studied satiety factor is a precursor for the mature but unstable neurotensin (NT), first cloned from canine intestine and bovine brain (149). The 170 amino acid pro-NT molecule is secreted from N-cells in the jejunum, ileum, duodenum, colon and the brain. Pro-NT/NT is released after food intake, especially fat, and regulates gastrointestinal motility and pancreatic and biliary secretion (150). In an earlier human study, NT levels in eight morbidly obese patients were undetectable during an oral glucose
tolerance test (OGTT) but three months post-gastrojejunostomy, 7/8 patients showed a significant release of NT during the OGTT (151).

Figure 3. Interactions between body organs, their secreted peptides and the brain. (Modified from Hanusch-Enserer 2005 Eur J Clin Invest).
Pancreatic peptides: insulin, pancreatic polypeptide (PP)

**Insulin** is produced in the β-cells of the islets of Langerhans in the endocrine glands of the pancreas. It is a vital growth hormone for human metabolism, especially glucose usage, and the blood glucose level is the main stimulus for insulin secretion. Insulin binds to a specific tyrosine protein kinase receptor and any mutation in this or the development of hyperinsulinemia may cause of downregulation of receptors, resulting in insulin resistance. In contrast, hypoinsulinaemia results in Type 1 diabetes, characterised by weight loss, ketosis, hyperglycemia, polydipsia, polyuria and if untreated, coma. When exhaustion of the β-cells occurs with failure, secondary to hyperinsulinemia, Type 2 diabetes evolves (152-153). A BMI >30 is the strongest predictor of hyperinsulinaemia, which will develop into Type 2 diabetes, if untreated (154).

Conventional weight reduction methods that induce 10 % weight loss lower insulin concentrations in obese people (155). Fasting decreased insulin levels in obese, normal weight and GH-deficient subjects (156). Lower fasting insulin levels were seen early (10-14 days) and late (14-22 months) after VBG (157). Similarly, insulin, glucose and leptin levels declined 3, 6 and 12 months after RYGBP (158).

**PP** is secreted by F-cells of the pancreatic islets of Langerhans, although levels are detectable throughout the gastrointestinal tract. PP is released into plasma during feeding, especially by protein and fat (159-160). It acts on Y4 and Y5 receptors in the brain and peripherally to inhibit exocrine secretion, biliary function, gastric acid secretion, gut motility and to augment insulin’s inhibition of hepatic glucose production. Chronic peripheral administration reduces food intake in lean and obese mice (161), whereas central administration augments it. Also seen with PYY, this disparity may be due to differential stimulation of Y4 receptors in the area postrema, which lower food intake, and Y5 receptors elsewhere that increase food intake. In humans, PP reduces appetite and food intake without affecting gastric emptying (162).

PP release is under cholinergic control as evidenced by a blunted response to a meal during atropine blockade (159) or vagotomy (163). Increases in PP concentrations have been used as a measure of vagal efferent function (cephalic and gastric phases) during sham feeding experiments (164-165). The cephalic phase is the central nervous system-driven anticipatory increase in gastric and pancreatic activity due to sensory stimulation by food. The gastric phase results from food distending the stomach (166). The cephalic phase can be measured using sham feeding, whereby a person thinks about,
smells and chews a meal but does not actually swallow it. PP levels rise after a meal from both phases, via the vagus nerve (164-167).

Only one study has shown that PP levels are low in obesity and increase after dietary weight loss (168). The few small surgical studies performed have found decreased (169-170), unchanged (87) or similar levels in GB and RYGBP groups postprandially (171).

Adipose tissue peptides: leptin, adiponectin

**Leptin**, an adipocytokine, has been investigated extensively since its discovery in 1994. The production of this 167 amino acid protein is regulated by the *ob* gene (172). Mutations in this gene or the leptin receptor *db* gene have been found in only a few obese people. Most obese individuals exhibit increased leptin expression indicating such genetic mutations are rare (173). Leptin has now been shown to regulate weight and thermogenesis in animals and humans (174). The circulating leptin level is directly proportional to the total amount of body fat (174-175), such that it is thought to act as a peripheral satiety factor, signalling the brain about the current state of energy stores. Subcutaneous fat is more closely related to serum leptin levels than visceral fat as may be explained by a significantly higher amount of leptin messenger RNA expression here in obese humans (173). An increased leptin level in obesity may indicate a form of leptin resistance (176). Administration into obese diabetic rodents, with reduced or absent leptin production, ameliorates impaired glucose tolerance by increasing energy expenditure, fat depletion and weight loss (177).

**Adiponectin** is a complex protein which circulates in high levels and appears to have multiple functions. It is suppressed in humans with lipodystrophy (178) and obesity (179-180), and it correlates inversely with insulin (179,181). The influence of BMI or body fat on adiponectin levels is unclear but it is involved in insulin signalling as it lowers insulin resistance by inhibiting gluconeogenesis (180), lowering TNF-α production (182), and increasing fatty acid oxidation (181). In *in vivo* insulin resistant models, adiponectin improves insulin sensitivity (182-183).

