On the Impact of Bariatric Surgery on Glucose Homeostasis

NICLAS ABRAHAMSSON
Obesity has grown to epidemic proportions, and in lack of efficient life-style and medical treatments, the bariatric surgeries are performed in rising numbers. The most common surgery is the Gastric Bypass (GBP) surgery, with the Biliopancreatic diversion with duodenal switch (DS) as an option for the most extreme cases with a BMI>50 kg/m².

In paper I 20 GBP-patients were examined during the first post-operative year regarding the natriuretic peptide, NT-ProBNP, which is secreted from the cardiac ventricles. Levels of NT-ProBNP quickly increased during the first post-surgery week, and later established itself on a higher level than pre-surgery.

In paper II we report of 5 patient-cases after GBP-surgery with severe problems with postprandial hypoglycaemia that were successfully treated with GLP-1-analogs. The effect of treatment could be observed both symptomatically and in some cases using continuous glucose measuring systems (CGMS).

In paper III three groups of subjects; 15 post-GBP patients, 15 post-DS, and 15 obese controls were examined for three days using CGMS during everyday life. The post-GBP group had high glucose variability as measured by MAGE and CONGA, whereas the post-DS group had low variability. Both post-operative groups exhibited significant time in hypoglycaemia, about 40 and 80 minutes per day <3.3mmol/l and 20 and 40 minutes < 2.8mmol/l, respectively, longer time for DS-group. Remarkably, only about 20% of these hypoglycaemic episodes were accompanied with symptoms.

In Paper IV the hypoglycaemia counter regulatory system was investigated; 12 patients were examined before and after GBP-surgery with a stepped hypoglycaemic hyperinsulinemic clamp. The results show a downregulation of symptoms, counter regulatory hormones (glucagon, cortisol, epinephrine, norepinephrine, growth hormone), incretin hormones (GLP-1 and GIP), and sympathetic nervous response.

In conclusion patients post bariatric surgery exhibit a downregulated counter regulatory response to hypoglycaemia, accompanied by frequent asymptomatic hypoglycaemic episodes in everyday life. Patients suffering from severe hypoglycaemic episodes can often be treated successfully with GLP-1-analogues.

Keywords: Hypoglycaemia, Gastric Bypass surgery, Biliopancreatic diversion with duodenal switch (DS), NT-ProBNP, Continuous glucose measuring system (CGMS), GLP-1-analog, glucose variability, MAGE, CONGA, counter regulation, incretin, Heart Rate Variability

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Cissa
Elliot & Caspian
This is what people don’t understand: obesity is a symptom of poverty. It’s not a lifestyle choice where people are just eating and not exercising. It’s because kids are getting sugar, fat, empty calories – lots of calories – but no nutrition.

/Tom Colicchio
List of Papers

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

I Niclas Abrahamsson, MD, Britt Edén Engström, MD, PhD, Magnus Sundbom, MD, PhD, F Anders Karlsson, MD, PhD “Gastric Bypass Surgery elevates NT-ProBNP levels” *Obesity Surgery* (2013) 23:1421–1426.

II Niclas Abrahamsson, MD, Britt Edén Engström, MD, PhD, Magnus Sundbom, MD, PhD, F Anders Karlsson, MD, PhD “GLP1 analogs as treatment of postprandial hypoglycemia following Gastric Bypass surgery: a potential new indication?” *European Journal of Endocrinology* (2013) 169:885–889.

III Niclas Abrahamsson, MD, Britt Edén Engström, MD, PhD, Magnus Sundbom, MD, PhD, F Anders Karlsson, MD, PhD “Hypoglycemia in Everyday Life after Gastric Bypass and Duodenal Switch” *European Journal of Endocrinology* (2015) 173:91-100.

IV Niclas Abrahamsson MD, Joey Lau Börjesson MScPharm PhD, Magnus Sundbom MD PhD, Urban Wiklund MD PhD, F Anders Karlsson MD PhD, Jan W Eriksson MD PhD “Gastric Bypass reduces Symptoms and Hormonal Responses to Hypoglycemia.” *Submitted.*

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### Abbreviations

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<th>Full Form</th>
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<tbody>
<tr>
<td>NT-ProBNP</td>
<td>N-Terminal Pro Brain Natriuretic Peptide</td>
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<tr>
<td>BNP</td>
<td>Brain Natriuretic Peptide</td>
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<tr>
<td>ANP</td>
<td>Atrial Natriuretic Peptide</td>
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<tr>
<td>GBP</td>
<td>Roux-en-Y Gastric Bypass Surgery</td>
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<tr>
<td>DS</td>
<td>BilioPancreatic Diversion with Duodenal Switch</td>
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<tr>
<td>CGMS</td>
<td>Continuous Glucose Measuring System</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon Like Peptide 1</td>
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<tr>
<td>GLP-2</td>
<td>Glucagon Like Peptide 2</td>
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<tr>
<td>PYY</td>
<td>Peptide YY</td>
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<tr>
<td>GIP</td>
<td>Glucose dependent Insulinotrophic Peptide / Gastric Inhibitory Peptide</td>
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<tr>
<td>PPHG</td>
<td>PostPrandial HypoGlycemia</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown Adipose Tissue</td>
</tr>
<tr>
<td>GNP</td>
<td>Gross National Product</td>
</tr>
<tr>
<td>LCD</td>
<td>Low Calorie Diet</td>
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<tr>
<td>VLCD</td>
<td>Very Low Calorie Diet</td>
</tr>
<tr>
<td>SOReg</td>
<td>Scandinavian Obesity Surgery Registry</td>
</tr>
<tr>
<td>MAGE</td>
<td>Mean Amplitude of Glycemic Excursion</td>
</tr>
<tr>
<td>CONGA</td>
<td>Continuous Overall Net Glycemic Action</td>
</tr>
<tr>
<td>GH</td>
<td>Growth Hormone</td>
</tr>
<tr>
<td>kCal</td>
<td>Kilo Calories</td>
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Introduction

“Everything in excess is opposed to nature” /Hippocrates

In the dawn of time, obesity seems to have been regarded as something admirable and positive, as the earliest sculptures known of humans are fat women (24,000 BC). In Greek-roman times this had changed to a more fit and muscular ideal, perhaps partly due to Spartan influence leading the way. Obesity has then more or less since the antiquity often been seen as a lack of character, and already the Greeks used fat people in plays to mock and ridicule. Later, early Christianity saw food as the entry port to the cardinal sins gluttony and lust, and excess intake of food is condemned by among others Thomas ab Aquino. This later changed and during medieval times and the renaissance obesity was regarded as a sign of wealth and power, with numerous portraits of obese kings and noblemen produced, e.g. Gustavus Vasa and Henry VIII. From the 1800’s, thinness has been the desirable condition, which can be exemplified by that the average weight of Miss America has decreased by 12% between years 1922-1999. In popular culture nowadays, obesity is often made fun of, with Monty Python’s Mr. Creosote as perhaps the foremost example of the 1900’s ridicule of obesity. This ridicule is a bit paradoxical in today’s society considering that approximately 50% of all adults are overweight or obese.