Multiple studies on adiponectin and leptin following weight loss from caloric restriction, medication use or bariatric surgery show varying results. Nine months after a weight-reduction program, leptin levels were lowered and adiponectin augmented, both of which moved towards initial levels after four months at home (184). Only leptin decreased after diet, exercise or a
combined program in postmenopausal women with Type 2 diabetes (185). Similarly, after a 4-6 week very low calorie diet, only leptin levels improved (186). Interestingly, a low calorie diet in healthy women lowered both leptin and adiponectin levels (64), the latter of which was unexpected and may reflect a change in insulin sensitivity.

Lipase inhibiting medication orlistat, affected both leptin and adiponectin levels positively, independent of changes in body fat and waist circumference (187). Furthermore, weight loss >5 % achieved with sibutramine was associated with a rise in adiponectin (188). RYGBP surgery improved adiponectin and leptin levels (191), as did an intragastric balloon and GB (190).

Inflammatory peptides: C-reactive protein (CRP), serum amyloid A (SAA), interleukin-6 (IL-6)

**CRP** has been well studied in the obese state, now referred to as a chronic low-grade inflammatory condition. Obesity is also associated with immune dysfunction (191-192) and elevated levels of proinflammatory cytokines such as IL-6 and tumor necrosis factor-α (TNF-α), which stimulate hepatocytes to produce acute phase reaction proteins including CRP (193). This state is reversible, with a decrease in markers after weight loss and exercise (194-196). Cytokines are not only produced by inflammatory cells in damaged tissues but are also secreted from adipocytes, making obesity a confounding factor in the association between acute phase proteins and cardiovascular disease (196-197). There are diverging reports regarding the importance of obesity *per se* and insulin resistance for driving the increase in inflammatory markers. In a study of CRP and IL-6 in women with polycystic ovary syndrome, obesity and not insulin resistance was found to be the major determinant of the inflammatory markers (198). In contrast, in a study of obese women in which CRP was measured before and after three months of caloric restriction, CRP concentrations and insulin resistance correlated, independent of obesity (199).

Weight loss programs for obesity lower CRP levels (195, 200-203). Medical treatment with sibutramine or orlistat for 6 months achieved a reduction in CRP after 2-5 % weight loss (204). A cardiac rehabilitation program resulted in a reduction in cardiovascular risk factors and CRP levels regardless of statin therapy or weight lost (205). Moreover, after 18 months of lifestyle intervention in overweight or obese subjects above 60 years, decreases in
CRP and IL-6 were observed (206). Likewise, surgically induced weight loss through GB lowered CRP to within the normal range (192, 207).

**SAA** is another acute-phase protein, where levels are elevated in obesity (198,208-209). It correlates directly with body fat and high mRNA expression of SAA in omental adipose tissue occurs (198, 210-211). Levels are higher in people with Type 2 diabetes and impaired glucose tolerance than in non-diabetic controls (212-213). Male subjects with coronary artery disease have markedly higher SAA levels than those without it (216) and SAA may interact with HDL-cholesterol to be involved in the accelerated atherosclerosis of diabetes (212). The presence of SAA in artery plaques supports a link between inflammation, lipoprotein metabolism and atherosclerosis (215).

Caloric restriction (210-211) during a 3 month low fat or low carbohydrate diet was associated with decreases in both SAA and CRP, an effect proportional to weight lost but independent of macronutrient composition (216). Similarly, 6 weeks of energy restriction resulted in weight loss associated with lower CRP, SAA and IL-6 levels. Adipose expression of IL-6 and adiponectin increased after weight loss. During weight maintenance, inflammatory markers increased and some returned to baseline (209).

After RYGBP, weight loss significantly decreased SAA concentrations (208). Furthermore, a reduction 3 months after gastric banding/bypass surgery was seen and remained stable beyond 6 months. mRNA expression of SAA isoforms (SAA 1, 2) in subcutaneous fat was higher than in visceral fat and liver and correlated significantly with BMI, SAA and CRP. SAA can be considered a marker of adiposity-induced low-grade inflammation but not of the metabolic status of obese subjects (217).

**IL-6** is secreted from macrophages, monocytes, endothelium and adipocytes, from which ~30 % of the serum concentration is derived (197). IL-6 stimulates liver production of acute phase proteins as well as having other pro- and anti-inflammatory roles. Levels increase with abdominal adiposity (197,209,218) because omental fat cells secrete 2-3 times more IL-6 than subcutaneous adipocytes (218). In patients with Type 2 diabetes, IL-6 levels are higher than in age-matched controls (212) and they correlate directly with percentage body fat and negatively with insulin action but not after adjusting for body fat (219). In a study of aboriginal men and women IL-6 was an independent predictor of CRP levels (220).