Pict 1. La Malade Imaginaire / the Imaginary Invalid, Moliere, 1673
Definition and Prevalence

Overweight is defined as body mass index (BMI) >25 kg/m² and obesity as BMI >30 kg/m². Obesity is subdivided into class I: BMI 30-34.9 kg/m², class II: BMI 35-39.99 kg/m², and class III: BMI >40 kg/m². BMI above 50 kg/m² is referred to as super-obesity. BMI is the simplest and without competition most commonly used measure of obesity, however it should be pointed out that it does not discriminate between muscular and fat tissue; i.e. a very muscular person (e.g. bodybuilder) would obtain a high BMI without being obese. Further the BMI measure has the built-in problem that it does not discriminate between the more dangerous fat depots stored around the waist – visceral adiposity – and the subcutaneous fat depots (Montague 2000). The BMI was developed to be used on a population scale and not on the individual scale; but because of its simplicity it has nevertheless become the most widespread measurement. Several other measurements have been proposed, such as waist-hip ratio, abdominal sagittal diameter, and waist circumference. These however demand more equipment and are slightly more troublesome to perform, and have not yet been used to the same extent as has BMI, making BMI the standard measurement.
Obesity has doubled since 1980, and in 2014 39% of adults were overweight and 13% were obese worldwide. On a global scale, obesity and overweight actually kills more people than underweight; 65% of the world’s population live in countries where more people are overweight/obese than underweight. In 2014, approximately 1.9 billion adults were overweight and 600 million obese on a global scale (WHO 2015). In Sweden 42% of adult males are overweight and 12% obese, while 28% of adult females are overweight and 11% obese (SCB 2013).

Causes

Massive changes in lifestyle the latest 100 years are thought to contribute to the obesity epidemic, with more fat and sugar rich food available, paired with less physical activity during both worktime and leisure. Additional, during most of mankind’s history the evolutionary pressure has pushed towards conserving energy to avoid undernutrition, and these mechanisms might be part of today’s problem (Morton 2006). The seasonal availability of food that has been the fact for most of our history favours the ability to store energy (Ulijaszek 2002). Quite interestingly though, the intake of fat and calories are by some authors reported to have decreased, and the intake of low-calorie products to have increased, from 1976 and onwards. If this is to be true, the amount of physical activity must thus have had to decrease dramatically (Heini 1997).

Complicating the eating behaviour even more, the nervous systems governing eating are under the influence of the reward systems (opioid and dopamine systems) in the central nervous system, closely connecting pleasure, relief and eating in the human experience (Berthoud 2008).

Obesity is often multifactorial, where diet is thought to contribute most to the development of obesity (Speakman 2012). On the other hand, from twin studies it has been proposed that 65% of the weight variation between individuals is genetically determined (Segal 2002). The genetic burden is however complicated to appreciate; from a genetic study involving ca 250,000 subjects, only 18 genes were identified and 14 known obesity-prone genes confirmed, together explaining merely 4% of the variation in BMI (Speliotes 2010). Consequently, the interaction between genes and environment is in the focus of explaining obesity (Speakman 2011). Physical activity is not strongly coupled to decrease in body weight (Tataranni 2003), and is in addition shown to stimulate food intake. Resting metabolic rate is associated with meal size, daily energy intake, and brown adipose tissue (BAT) activity, but not BMI (Blundell 2012, Cypess 2009). Finally, and not surprisingly, sedentary lifestyle (e.g. television-watching) is connected to increase in weight (Hu 2003).
Mutations in satiety-signalling hormone leptin (either production or receptor) make patients extremely obese (Farooqui 2008 and 2009). Leptin agonists have however not been effective pharmacologically on non-leptin-deficient individuals, illustrating the complex pattern of hunger-satiety signalling in humans.

The effect of in utero nutrition is complex, under-nutrition children (born during famine) have a higher risk of diabetes and obesity, and at the same time foetal over nutrition is linked to later life obesity (Ravelli 1999, Symonds 2009 and 2011, Rooney 2011).

The intestinal flora is in some studies suggested to influence weight, since the microbial populations of obese and lean individuals are reported to differ (Turnbaugh 2006). Lean mice that were transplanted with obese mice’s faecal microbial are reported to gain 50% weight (Bäckhed 2004). In humans it is reported that faecal transplant from lean donors to obese recipients increase insulin sensitivity in just 6 weeks (Vrieze 2012).

Also medications are thought to contribute to the rise in obesity, with e.g. glucocorticoids and anti-psychotics contributing heavily to some patients’ weight-gain.

Health Economy

The cost for obesity and its related diseases is quite significant although hard to precisely calculate; in Europe it is however calculated to be 11 billion euros yearly (Müller-Riemenschneider 2008) equivalent of 0.1-0.6% of the GNP (Gross National Product) of all Europe. In Sweden the cost is calculated to be 1.9% of GNP, 390 million euros (Odegaard 2008).

Associated mortality and morbidity

“Corpulence is not only a disease itself, but the harbinger of others” /Hippocrates

Obesity is linked to a wide variety of diseases and mortality. A 25-year old obese male loses about 13 years and female 8 years of life expectancy (Fontaine 2003). This shorter lifespan has been shown for all groups of obesity but not for the overweight (Flegal 2013). In the largest study so far, the mortality for grade III obesity and above (BMI 40 and above) was significantly raised, associated with a shorter lifespan of 6.5-13.7 years. Most subjects die of cardiovascular disease and cancer. A BMI over 50 actually shortens life more than smoking one pack of cigarettes per day (Kitahara 2014). Furthermore, when examining the metabolically healthy obese, an increased risk of
cardiovascular disease and higher all-cause mortality was found (Kramer 2013). Recently, an increased risk for chronic kidney disease was found in the metabolically healthy obese and overweight (Chang 2016).

Obesity is in the USA reported to cause 14% of all deaths from cancer in men and 20% in women (Calle 2003). Having a BMI >32 increases the risk of cardiovascular death threefold (Calle 1999).

Obesity and overweight increases the risk for several diseases; hypertension, hyperlipidaemia, insulin-resistance, coronary heart disease, stroke, type 2 diabetes, and cancer of the breast, colon, prostate, endometrium, kidney, and gallbladder (WHO Obesity, Kitahara 2014).

Treatment
The treatment for obesity can be diet, exercise, behavioural, pharmacological, surgical, or any combination of these.

Diet/Exercise/Behavioural
Dietary intervention is always hard to achieve and control in studies which is reflected in many conflicting reports. Diets are abundant and new ones are invented in a never-ending flow, often endorsed by various celebrities. The diets most specifically aimed at weight loss are the LCD (Low Calorie Diet) and the VLCD (Very Low Calorie Diet) which contains 800-1100 kCal/day and <800 kCal/day respectively. When reviewing the randomised clinical trials on dietary interventions (Avenell 2004), low fat diets showed a weight loss of 5.3 kg at one year, and 3.6 kg for 36 months, with lipids, glucose, and hypertension improving after one year. Very few studies unfortunately go beyond one year. Even fewer studies report of long-term results from LCD/VLCD; -6.2 kg at one year (LCD) and -13.4 kg at one year (VLCD). The authors conclude that most evidence supports the use of low fat diets in obesity (Avenell 2004).

Sacks et al randomised patients in 4 groups for 2 years, with dietary instructions on differing amounts of fat, carbohydrate, and protein intake for the groups. All groups however performed the same, with a mean weight loss of 4 kg (Sacks 2009).