Caloric restriction studies lower IL-6 levels (221). A large randomized controlled trial for 18 months (222), as well a shorter study including exercise (223), showed reduced levels. Another study wherein women lost 11 % of their weight, demonstrated that genes related to insulin and IL-6 were down
regulated and that changes in skeletal muscle gene expression shifted towards oxidative metabolism (224). Surgical weight loss also appears to decrease IL-6 levels, along with other inflammatory and metabolic markers.
Aims of the investigation

To examine the adipose tissue regulatory peptides ghrelin, adiponectin, leptin and insulin, as well as glucose and lipids, and the interplay among them basally and 6 and 12 months after RYGBP in morbidly obese subjects.

To explore interactions between obesity, insulin resistance and inflammatory markers; CRP, SAA and IL-6 prior to and 6 and 12 months postoperatively.

To investigate early postoperative changes in gut peptides ghrelin, gastrin, pepsinogen I and pancreatic polypeptide after RYGBP surgery, and also at 1, 6 and 12 months.

To observe changes in satiety peptides in young and middle-aged lean, obese and surgically operated subjects during an extended fast, and to determine the influence of a meal on these peptide hormones.
Subjects

Paper I and II
Subjects were recruited from the obesity register at Samariterhemmet Hospital, Uppsala. Sixty-six morbidly obese patients planned for RYGBP: 12 men and 54 women with mean age 39 years (range 24-50), mean weight 127 kg (range 96-195) and mean BMI of 45 kg/m² (range 33-64) were included. Previous attempts at weight loss were made, as eight had prior surgery: three adjustable-GB and five VBG. Two patients had Type 2 diabetes; one received metformin, the other glipizide and insulin. All patients gave written informed consent.

Patients underwent RYGBP at the Department of Surgery, University Hospital, Uppsala. A small proximal gastric pouch (15 ml) was created along the lesser curvature and totally separated from the main stomach by cutting linear staplers. The small bowel was divided 20 cm distal to the ligament of Treitz and a Roux-limb, at least 50 cm long, made. The Roux-limb was placed retrocolic, retrogastric and anastomosed to the small gastric pouch directly below the oesophagus. The small bowel continuity was maintained by a linearly stapled side-to-side entero-enterostomy (61). Preoperatively, and 6 and 12 months postoperatively, morning fasting blood samples were drawn and body height and weight measured to determine BMI. Sera were prepared and stored at -70°C until analysis.

Paper III
Fifteen patients (median age 43 (27-54) years and BMI 45 (36-63) kg/m², 14 women), planned for RYGBP (outlined in Paper I) as their first bariatric procedure were included. Blood samples for ghrelin, PP, PGI and gastrin were taken 2 weeks before RYGBP and on postoperative days 1, 2, 4, and 6. Also at 1, 6 and 12 months after the procedure, samples were taken at planned outpatient visits in the morning after an overnight fast.

Paper IV
Forty subjects were recruited for the study and divided into four groups each comprising 5 men and 5 women: 10 morbidly obese (mean age 41 ± 7 years; mean BMI 44 ± 3 kg/m²), 10 RYGBP-operated (age 45 ± 5 years; BMI 35 ± 6 kg/m²), 10 lean age-matched (age 44 ± 5 years; BMI 24 ± 3 kg/m²) and 10 young lean subjects (age 26 ± 2 years; BMI 23 ± 2 kg/m²). The RYGBP procedure was performed a mean of 43 months (range 25-53) prior to this study. Subjects presented to the Outpatient clinic for Obesity Care after an
overnight fast and continued fasting until a standardized meal was served at 13:00. The meal consisted of 574 kcal (21% fat, 18% protein and 61% carbohydrates). Blood samples were drawn at 08:00, 10:00, 12:00, 13:00, 13:30, 14:00, 14:30, 15:00 and 16:00.

Methods

**Paper I**
Human serum ghrelin was measured using a commercial radioimmunoassay (Phoenix Pharmaceuticals, Inc., Belmont, CA). It employs $^{125}$I-labelled bioactive ghrelin as a tracer and a polyclonal antibody from rabbits against the full-length, octanoylated human ghrelin. The intra- and inter-assay coefficients of variance (CV) were 5.3% and 13.6%, respectively (62). The ghrelin values from all three serum samples from each individual were analyzed in the same assay run. Adiponectin serum levels were measured using the Human Adiponectin RIA (Linco Research, Inc. Missouri, USA) which utilises $^{125}$I-labelled murine adiponectin and a multispecies adiponectin rabbit antiserum. The assay’s sensitivity limit was 1 ng/ml and the intra- and inter-assay CV 6.1% and 9.3%, respectively. Leptin was tested with a RIA kit (Linco Research, Inc. Missouri, USA) that employed a rabbit antiserum against human leptin and $^{125}$I-labelled human leptin. The assay’s sensitivity limit was 0.5 ng/ml and intra- and inter-assay CVs 6.2% and 8.3%, respectively. Fasting plasma glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides (TG) and free fatty acids (FFA) were measured with routine techniques and insulin with an AutoDELFIA immunoassay (Wallac Oy, Turku, Finland).

**Paper II**
CRP was analysed in serum with latex enhanced reagent (N Latex CRP, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec immunonephelometry. Monoclonal antibodies to CRP coat polystyrene particles which agglutinate when mixed with human serum samples containing CRP. The CRP concentration in the sample is reflected by the intensity of scattered light in the nephelometer. The intra-assay CV of the CRP method was 1.4% at both 1.23 mg/L and 5.49 mg/L. The limit of detection was 0.16 mg/L. SAA levels were determined in serum with a latex enhanced reagent (N Latex SAA, Dade Behring, Deerfield, IL, USA) and a Behring BN ProSpec analyser. The limit of detection was 0.74 mg/L. Intra-assay CV of the SAA method was 5.9% at 12.8 mg/L and 3.2% at 81.7 mg/L. Serum IL-
6 levels were analysed with the Quantikine HS IL-6 ELISA kit (HS600B, R & D Systems, Minneapolis, MN, USA). IL-6 from the standards and samples bind to the specific, immobilised monoclonal antibodies pre-coated on microtitre plates during the first incubation. Enzyme-linked polyclonal antibodies are then added prior to the second incubation, after which substrate and amplifier solutions are used to achieve colour development as measured by photospectrometry. The lower detection limit was 0.15 ng/L and CVs intra-7 % and interassay-5 %.

**Paper III**

Total serum ghrelin was measured using a commercial RIA (Linco Research, St. Charles, MO, USA) which utilizes $^{125}$I-labelled ghrelin as a tracer, polyclonal antibody raised in rabbits against full-length octanoylated human ghrelin and the double antibody/PEG technique. Kit sensitivity is 93 pg/ml and intra- and inter-assay CVs <10 % and 18 %, respectively. PP was assayed in serum with a competitive radioimmunoassay (Euro-Diagnostica AB, Malmö, Sweden) using rabbit antiserum, $^{125}$I-labelled human, PP and the double antibody-polyethylene glycol precipitation technique. The lowest detectable concentration is 3 pmol/L and CVs <3.5 %. PGI was measured in serum using an ELISA kit (Biohit Plc, Helsinki, Finland), with a monoclonal antibody, specific to human PGI, adsorbed on a microplate which binds PGI in the sample. An HRP-conjugated monoclonal detection antibody is added to the walls and binds PGI molecules. Intra- and inter-assay coefficients of variance were <6 % and kit detection limit 0.6 µg/L. Gastrin in serum was assayed by a competitive radioimmunoassay (Euro-Diagnostica AB, Malmö, Sweden), that employs rabbit antiserum and $^{125}$I-labelled gastrin which is separated from the unbound fraction by the double antibody-polyethylene glycol precipitation technique, with measurement of radioactivity. Lowest limit of detection is 5 pmol/L and CVs <8 %.

**Paper IV**

A commercial RIA kit (Linco Research Inc. Missouri USA) measured serum concentrations of total PYY, using radioactive iodine labeled PYY, guinea pig PYY antiserum and goat anti-guinea pig antibody/PEG technique. The assay’s sensitivity was 10 pg/ml and specificity 100 % for human PYY 1-36, PYY 3-36, [Pro34] PYY and [Leu31, Pro34] PYY and <0.1 % for rat/porcine PYY 1-36, PYY 3-36, and NPY. Inter- and intra-assay CVs were <9 %. Serum GLP-1 was determined with an ELISA kit (Linco Research Inc. Missouri USA). The kit only detects active GLP-1 forms including GLP-1_{7-36} and GLP-1_{7-37}. The detection limit was 2 pM, specificity 100 % for human GLP-1 and GLP-1_{7-37} while <0.1 % for human GLP-1_{7-36}. CVs
<13 %. PP was assayed in serum with a RIA kit as in Paper III. Pro-NT was measured in serum by Ernst et al. (Peptides 2006) at SphingoTec GmbH, Borgsdorf, Germany, using a recent chemiluminometric sandwich immunoassay to detect a proneurotensin precursor fragment (proneurotensin 1-117). Sensitivity of the assay was 10 pmol/L, CV <20 % for inter- and <10 % for intra-assay. Measurements of pro-NT had to be limited to three serum samples from each individual in the age-matched groups; drawn at 13:00 prior to the test meal and 30 and 90 minutes after the meal. The time points chosen were based on observations from prior assays of PYY, GLP-1 and PP.