Adding exercise to diet, and comparing that to diet alone showed an extra decrease in mean weight of 1.95 kg at 12 months. Adding behavioural therapy to diet was associated with a weight decrease of 7.7 kg at one year, and 2.9 kg at 36 months in the studies included (Avenell 2004).

Concerning behavioural psychological treatments, results are not convincing over time as participants tend to drop-out and/or regain weight after a few
years of follow-up (Cooper 2010). The Swedish council on health technology assessment (SBU) drew in 2002 the conclusion that no conclusion could be drawn regarding the efficacy of behavioural therapy (SBU report 2002).

Pharmacological

The only prescribable and subsidised drug presently available on the Swedish market with the indication obesity is Orlistat (Xenical© /Alli©). Orlistat inhibits pancreatic lipase and thereby hinders fat degradation and triglyceride uptake in the intestine, but unfortunately leads to troublesome side-effects such as steatorrhea and diarrhoea. These side-effects often lead to discontinuation of treatment. Orlistat is shown, under optimal study circumstances, to lead to a weight loss of about 10% in one year (Padwal 2007).

During the latter part of 2016, the GLP-1-analogue Liraglutide (Saxenda©) will be available for the treatment of obesity, presently used for the treatment of diabetes. It is an injectable, taken once daily, and has been shown to lead to a weight-loss of approximately 6% in one year (Pi-Sunyer 2015). The main mechanisms are reduced gastric emptying leading to earlier satiety, and decreased hunger centrally.

Surgical

Due to lack of effect of diet and medical treatment, bariatric surgery has established itself as the preferred treatment of obesity in patients with a BMI>35 kg/m². It is also in fact the only treatment with evidence of effect on morbidity and mortality for weight loss. In Sweden, the absolute majority of surgeries performed are Roux-en-Y gastric Bypass (GBP), 82% of the annually 6800 bariatric surgeries in Sweden are GBPs, most often performed laparoscopically. The Biliopancreatic diversion with duodenal switch (DS) is reserved for the extremely obese cases (BMI >50 kg/m²), and in 2014 47 DS-surgeries were performed. The sleeve gastrectomy (SG) surgery is steadily on the rise, mostly due to patient demand, from being performed on 1.4% of patients in 2012 to 16.5% in 2014 (SOReg 2014). The long-term effects of the SG are however much less known.

Importantly, a bariatric surgery demands an extensive lifestyle change of the patient undergoing it; patients have to post-surgery eat frequent meals 6–7 times a day with low carbohydrate and high protein content, and not drink during meals but rather in between.
The Roux-en-Y Gastric Bypass

The GBP surgery (Fig 2), most often performed laparoscopically, creates a small gastric pouch (15-30 ml), and a bypass of the duodenum consisting of an approximately 30 cm long biliopancreatic limb, which connects to the approximately 100 cm long Roux-limb that is connected to the gastric pouch. This thus leads to a smaller capacity for large portion size, but this restrictive effect is mainly seen only initially, with adaptation to larger portion size with time. The bypass of the first absorptive parts of the intestine leads to later mixing of nutrients, bile and pancreatic enzymes, and thereby later degradation and possible uptake. It consequently exposes the jejunal mucosa to undigested nutrients, and the duodenal and first part of the jejunum to undiluted bile and pancreatic enzymes. Furthermore, the biliopancreatic limb that is disconnected from the usual through-flow of the intestine becomes a locale for an altered bacterial flora.

The surgery further affects the incretin balance, with higher levels of incretins, e.g. GLP-1, GLP-2, PYY, and GIP post-surgery. The peak GLP-1 level increase about six times compared to preoperative levels (even though there is a wide variety in reports ranging from 2-30 times increases) and GIP and PYY doubles postprandial (Le Roux 2006, Laferrere 2008). It is noteworthy that the incretins thus seem to increase to different degrees, thereby also altering the balance between the incretins and not only the absolute levels. These increased levels of incretins are believed to contribute a great part to the remission of diabetes and weight loss post GBP/DS-surgery.

Increased levels of bile acids post-GBP (Pournaras 2012) can increase L-cell secretion via the TGR5 receptor, and thereby increase the levels of GLP-1 and 2 (Parker 2011). As mentioned, also the bacterial flora seems to change post-surgery, with increased numbers of the so called Gammaprotobacteria and Verrucomicrobia. Interestingly, when transferring this gut microbiota from mice post-GBP to control mice, controls lose weight and decrease fat mass (Liou 2013). This is suggested to be related to altered microbial production of short-chain fatty acids, since short-chain fatty acids are reported to increase secretion of GLP-1 (Tolhurst 2012).

Greater energy expenditure, seen both totally over 24 hours and after meals, as compared with patients after Vertical Banded Gastroplasty (VBG)-surgery are reported, possibly also contributing to weight loss (Werling 2013). When comparing patients before and after GBP-surgery, energy expenditure increased after meal but not totally over 24h (Werling 2015).

Finally, there are reports that the GBP-surgery affects food preference and amount of food eaten, with a lessened drive to eat high-sugar and high-fat
content food (Behary 2015). The mechanism for this is not fully understood, although it is speculated that it is related to the increased incretin levels and their central nervous effects on hunger and satiety-centres.

**Figure 2.** Left: Normal anatomy. Right: Roux-en-Y Gastric Bypass. Courtesy of M Sundbom.

In the SOS-study (Swedish Obese Subjects), the longest running bariatric surgery study so far, the diabetes remission rate was 30.4% in the surgery group 15 years post-surgery, compared to 6.5% in the control group. Weight decrease was 22.5 kg in the GBP group compared to 4.4 kg in the control group 10 years after surgery, and diabetic complications retinopathy, neuropathy and nephropathy are about halved (Sjöström 2014). Mortality of all causes is significantly decreased except that for non-disease causes (Sjöström 2007); i.e. accidents and suicide, which are in fact 58% higher (Adams 2007). If this might be hypoglycaemia-associated accidents is not known. Further, cancer incidence is shown to decrease post bariatric surgery (Adams 2009, Sjöström 2009). About 5-10% of patients are reported to regain their pre-surgery weight in a few years (Hörchner 2013). Life-quality, as measured 12 years post-surgery was improved compared to an obese con-
control group, and improvement is in relation to the medical outcome of the surgery (Raoof 2015).

When randomising patients with uncontrolled diabetes and comparing bariatric surgery versus best medical therapy, the GBP group met primary criteria (HbA1c< 6.0 %) in 38 % and in the medical group to 5 %, with weight reductions of 24 kg vs 4 kg respectively, 3 years post-surgery (Schauer 2014). Likewise, when comparing GBP to intensive lifestyle treatment/medical management in obese type-2 diabetes (BMI 30-39.9), the surgery group exhibited greater weight decrease, 26.1 % compared to 7.9 %, and greater remission of diabetes, hypertension, and hyperlipidaemia, 49 % compared to 19 %. Concerning deficiencies of iron and vitamin D, the surgery group showed 7 % vitamin D and 22 % iron deficiency (medical group 8 % and 0 % respectively) (Ikramuddin 2013).

Moreover, comparing GBP with usual care and usual care plus GLP1-analogue Exenatide in patients with BMI>28kg/m², the surgery group exhibited after one year a 90 % remission in type-2-diabetes and a significant decrease in requirements of anti-hypertensive drugs, being superior to both groups of controls. Weight loss was greatest in the surgery group, even though the usual care plus GLP-1-analogue group also lost more weight compared to the usual care group (Liang 2013).

When reviewing and comparing different surgical techniques, Levy et al concluded that the malabsorbtive procedures, GBP and DS, were superior to the restrictive procedures, Gastroplasty and Gastric banding, in outcome on weight and type 2 diabetes remissions (Levy 2007).

Side-effects include leakage and stricture at the anastomoses, ulcers, hernias, nutritional deficiencies (particularly vitamin B12, vitamin D, Folate, Zinc, Iron, Calcium and protein), dumping syndrome, and late postprandial hypoglycaemia.

Dumping is characterized as an attack within 10–30 min after eating with symptoms of dizziness, diaphoresis, flushing, nausea, bloating, and fatigue. Osmotic fluid-shifts from blood to lumen accompanied by symptoms from activation of the sympathetic nervous system are believed to be chiefly responsible for the dumping syndrome (Hammer 2012).

Late hypoglycaemia is a reflective hypoglycaemic episode occurring 1-5 hours after meal, particularly after a high carbohydrate meal and is described more in detail below.
Biliopancreatic diversion with duodenal switch

The Biliopancreatic diversion with duodenal switch (DS, Figure 3) procedure includes creating a gastric sleeve, i.e. removing the main part of the stomach, and bypassing the larger part of the small intestine with a 250 cm long alimentary limb, leaving only the last 100 cm of the ileum as a common channel for absorption of fat-soluble nutrients. The mechanisms for weight loss are in many parts similar to the above discussion regarding GBP; nevertheless malabsorption post-DS plays a more important role, with especially fat malabsorption contributing to weight loss.

When randomizing patients to either GBP or DS, patients lost a mean 23 BMI-units post-DS and 16 BMI-units post-GBP, making the DS significantly more effective concerning weight loss. The DS-group also exhibited lower HbA1c and glucose 3 years post-operatively (Hedberg 2012). DS has a great impact on diabetes remission, with an almost 95% remission of diabetes post-DS (Buchwald 2004). When comparing GBP and DS subjects 6 months post-surgery, DS patients have lower HbA1c and lower 1h insulin peak following oral glucose challenge test (Roslin 2012). This surgery is reserved for
patients with a BMI>50 kg/m², considering its higher risk of side-effects coupled to the marked weight decrease and malabsorption. Side effects include flatulence, diarrhoea, and nutritional deficiencies of in particular calcium, iron, zinc, protein, and fat soluble vitamins.

**Glucose Homeostasis and GLP-1 post-surgery**

GLP-1 is a neuropeptide excreted from enteroendocrine L-cells and neurons. It is derived from proglucagon, and is co-secreted with GLP-2, Oxyntomodulin, and Glicentin. While glucagon, GRPP (Glicentin related pancreatic polypeptide), and major proglucagon fragment are derived from the similar proglucagon precursor in the α-cells (Holst 2007). The exclusivity of the L-cells, neurons, and α-cells in secreting its respective proglucagon-products is unclear since there are reports of physiological levels of glucagon in totally pancreatectomised patients (Lund 2015). L-cells and neurons are believed to use prohormone convertase PC1/3, and α-cells PC2 to yield their products, but there are as mentioned more to study concerning their precise expression in different cell-types. GLP-1 has a half-life of 1-3 minutes, and is eliminated via the kidneys after degradation by the dipeptidyl peptidase-4 (DPP-4), which is expressed on endothelial cells (Baggio 2007).

The L-cells are located in the small intestinal mucosa in increasing numbers from the jejunum to the rectum. GIP is on the other hand secreted from K-cells, situated in decreasing number from the duodenum to the rectum. These differences in cell-positioning probably affect their adaptation post bariatric surgery. Incretins are secreted minutes after food intake in response to food in general and carbohydrates in particular, most probably involving a nervous mechanism from early to late portions of the intestine, since the GLP-1 response is much quicker than the nutrients’ passage to the lower intestine (Wang 2015). Concerning nervous stimulus, the secretion of GLP-1 is reported to be stimulated by acetylcholinergic stimuli and isoproterenol (adrenergic agonist) but not by norepinephrine (Hansen 2004).

The GLP-1-receptor is a G-protein coupled receptor and is expressed in the pancreas, brain, heart, kidney, stomach, liver and intestinal system (Mayo 2003). GLP-1 and GIP stimulate insulin release from the pancreatic beta cells, constituting the main part of the incretin effect in insulin release (Nauck 1996). GLP-1 has a multitude of effects apart from stimulating insulin release; it inhibits glucagon release from α-cells when glucose >4.0 mmol/l, inhibits hepatic gluconeogenesis, slows gastric and intestinal emptying, decreases acid secretion in the stomach, lowers blood pressure, improves β-cell proliferation, prevents β-cell apoptosis, and suppresses appe-
In addition, GLP-1 exists in the central nervous system where it can penetrate the blood-brain barrier (Baggio 2007), and can exert an inhibitory effect on appetite and drinking (Larsen 1997). GLP-1 increases dopamine turnover in the amygdala, which via the D2 receptor increase satiety (Anderberg 2014). GLP-1-receptor analogues activate GLP-1-receptors in the arcuate nucleus to induce weight loss (Seecher 2014), and further both GLP-1 and PPY affect POMC (Pro-Opio Melano Cortin)-neurons in the arcuate nucleus to induce satiety via inhibition of AGRP/NPY (AGouti Related Peptide / Neuro Peptide Y) secretion (Larsen 1997). GLP-1’s weight reducing effect is thought to be due both to this increased central satiety, and slowed gastric emptying. Additionally, GLP-1 activates the corticosteroid axis, and sympathetic nervous system (Larsen 1997).

In the portal vein GLP-1 stimulates the hepatic afferents and subsequently the pancreatic efferents, and GLP-1-signal is thought to constitute 60% of the insulin response to oral glucose (Nishizawa 2013). It has been reported that the hepato-portal gluco-sensing is dependent on the co-effect of glucose and GLP-1 and to act via the GLUT-2 channel (Burcelin 2001).

The levels of GLP-1 are reported to be lower both in type-2 diabetics and in the obese (Toft 2001, Holst 1983).

Hypoglycaemia

Hypoglycaemia is defined by the American Diabetes Association (ADA) as plasma glucose <3.9 mmol/L, with or without symptoms (ADA 2005). Clinically Whipple’s triad is commonly used; symptoms of hypoglycaemia, low plasma glucose, and regress of symptoms when plasma glucose levels are raised. Most studies use 3.3 mmol/L as the lower limit, but 2.8 mmol/L and 3.9 mmol/L are also frequently used.

The risk of developing late reactive postprandial hypoglycaemia, a rare but severe complication of bariatric surgery, would be the most extreme effect of improved glucose homeostasis.