Statistics (Papers I, II, III and IV)

Data were given as means and standards deviations (SD) and skewed data as geometric mean and standard error mean (SEM). A statistic computer package (Statview 5 for Windows, SAS Institute Inc., USA) was employed to carry out ANOVA’s for all variables, simple regression analyses to determine univariate relationships and forward stepwise regression analyses to explore interrelations between variables. Factorial ANOVA and ANCOVA were used to evaluate differences between groups, while paired t-test was used to calculate effects of surgery. The level of significance was taken at p<0.05. A subgroup comparison was made, using the unpaired t-test, for BMI-matched surgical and non-surgical patients to assess any difference in ghrelin level due surgery. To illustrate the relationship between insulin resistance and changes in CRP, the material was divided into three groups, HOMA <4, HOMA 4-9 and HOMA >9. The homeostatic model of assessment index for insulin resistance (HOMA) is determined by the equation: fasting plasma glucose (mmol/L) x fasting serum insulin (mU/L)/22.5. Calculated from a study of healthy individuals given an euglycemic hyperinsulinemic clamp, a mean value of insulin-mediated glucose uptake of 4.2 mg/kg/min (mean – 2SD) or above was considered normal insulin sensitivity and corresponded to HOMA <4, while HOMA >9 identified individuals with pronounced insulin resistance (<2 mg/kg/min at clamp).
Results and Discussion

**Paper I**

Preoperatively, men weighed 145 kg (range 107-195) and women 123 kg (range 96-190). RYGBP resulted in marked changes at 6 and 12 months in body weight, BMI and circulating levels of total cholesterol, LDL-cholesterol, triglycerides, glucose, ghrelin, insulin, adiponectin and leptin. By one year, HDL-cholesterol increased and FFA decreased.

Basally, BMI correlated inversely with ghrelin, and positively with insulin and leptin. The changes in ghrelin, insulin, adiponectin and leptin all correlated with the change in BMI 12 months after RYGBP (Fig. 4). Ghrelin and insulin correlated negatively and consistently (Fig. 5). Adiponectin and BMI changes correlated inversely at 12 months. Changes in insulin and adiponectin related to that in leptin at 12 months, indicating appearance of this relationship with weight loss. The change in insulin predicted that in ghrelin at 12 months, independent of BMI. Finally, there was no effect of RYGBP surgery *per se* on circulating ghrelin levels.

We found that RYGBP decreased body weight, metabolic markers, insulin and leptin, whilst it increased ghrelin and adiponectin. Changes in ghrelin and BMI correlated, supporting earlier reports on ghrelin following dietary weight loss (72,226-229). Moreover, ghrelin levels are reported to be low in obesity (57,69) and high in anorexia nervosa (69,228). Ghrelin and insulin are probably physiologically linked as evidenced by low ghrelin and high insulin levels following feeding (56) and reduced ghrelin concentrations during an insulin infusion in humans (78) under hypo- and hyperglycaemic states (79). Moreover, parenteral ghrelin reduced insulin levels within 30 minutes (229), indicating insulin’s indirect effect on ghrelin-secreting cells.

The first report of five gastric bypass surgery patients detected lower ghrelin levels than in BMI-matched subjects who completed a 6 month dietary weight loss program (72). The authors hypothesised that the hypoghrelineemia resulted from disconnection of the stomach with a subsequent lowering of appetite and weight loss. Multiple studies have found similar results (230-234) whilst we and others (189,235) report a postoperative rise in ghrelin, associated with marked weight loss.
A recent study by Garcia-Fuentes et al. also observed a rise in ghrelin after RYGBP but not after BPD surgery, probably because this group lost 5% less weight than RYGBP subjects. They concluded that the type of surgery was the only variable that could explain variance in ghrelin levels (238). In support of prior studies, serum ghrelin augmented after dietary and surgical weight loss with VBG, but declined 18 months after BPD-duodenal switch, the procedure with the greatest weight loss (43%) (239). In contrast, Stratis et al. (240) saw a 40% increase 12 months post-BPD-RYGBP. Others reported similar ghrelin levels in operated and BMI-matched subjects (241) and pre- and post-RYGBP in a randomised study (242). Limitations of the performed studies include no preoperative ghrelin levels, comparison only with obese-matched controls, small subject samples and the fact that most subjects remain obese despite impressive weight loss. Importantly, Faraj et al. only observed increased ghrelin levels in subjects actively losing weight and not in weight-stable patients, defined as <10% weight loss in last 6 months (237). We propose that ghrelin levels in RYGBP patients continue to be primarily produced by the disconnected stomach and that this ghrelin secretion is unaltered.
Adiponectin concentrations are low in obesity, Type 2 diabetes patients and in lipodystrophy, indicating levels are not directly related to adipose tissue mass but rather to an insulin resistant state. However, an inverse relationship between adiponectin with BMI has been observed in animals and humans (243-244) and we confirmed this as changes in adiponectin and BMI related inversely at 12 months. Also, changes in adiponectin and insulin were indirectly correlated 1 year post-RYGBP, independent of BMI. Adiponectin improves plasma glucose levels by suppressing hepatic glucose production (206,219) and increasing muscle FA oxidation (218-218). Low plasma adiponectin levels may precede a reduction in insulin sensitivity by lowering phosphorylation of skeletal muscle insulin receptor (245). Very recently, high adiponectin levels were associated with a metabolically healthy obese phenotype (246). In mice, administration of adiponectin reverses insulin resistance in models of obesity and lipoatrophy (183). Last year, it was demonstrated that adiponectin levels declined with increasing doses of insulin during a clamp study in diabetics but not non-diabetic subjects (243).
Leptin correlated consistently with BMI, with the reduction in leptin partly explained by the decrease in body mass as leptin is a marker of fat mass. An in vivo and in vitro study suggested that insulin regulates ob/ob gene expression and leptin production through trophic effects on adipocytes (180). Leptin and insulin correlate directly in cohorts including biliopancreatic diversion patients, overweight controls (247), and those with Type 2 diabetes (179). Recently in 19 women 12 months after RYGBP, the change in fat mass correlated with changes in leptin and adiponectin, with these, age and initial BMI explaining 71% of the change in fat mass. The change in weight was accounted for 64% by leptin and adiponectin (248).

**Paper II**

The measured inflammatory markers were elevated in this morbidly obese cohort and decreased postoperatively. CRP declined 57% and 82%, followed by SAA by 43% and 57% and IL-6 30% and 50% at 6 and 12 months, respectively. CRP correlated directly with BMI and HOMA index preoperatively. CRP reduction correlated inversely with basal HOMA index, independent of weight change and basal CRP levels. Subjects were grouped according to HOMA indices (< 4, 4-9, and > 9) and the largest relative CRP reduction occurred in the insulin-sensitive group (HOMA < 4), despite similar BMI changes in all groups (Fig. 6).

The reduction in CRP following surgery, but not the changes in body weight, fatty acids or triglycerides, were related to HOMA index. Therefore, CRP is regulated with greatest flexibility in insulin sensitive subjects, who may gain more from surgery than their resistant counterparts, in terms of reducing inflammatory marker levels. In accordance with several studies, we found close associations between CRP, SAA and IL-6. Following RYGBP, changes in SAA and IL-6 paralleled those in CRP, but were of lesser magnitude. The importance of insulin sensitivity rather than body weight is supported by observations of CRP changes in patients receiving various treatments with a stable body weight. Metformin for polycystic ovary syndrome (249) and lipid-lowering statin regimens (216, 221) lowered CRP without changing body weight, while oestrogen supplementation for postmenopausal women increased CRP with no effect on body weight (250). Peroxisome proliferator-activated receptor-agonists, such as rosiglitazone, lowered CRP, improved insulin sensitivity and increased body weight (251).

There are various reports that support our findings. An impressive drop in weight 3 and 6 months after RYGBP was seen in 20 subjects, as well as a decline in CRP at 3 but not 6 months (252). In 65 subjects before and after RYGBP, massive weight loss occurred along with a decrease in CRP and augmentation in adiponectin (253). Furthermore, in a study of 10 adults, half
with Type 2 diabetes, it was observed that 6 months after RGYBP the entire group had similar weight loss. CRP dropped in Type 2 diabetes patients only. Also, BMI, CRP and IL-6 were lower postoperatively in 13 subjects, with a correlation between changes in BMI and IL-6 found (254). These improvements in BMI and inflammatory markers were associated with better quality of life and reduced eating disorders and depressive symptoms. More recently, 12 months after RYGBP and GB have been shown to have same effect by reducing CRP by 70 % in 640 patients. Before surgery, CRP correlated directly with BMI. Fasting plasma glucose and haemoglobin predicted CRP (255). In another study of GB patients, CRP and HOMA were reduced at 6 months, with a correlation between HOMA and visceral adipose tissue detected (256). A similar study found a decrease in IL-6, which correlated with waist hip circumference, insulin and HOMA at 12 months. The variation in percentage change in HOMA was explained 59 % by age, changes in adiponectin, NEFA and IL-6 (248).

[Figure 6. The percentage changes in CRP (left) and in BMI (right) 12 months after RYGBP in groups with different degrees of insulin sensitivity (HOMA).]