The prevalence is uncertain and dependent on study-methods used and definition of hypoglycaemia; in 2010 Marsk et al used the Swedish National Patient Registry to report an absolute risk of 0.2 % for a post-surgery bariatric patient to require emergency hospital care due to postprandial hypoglycaemic episodes, compared to 0.04 % in the general population (Marsk 2010). Sarwar et al used the American BOLD (Bariatric Outcomes Longitudinal Database) registry of over 100,000 gastric bypass patients to estimate a
self-reported prevalence of 0.02-0.1 % of hypoglycaemic symptoms (Sarwar 2014). On the other hand, Lee et al, using the Edinburgh Hypoglycaemia score questionnaire report that 34 % of patients had high suspicion for post-prandial hypoglycaemia, and 11 % were suspected of suffering from severe hypoglycaemia. The risk was increased in subjects with pre-operative hypoglycaemic problems, female gender, longer time since surgery, and lack of diabetes (Lee 2015). An even higher incidence was found when investigating 40 patients 86 months post-GBP with CGMS and mixed meal tests, when 75 % of patients had hypoglycaemic episodes below 3.05 mmol/L using the CGMS (5-day recording) and 29 % using the Mixed Meal Test (Kefurt 2015). There were no reports of symptoms in this study. Further, 69 % of post-GBP patients examined at least six months post-surgery exhibited glucose levels <3.3 mmol/l when tested with 100g oral glucose liquid load (Roslin 2011).

There is thus a huge difference in prevalence of postprandial hypoglycaemias, depending on method used in study and if searching for asymptomatic, symptomatic or severe episodes. Mostly these are reported together, diffusing the results. Further, the risk of confusing symptoms with dumping always exists, especially when using self-reports and questionnaires. However, it seems to be common with measurable hypoglycaemia, and hypoglycaemia-like symptoms, but to what extent these troubles patients, and how large the prevalence of severe hypoglycaemia is, is largely unknown.

The reactive late postprandial hypoglycaemic syndrome arrives 1-5 h after meal, and several mechanisms have been proposed to explain this. Mainly, the hypoglycaemia is believed to stem from hypersecretion of incretins, primarily GLP-1 and GIP, and consequently insulin, in relation to amount of glucose ingested. The symptoms include weakness, sweating, dizziness, and ultimately fainting and seizures derived from neuroglycopenia. This problem is known to also occur after other surgeries involving the stomach and small intestine, e.g. after partial and total gastrectomy when 1/3 of patients post gastric cancer gastrectomy surgery developed postprandial hypoglycaemia (Mine 2010). The incretin hyper secretion is proposed to induce β-cell expansion (Rabiee 2011, Vella 2007), which by some is considered as an adult version of nesidioblastosis (Service 2005), and has led to the suggestion that partial pancreatectomy might be the cure (Yunfeng 2011).

This proposition has been questioned; Meier et al studied pancreatic tissues from patients and controls and found no β-cell hyperplasia but found a correlation between β-cell nucleus diameter and preoperative BMI (Meier 2006). When investigating porcine pancreas post-GBP compared to sham-operated pigs, the GBP-group exhibited increases in number of extra-islet cells, number of islets and increase in β-cell mass. There were also increased expres-
sion of insulin, glucagon, and cells expressing the GLP-1-receptor in GBP-pigs (Lindqvist 2014). Recently, Patti et al examined post-GBP-patients with postprandial hypoglycaemic problems with intravenous glucose test and oral mixed meal tolerance test, demonstrating that the exaggerated insulin peak appeared after the oral test, but not after the intravenous. This would indicate that the genesis to hyperinsulinemia probably not originates within the β-cell but rather in its hyper stimulation by incretins, since authors conclude that the β-cell response to glucose was adequate (Patti 2015). In this study no indication of difference in insulin-clearance was found, which however was reported in an earlier study (Salehi 2014). The feedback mechanisms might also be of importance, insulin is shown to inhibit the gene expression of proglucagon gene mRNA in α-cells (Philippe 1989), but one study reports that insulin in high doses increase expression of proglucagon gene in L-cells, and thereby positively feedback on GLP-1 secretion (Yi 2008). In the latter study also IGF-1 likewise stimulated proglucagon gene expression, raising the question as to what receptor is stimulated by these high doses of insulin.

Salehi et al used GLP-1-antagonist infusion during mixed meal tests in patients with postprandial hypoglycaemia, and showed that the hyperinsulinemia and hypoglycaemia were eliminated when infusing GLP1-antagonist; thus showing to the importance of GLP-1 in the pathogenesis of postprandial hypoglycaemias (Salehi 2014). In the same study the rate of appearance of meal-derived glucose was further demonstrated to be faster in the group with patients suffering from hypoglycaemic episodes compared to the asymptomatic post-GBP-group. This could speculatively be due to increased levels of GLP-2, known to induce intestinal mucosa-cell proliferation (Rowland 2011), since GLP-2 is co-secreted with GLP-1, and GLP-1 levels are higher in patients with hypoglycaemic problems. The latter being demonstrated when studying GBP patients with neuroglycopenic symptoms, and reporting higher levels of insulin and incretins after a mixed meal test compared to GBP-patients without symptoms. Fasting morning values were similar (Goldfine 2007).

Counter regulation to Hypoglycaemia

The body has several mechanisms to keep blood glucose at an adequate level, especially to protect it from low levels. Glucose sensing neurons are placed in the brain (hypothalamus) and in the carotid body, oral cavity, gut, and the hepatic portal vein (Verberne 2014). The brain can act fast and efficiently on hypoglycaemia via neurons to influence cells in the pancreas (α and β-cells), adrenal medulla (chromaffin cells), and anterior pituitary (corticotrop and somatotrop cells) to increase levels of glucagon, cortisol, epinephrine, norepinephrine, and growth hormone (GH) (Watts 2010). Counter
regulatory hormones glucagon, GH, epinephrine and norepinephrine are secreted when glucose reaches 3.6-3.8 mmol/L, and cortisol at 3.0 mmol/L. Autonomic symptoms (anxiety, palpitations, irritability, sweating, and tremor) begin at 3.2 mmol/L, while neuroglycopenic symptoms (hunger, dizziness, tingling, blurred vision, difficulty thinking and faintness) and deterioration in cognitive function tests begin at 2.8 mmol/L (Mitrakou 1991). Some branches of the vagal nerve, conveying signals to target glands, might be damaged during GBP surgery (Sundbom 2007).

Brain Adaptation and unawareness to hypoglycaemia

The brain relies primarily on glucose as source of energy, with ketone bodies used during starvation. To cope with lowered blood glucose levels, the brain upgrades glucose sensing and uptake. Hypoglycaemia can induce a 25-45 % increase in Blood-Brain-Barrier permeability for glucose, a 23 % increase in total glucose-transporter GLUT-1/mg of micro vessel protein, and a 52% increase in luminal GLUT-1 in rat (Simpson 1999). Three hours after hypoglycaemia, brain gene expression for six genes (angiotensinogen, GLUT-1, inhibitor of kB, ID-1 (Inhibitor of DNA binding 1), Ubp41, and MKP-1 (mitogen-activated protein kinase phosphatase-1)) was increased (Mastaitis 2005). Of these, four are known to enhance glucose availability (angiotensinogen, GLUT-1, ID-1, and MKP-1). This adaptation to a lowered glucose level to ensure the brain’s need for glucose would probably secondarily downregulate the level when the central nervous system responds to hypoglycaemia. Such downregulation is in fact known to occur under different physiological situations, such as insulin treated diabetes patients (Berlin 1987), pregnant diabetic women (Diamond 1992), and in normal subject following exercise (Galasetti 2001).