**Paper III**

BMI decreased to 40, 33 and 30 kg/m² at 1, 6 and 12 months, respectively. Serum ghrelin dropped by 43 % 24 hours after surgery and remained so until the end of week 1, before reaching the preoperative level at one month and continuing to increase beyond this by 25 % at 12 months (Fig. 7). PP level declined from 27 to 16 pmol/L on day 1 after surgery and on day 2, serum PP was similar to the preoperative level. Before RYGBP, PGI was 96 and 45 ug/L at 1 month, where it remained throughout the study. Gastrin concentrations decreased from 36 to 28 and 24 pmol/L at 1 and 12 months, respectively after RYGBP.
We found markedly reduced ghrelin concentrations immediately after RYGBP and then a return to the preoperative level 1 month after surgery, prior to increasing further at 12 months. This reduction in ghrelin after surgery has been confirmed by numerous other studies but only few teams have observed a postoperative rise, including our earlier report, in which 60 of 66 subjects had a higher ghrelin level 12 months after RYGBP (257). PP levels also declined postoperatively and increased from day 2 onwards, and thereafter remained at preoperative levels. Similarly, Swarbrick et al. (248) de-
ected lower levels 1 and 12m after RYGBP but not at 3 and 6 months. Also, in other studies PP decreased postoperatively (258-259). The acute decreases in ghrelin and PP levels potentially indicate temporary vagal nerve damage. Since different proportions of the gastric fundus are disconnected depending on surgical technique with varying impact on the vagus nerve structure and function, we suggest this may explain published differences in postoperative ghrelin levels, in addition to Roux limb length and adaptive responses to body weight homeostasis. Lin et al. (232) also observed an initial drop in ghrelin level during RYGBP, occurring when the stomach was divided to create a pouch, pointing to damage of the fundus’ vagal supply.

Recent studies by Perathoner et al. (260) found no difference in ghrelin or gastrin level 3 years after RYGBP, where subjects were separated into pouch and no pouch groups. In a later study, again, there were no differences in ghrelin and gastrin levels between groups with vagus nerve preservation or dissection at 3 years (261). The authors suggested that ghrelin levels remain unaffected by vagotomy if the gastric fundus is excluded, whereby the new pouch should be formed by simple dissection of perigastric fat which interferes with the anterior vagal trunk without affecting overall vagal function. The existence of vagal collaterals may also explain normal vagal function after potential surgical damage.

Markers of gastric mucosal activity include PGI and gastrin, the levels of which were low 1 month postoperatively suggest a resting mucosa. PGI rose acutely postoperatively probably due to surgical trauma and then declined further. Previously, we showed a reduction in PGI 1 year after RYGBP (90) and since gastrin secretion is from the antrum upon food stimulation, the continuous decrease in gastrin secretion is most likely from lack of nutrients stimulating the excluded stomach. Gastrin levels are similar in surgeries where the gastrointestinal tract is maintained, such as in VBG and GB (262). The low gastrin stimulation after RYGBP leads to reduced production of hydrochloric acid. However, patients with low basal acid output 7 years after RYGBP produced an almost normal maximal acid output after administration of iv pentagastrin (263), illustrating the gastric mucosa is simply resting.

**Paper IV**

Preprandially, PYY concentrations continuously declined in obese and lean subjects. The extended fast resulted in higher PYY concentrations in the RYGBP than in other groups. An exaggerated postprandial rise in PYY concentrations occurred in RYGBP subjects. Preprandial GLP-1 concentrations were similar across the groups. After the meal, levels were markedly elevated in the RYGBP group, with a small rise also in older lean subjects. PP
levels were similar in all groups after the extended fast and postprandially, with concentrations increasing to a lesser extent in the RYGBP group. Preprandially, RYGBP subjects had 4-6-fold higher pro-NT concentrations than obese and lean subjects. Pro-NT concentrations were not affected by food intake in RYGBP patients but rose in lean and obese subjects.

There are multiple reports that have studied RYGBP subjects and found similar postprandial PYY and GLP-1 hyperresponses to those found in our study, such as after an oral glucose tolerance test (264), an oral glucose load (265) and a liquid meal (241). All groups in our study responded with an increase in PYY concentrations to a standardised solid meal (574 kcal). The most pronounced, 3-fold greater, response was seen in the RYGBP group. Their larger responses in RYGBP patients could be explained by the rapid passage of nutrients into the jejunum and ileum, stimulating nervous and endocrine signals, resulting in PYY secretion, most pronounced in subjects given liquid meals, possibly reflecting the most rapid absorption. The Rodeigue study (265) had the lowest response, possibly due to the relatively low energy content in the glucose load.

PYY concentrations and measures of satiety have been investigated in few surgical studies. Six weeks after RYGBP, postprandial PYY and GLP-1 areas under the curve (AUC) were higher, than in the BMI-matched group, and were accompanied by a decrease in both fasting and postprandial hunger, and increased satiety (266). Only four weeks postoperatively, the post-meal AUC for PYY and GLP-1 was elevated and increased further at 6 months, along with a rise in satiety scores at 1 month, also maintained for 6 months. The lack of an augmented appetite and food intake after RYGBP may occur as a counter-regulatory response, possibly explained by gut adaptation and concurrent rises in PYY and GLP-1 concentrations resulting in earlier satiety (267). Recently, a report of 17 subjects who had sleeve gastrectomy, omentectomy and initial jejunectomy, demonstrated rises in PYY and GLP-1 postprandially as well as earlier satiety, no adverse effects and better quality of life 5 years after surgery (268).

PYY levels in obesity are conflicting. Obese humans appear to need double the meal calorie content of lean subjects to achieve similar PYY levels (269). In a group of anorexic, obese and lean females no basal differences were detected but a positive correlation between PYY concentration and satiety 15 minutes postprandially (liquid meal-360 kcal) was observed (123). In addition, PYY levels in lean and obese subjects were similar following high-protein, high-fat, and high-carbohydrate isocaloric meals (1088 kcal) (108). Hunger scores changed in parallel with PYY levels. In contrast, a high-protein (25 %) meal did not change PYY levels in subjects who achieved elevated satiety 30 and 120 minutes postprandially. Interestingly, isoener-
getic different fibre meals did not affect satiety but a decline in the PYY level 30 and 60 minutes after a wheat meal was detected (270). A recent study could not correlate PYY levels and satiety in 12 lean subjects who consumed a 417 kcal drink comprising 95 % long chain fatty acids was found. However, when the subjects were divided into high and low PYY responders, the high responders had a suppressed appetite 60-180 minutes postprandially (271). A clear picture has not been drawn with regard to PYY and its physiological role in satiety.

GLP-1 secretion in obesity is also not well understood. A very recent study of overweight male adolescents found lower levels in leans as well as a greater postprandial response achieved after acute exercise, whilst no correlation was seen with hunger or satiety (272). Dietary weight loss appears to result in a decline in GLP-1 levels (145-146) and an increase in postprandial secretion, more closely resembling the change in lean subjects (142). This study also found an increase in GLP-1 concentrations after 24 weeks of intervention and a 15 % decrease in body weight.

Surgical studies involving RYGBP all display a rise in GLP-1 basally and postprandially (241,263-267). These changes are related to decreased hunger or increased satiety. More recently, an increase in GLP-1 to accompany that in ghrelin 6 months after GB surgery was reported (255). BPD had a greater effect than VBG on basal levels and those during an oral glucose tolerance test 3 months after surgery (273). Collectively, surgical and in particular RYGBP studies, illustrate that GLP-1 levels rise postoperatively after a meal and this may be linked with the earlier satiety experienced by these patients and contribute to their impressive weight loss.

PP concentrations were similar among all subjects throughout the study and rose postprandially. This result was also observed in patients operated with GB or RYGBP (269). PP increased slightly less in the RYGBP group and although insignificant, this may point to a degree of vagal nerve dysfunction, more than 3 years after surgery.

Pro-NT preprandial concentrations were highest in RYGBP subjects. Postprandial rises occurred in lean and obese groups while operated subjects had no change. Lower pro-NT concentrations 3 and 6 months after GB compared with RYGBP were found, whereas at 24 months, concentrations increased further in RYGBP patients and no change was detectable in GB group (274).
Conclusions

Paper I

The marked weight loss and new state of energy balance achieved after RYGBP was reflected by increased ghrelin and decreased insulin secretion. RYGBP *per se* did not affect ghrelin levels. The decline in leptin and rise in adiponectin may contribute to long-term energy homeostasis.

Paper II

Weight loss induced by RYGBP markedly decreased levels of inflammatory markers. In obesity, factors inducing insulin resistance, rather than obesity *per se*, appear to elevate basal production of CRP. The CRP reduction after surgery was most pronounced in insulin sensitive subjects.

Paper III

After RYGBP, ghrelin declined rapidly, possibly due to temporary vagal dysfunction. This reduction was transient and as weight loss occurred, ghrelin levels augmented to higher than those preoperatively, indicating that the weight loss effect is not dependent on disturbed ghrelin signalling.

Paper IV

RYGBP subjects displayed exaggerated postprandial PYY and GLP-1 responses, as well as higher pro-NT concentrations pre- and postprandially. The findings suggest that changes in these potential satiety peptides may contribute to the impressive weight loss and improvements in metabolic markers observed after RYGBP.
Future perspectives

Despite the discovery of most of the studied gut peptides decades ago, their role in appetite regulation and energy homeostasis is only starting to unravel. Clearly there are a multitude of peptides responsible for these functions, including those from adipose tissue but even these appear to have numerous functions such as the anti-atherogenic properties of adiponectin. With the obesity epidemic blindly leading us and healthy lifestyle interventions proving successful but difficult on a wide scale, we need to further explore the status quo of these peptides in lean and obese persons to truly illustrate the existence of any disturbed physiology that can be potentially restored with conventional, medical or surgical treatment.
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