Hypoglycaemia -Treatment

No evidence-based treatment exists for postprandial hypoglycaemias. First and foremost, attention is put on the diet, and to avoid nutrients that induce a sharp rise in glucose, GLP-1 and thereby insulin, i.e. to avoid fast-absorbed carbohydrates (Botros 2014). If that fails, pharmacological treatment can be tried. Proposed treatments in case-reports include; Acarbose (α-glucosidase inhibitor) to reduce uptake and speed of uptake of glucose (Valderas 2012); Calcium channel inhibitors Nifedipine and Verapamil to decrease insulin secretion (Moreira 2008); somatostatin analogue Octreotide to dampen insulin secretion (Myint 2012); and β-cell inhibitor Diazoxide (Gonzalez-Gonzalez 2011). Regarding surgical treatment, some reports advocate partial pancreatectomy (Yunfeng 2011), even though presently that is commonly
not recommended. Glucagon has been tried but unfortunately secondarily raised insulin, rendering it not efficient as treatment (Halperin 2010). Preprandial doses of Insulin aspart (rapid acting insulin analogue) has been tried successfully in a diabetic patient with postprandial hyperglycaemia followed by hypoglycaemia (Schoenberger 2012). Under experimental circumstances, infusion with GLP-1-antagonist could abolish postprandial hypoglycaemia in 9 post-GBP-patients, but this has however not been tried under clinical circumstances (Salehi 2013).

**Continuous Glucose Measuring System (CGMS)**

Measuring glucose with the CGMS yields an interstitial glucose value every 5 minutes (de facto measured every 10 seconds, mean value given every 5 min). The earlier problem with nocturnal measurements seems to have been corrected with the latest generation systems. Glucose time lag for plasma to interstitial fluid is reported to be about 4-10 minutes (Boyne 2003, Steil 2003), and the interstitial glucose level is reported not to decrease before plasma levels in hypoglycaemia (Steil 2005). However, the latest generation CGMS IPRO-2 is in fact just recently reported to possibly underestimate hypoglycaemia, i.e. reporting a slightly (+1 mmol/l) higher value than measured simultaneously in plasma (Nielsen 2016).

![Continuous Glucose Measuring System Medtronic IPRO-2](image)

**Figure 4.** Continuous Glucose Measuring System Medtronic IPRO-2, used in Paper II and III.

**Bariatric Surgery and NT-ProBNP**

The B-type Natriuretic Peptide (BNP) and its inactive by-product N-Terminal Pro Brain Natriuretic Peptide (NT-ProBNP) is synthesised in cardiac ventricle myocytes in response to wall distension (volume load) and to
neuro-hormonal stimulation (Maisel 2002). Measuring of BNP/NT-ProBNP has established itself as the best diagnostic tool regarding heart failure. The nearby related Atrial Natriuretic Peptide (ANP) is synthesized in a likewise manner in the atrial myocytes.

BNP is elevated in response to most heart distresses as; acute myocardial infarction, congestive heart failure, hypertrophic cardiomyopathy, diastolic dysfunction, and left ventricular dysfunction (Nishikimi 2011, Levin 1998, Martinez-Rumayor 2008). Increased plasma BNP concentration leads to diuresis, natriuresis, vasodilation, improved myocardial relaxation, inhibition of the renin-angiotensin system, and inhibition of adrenergic activity. The natriuretic peptides are strongly correlated to mortality and morbidity following all forms of acute coronary syndrome (de Lemos 2001) as well as to morbidity and mortality in non-cardiac surgery (Ryding 2009). In mice, a lipid accumulated heart has been shown to have a decreased ability to synthesize natriuretic peptides (Bartels 2010). The NPR-C (Natriuretic Peptide Receptor C), a membrane bound receptor on adipocytes, clears BNP while NT-ProBNP is mainly cleared by the kidney (Sarzani 1996).

In the Framingham study BNP was inversely correlated with obesity (Wang 2004), and obese subjects had decreased levels of natriuretic peptides with 6-20% (Khan 2011). Furthermore, patients with the same degree of heart failure differed in BNP levels in relation to BMI levels; with the obese having the lower values (Mehra 2004). Overweight patients with acute myocardial infarction (AMI) have a 20% lower NT-ProBNP level and obese have a 60% lower level than non-obese patients with AMI (Lorgis 2011). Losing weight by diet or by adjustable gastric band operation seems to lower the NT-ProBNP levels (Hanusch-Enserer 2003, Minami 2000) whereas after GBP surgery, NT-ProBNP is reported to increase and to correlate with weight reduction (Changchien 2011).
Aim

The post bariatric surgery state differs quite significantly from the pre-surgery state concerning some major hormonal systems, and offers the opportunity to study these systems during these altered conditions. The most evident and recognized condition would be the incretin-insulin-glucose homeostasis axis that undergoes dramatic changes almost immediately post-surgery in response to the changed anatomy and nutrient delivery. Also the natriuretic system seems to modify its normal response to stimulators. The purpose of the present work is to explore some important modifications in these two hormonal systems post bariatric surgery, with most impact on adaptations in the regulation of the glucose homeostasis.
Hypotheses

**Paper I** To test the hypothesis that GBP-surgery affects the level of NT-ProBNP immediately post-surgery, i.e. that it is not weight-related, and to establish whether these changes are chronic.

**Paper II** On the grounds of the glucose-stabilising effect of GLP-1 analogues in diabetic patients, GLP-1 analogue treatment was used in five patients.

**Paper III** From clinical experience we hypothesized that hypoglycaemias were more commonly occurring than previously known, and wanted to investigate the extent of both de facto hypoglycaemias as defined biochemically, as well as the symptomatology of hypoglycaemias post bariatric surgery.

**Paper IV** In view of the high frequency of asymptomatic hypoglycaemias post-bariatric surgery found in paper III, we hypothesized that the surgery affected both the counter-regulatory response and symptomatology post-surgery.
Subjects

**Paper I**  Twenty patients; 18 women, mean age 41 years, mean pre-operative BMI 44.6 kg/m² were recruited before their planned GBP surgery. These subjects were examined biometrically and blood samples (NT-ProBNP) were drawn preoperatively, and at day 6 and months 1, 6 and 12. Fourteen of the 20 patients were also examined at day 1, 2 and 4 to in detail study the first postoperative week.

**Paper II**  As the GLP-1 analogues entered the market as anti-diabetic treatment, their glucose stabilising effect could be noted. We therefore tried GLP-1-analogues as off-label treatment in patients with severe postprandial hypoglycaemia. All five patients had had frequent meetings with the clinic’s specialist dieticians to optimize their eating regimen prior to medication with GLP-1-analogue, and had also tried different other suggested treatments with negative results.

**Paper III**  Three groups of participants; 15 patients post GBP-surgery (12 women, mean age 44 years, mean BMI 32.6 kg/m², mean 2.6 years since operation), 15 patients post DS-surgery (7 women, mean age 47 years, BMI 30.7 kg/m², 1.4 years since operation), and 15 non-diabetic obese BMI-matched controls (12 women, mean age 52 years, BMI 31.1 kg/m²) were recruited.

**Paper IV**  Morbidly obese non-diabetic patients were consecutively invited to participate after being accepted for bariatric surgery at the Metabolic out-patient clinic of the Uppsala University Hospital. A total of 15 patients were enrolled, but three discontinued study after first clamp, one due to pregnancy and two due to lack of time. 12 patients (8 women and 4 men) were thus examined 3 months before and about 4-5 months after surgery using a hyperinsulinemic hypoglycaemic clamp.
Methods

Roux-en-Y-Gastric Bypass surgery (GBP) (Papers I-IV)
Patients underwent Roux-en-Y-Gastric Bypass surgery, including a 100-cm Roux-limb connected to a small proximal gastric pouch, and a 50-cm biliopancreatic limb. The GBP-surgery was preceded by a four week low-calorie diet treatment, according to clinical routine, in order to reduce liver size and intestinal fat. See Introduction for more detailed information regarding this surgical technique.

Biliopancreatic Diversion with Duodenal Switch (DS) (Paper III)
A gastric sleeve was connected to a 250 cm alimentary limb that consisted of the distal part of the ileum. The remaining small bowel (the biliopancreatic limb) which consists of mainly the jejunum was thus excluded from the passage of food. The distal part of the biliopancreatic limb was connected to the last 100 cm of the alimentary limb, to allow absorption of fat soluble nutrients. The surgery was preceded by at least four weeks low-calorie diet treatment, according to clinical routine, in order to reduce liver size and intestinal fat. See Introduction for more detailed information regarding this surgical technique.

Biochemical Analyses
All blood samples, except noted below, were analysed at the Department of Clinical Chemistry at the University Hospital of Uppsala, according to clinical routine. The laboratory is certified by the Swedish authorities, Swedac. Hormonal analyses used were; Insulin (automated analysis, Cobas E, Roche), Cortisol (automated analysis, Cobas E, Roche), GH (automated analysis, Immulite XP, Siemens Healthcare Global), and C-peptide (automated analysis, Cobas E, Roche). Catecholamine analyses (Chromatography (LC) were performed at the Laboratory of Clinical Chemistry at the Karolinska Universitetssjukhuset, Stockholm, Sweden.
Specific hormonal analyses for paper IV were performed at the Clinical Diabetes Research Laboratory at the University Hospital of Uppsala. Glucagon was measured with ELISA (Mercodia ELISA Glucagon #10-1271-01, Mercodia, Uppsala, Sweden). GLP-1 and GIP were measured with ELISA (Merck Millipore ELISA EZGLP1T-36K, and EZH-GIP54K respectively, Merck, Darmstadt, Germany). Free Fatty Acids were analysed using Free Fatty Acid Fluorometric Assay Kit (Cayman Chemical company, Ann Arbor, MI, USA. item no 700310), and Glycerol was analysed using Free Glycerol reagent (Sigma #F6428, Sigma Chemical, St. Louise, MO, USA).

**Paper I**

Blood samples NT-ProBNP, plasma glucose, and serum insulin were measured at all time points (preoperatively, day 6 and months 1, 6 and 12, and in 14 of the 20 patients day 1, 2 and 4). HOMA-IR was calculated (according to Matthews 1985).

Blood loss, fluid balance and intravenous (IV) fluid given can be seen in table 1. Fluid balance was calculated as intravenous fluids and oral intake reduced by urine output and insensible losses. Insensible losses were calculated as 5 mL/kg/surgical hour and 40 mL/nonsurgical hour, with an additional 500 mL per degree of fever (Finsterer 1980).

Patients were offered 500 mL to drink on the first postoperative day and then to increase their oral intake gradually, with free intake from day 3, all in accordance to clinical protocol.

<table>
<thead>
<tr>
<th>Blood Loss</th>
<th>Fluids IV</th>
<th>Fluid Balance</th>
<th>PO intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of surgery</td>
<td>300(0-700)</td>
<td>4700(2500-11600)</td>
<td>1328(-1322-6178)</td>
</tr>
<tr>
<td>Post-op day 1</td>
<td>-</td>
<td>2500(1500-4800)</td>
<td>-1000(-3900-1900)</td>
</tr>
<tr>
<td>Post-op day 2</td>
<td>-</td>
<td>1750(450-4100)</td>
<td>475(1300-1200)</td>
</tr>
</tbody>
</table>

NT-ProBNP levels were compared with ANOVA test rendering significant difference with a p=0.02. The NT-ProBNP levels from preop, day 2 (day of peak value during week one), and months 1, 6, and 12 were compared with univariate t-tests. Bonferroni corrected p-value for multiple testing was 0.0125. Linear regression was used to evaluate if change in NT-ProBNP was related to change in BMI, glucose, insulin or HOMA-IR.

**Paper II**

All patients initially had frequent meetings with the clinic’s specialist dieticians to optimize their eating regimen, and also tried other
suggested published treatments with negative results. For lack of other treatment and in view of their known glucose-stabilising effect we therefore used GLP-1-analogues as off-label treatment in these patients with severe postprandial hypoglycaemia. Patient characteristics of the five cases are given in table 2.

Table 2. Patient characteristics. 5 post GBP patients with late postprandial hypoglycaemias. W = weekly, eod = every other day, nd = not defined, BMI in kg/m2, f=fasting, Glucose in mmol/L, Insulin in mU/L, HbA1c in mmol/mol and %.

<table>
<thead>
<tr>
<th>#</th>
<th>Age</th>
<th>Admitted years after GBP</th>
<th>BMI preop/ admittance</th>
<th>f Glucose</th>
<th>f Insulin</th>
<th>HbA1c</th>
<th>Lowest P-Glucose measured with symptoms</th>
<th>Freq of PPH G</th>
<th>Relapse of symptoms when lowered dose or off drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>3</td>
<td>42/29</td>
<td>5.2</td>
<td>nd</td>
<td>5.5</td>
<td>5.5 (37)</td>
<td>nd</td>
<td>w yes</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>1</td>
<td>46/27</td>
<td>5.1</td>
<td>nd</td>
<td>5.1</td>
<td>5.1 (32)</td>
<td>2.7</td>
<td>eod yes</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>2</td>
<td>47/35</td>
<td>5.4</td>
<td>nd</td>
<td>5.4</td>
<td>5.4 (36)</td>
<td>2.5</td>
<td>daily nd</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>1</td>
<td>51/32</td>
<td>4.6</td>
<td>1.26</td>
<td>6.2</td>
<td>6.2 (44)</td>
<td>1.8</td>
<td>daily yes</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>3</td>
<td>38/27</td>
<td>4.8</td>
<td>1.86</td>
<td>5.3</td>
<td>5.3 (34)</td>
<td>1.6</td>
<td>w yes</td>
</tr>
</tbody>
</table>

Paper III Patients and controls were instructed to live and eat according to their ordinary routines, and to keep a food diary. Since post-bariatric surgery meals are significantly smaller than normal meals, a portion consisting of at least 125 kilocalories (equivalent of 150 g of pasta or a banana) was defined as a meal (modified after Brolin 1994 when a meal for a normal eating person was defined as 150 kcal). Characteristics in table 3.
Table 3. Basic characteristics of patients and controls. Means (SD).

<table>
<thead>
<tr>
<th></th>
<th>GBP</th>
<th>DS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.6 (7.1)</td>
<td>46.7 (9.1)</td>
<td>52.3 (10.7)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>12/3</td>
<td>7/8</td>
<td>12/3</td>
</tr>
<tr>
<td>Preop BMI (kg/m²)</td>
<td>42.9 (4.1)</td>
<td>54.3 (3.8)</td>
<td>n/a</td>
</tr>
<tr>
<td>Years since surgery</td>
<td>2.6 (0.4)</td>
<td>1.4 (1.7)</td>
<td>n/a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.6 (4.0)</td>
<td>30.7 (5.8)</td>
<td>31.1 (2.5)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>122 (15)</td>
<td>122 (12)</td>
<td>128 (14)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>73 (9)</td>
<td>78 (8)</td>
<td>82 (6)</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>66 (6)</td>
<td>66.6 (6)</td>
<td>67 (4)</td>
</tr>
</tbody>
</table>

Continuous Glucose Measuring System (CGMS)

In the present study the blinded CGMS Medtronic Minimed IPRO-2 was used, to prevent patients from adapting their lifestyle to glucose measurements. The CGMS was inserted according to instructions, patients wore it for at least 3 days (only first 72 hours of data used), and measured capillary glucose 4 times daily for calibration. Glucose meter Bayer CONTOUR® was used, and all data was uploaded to the Carelink® software program. Shapiro-Wilk’s tests were performed for control of normality, and values were compared with Student’s t-test or Mann-Whitney U-test (Wilcoxon’s rank sum test).

Glucose Variability measurements

Among the wide variety of measurements of glycaemic variability that exist we chose to use the MAGE (Mean Amplitude of Glycemic Excursion) and the CONGA (Continuous Overall Net Glycemic Action). The MAGE because it is the most widely used glucose variability measurement in published reports. The MAGE calculates the average size of fluctuations between adjacent peaks and nadirs in glucose level, with peaks and nadirs defined as at least +/- 1 SD from mean glucose (Service 1970). Additionally the CONGA-method was used since it is, in contrast to most other analyses, designed specifically to analyse the complex variability of CGMS-curves. The CONGA-measurement is defined as the standard deviation of the differences between the glucose values during the day. (McDonell 2005, Cameron 2010) EasyGV® (Ver 9.0 by Nathan R Hill, ©University of Oxford) was used for calculations.
Paper IV  The 12 patients were examined 3 months before and 4-5 months after surgery using a hyperinsulinemic hypoglycaemic clamp. Symptoms were recorded using the Edinburg hypoglycaemia scale and autonomic efferent activity using the heart rate variability analysis method on ECG-recordings taken during clamp. Patient characteristics are shown in table 3.

Table 4. Anthropometric measures, Means (SD). ^ Weight at day of surgery 112kg, not significantly changed compared with pre-surgery weight at clamp investigation. Body surface calculated according to DuBois and DuBois (1916).

<table>
<thead>
<tr>
<th></th>
<th>Pre-surgery</th>
<th>Post-Surgery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.1 (10.8)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 (0.09)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)^A</td>
<td>116.5 (15.7)</td>
<td>86.4 (16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>40.6 (3.1)</td>
<td>30.1 (4.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>43.3 (7.4)</td>
<td>36.0 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Surface (m²)</td>
<td>2.25 (0.2)</td>
<td>1.96 (0.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Hyperinsulinemic hypoglycaemic clamp

A hyperinsulinemic hypoglycaemic clamp was performed following an overnight fast (water intake allowed). The clamp was conducted in a step-wise manner for 165 minutes, followed by a recovery period (modified after Norjavaara et al (2012). 40 U of human regular insulin (Actrapid, (Novo-Nordisk, Bagsvard, Denmark) 100 U/mL and 12 ml of Albumin (200 mg/mL (20%)) were added to Saline (NaCl 0.9%), final volume 500 ml, giving the concentration of 0.08 U/mL. Following a priming infusion, insulin was infused at a fixed rate, 80 mU/m² body surface/min together with a variable glucose (200 mg/mL) infusion to maintain plasma glucose at levels of 5 mmol/L (90 mg/dL) 0-60min, 4 mmol/L (72mg/dL) 60-90min, 3.2 mmol/L (58 mg/dL) 90-135min and finally 2.7 mmol/L (49 mg/dL) 135-165min.

At 165 minutes insulin-infusion was stopped, and glucose-infusion continued to achieve normal steady glucose above 4.0 mmol/L (72 mg/dL). Plasma glucose was measured every 5 minutes during the clamp with the glucose meter Bayer Contour (Bayer Healthcare, Leverkusen, Germany). Levels of insulin, C-peptide, cortisol, glucagon, growth hormone (GH), epinephrine, norepinephrine, GLP-1, and GIP were measured at 0, 60, 90, 120, 135, 150, and 165 minutes during clamp, whilst free fatty acids and glycerol were measured at 0, 135 and 165 minutes.
Heart Rate Variability (HRV) analyses

As a marker of efferent activity in the autonomic nervous system, heart rate variability (HRV) analyses were performed in the 6 of the 12 subjects who had complete recordings (in 6 subjects there were incomplete recordings). HRV analyses were based on continuous recordings of a single-lead ECG. HRV indices were determined based on power spectrum analysis, using the Welch method (Wiklund 2008). The HRV-indices total spectral power ($P_{tot}$), power of the low-frequency (PLF, 0.04-0.15 Hz), and power of the high-frequency (PHF, 0.15-0.50 Hz,) all log-transformed, were calculated over consecutive 5-minute periods from the complete recording. PHF mainly reflects the parasympathetic activity, PLF reflects a combination of sympathetic and parasympathetic activity, while the ratio PLF/PHF reflects the balance between sympathetic and parasympathetic activity (Task 1996). The HRV analysis was performed by use of the Matlab Software (MathWorks, Natick, Mass., USA).

Edinburgh Hypoglycaemia Symptom Score

The Edinburgh hypoglycaemia score, composed of 11 symptoms statistically validated to closely associate with hypoglycaemia, divided into three areas (autonomic, neuroglycopenic, and malaise) was used throughout the study (Deary 1993). The 11 hypoglycaemic symptoms are; four autonomic: sweating, palpitation, shaking, hunger, five neuroglycopenic: confusion, drowsiness, odd behaviour, speech difficulty, incoordination, and finally two malaise: nausea, and headache. Symptoms are graded between 1 (no symptoms) to 7 (maximum symptoms) at 0, 60, 90, 120, 135, 150, and 165 minutes. To compensate for the possible different symptom-level at start of clamp, symptom score-difference during clamp ($\Delta$-score) was evaluated while absolute levels were not.
Results

**Paper I** The NT-ProBNP levels changed considerable during the first six postoperative days, with a 6-fold (compared with pre-surgery level) peak at day 2 (p=0.003) (Table 5, Figure 4). Levels continued to be increased at one month, 2-fold (103 pg/mL, p=0.008 vs baseline) and finally at 12 months they were increased by 125% (122 pg/mL) (p=0.012 vs baseline).

The change in NT-ProBNP was not correlated to the changes in BMI, glucose, insulin, or HOMA-IR, as examined with linear regression. In figure 5 changes in BMI and NT-ProBNP levels are demonstrated, to show their separate dynamics.

**Table 5.** Baseline characteristics, mean (SD) of 20 morbidly obese subjects (mean age 41 (SD 5.5)) undergoing GBP surgery, and changes in the postoperative period and during follow-up 1 year. P-values compared with preoperative values. D=Day, M=Months.*=p<0.05, **=p<0.01, ***=< 0.001 significance.

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>NT-ProBNP ng/L</th>
<th>Glucose mmol/L</th>
<th>Insulin mU/L</th>
<th>HOMA-IR</th>
<th>BMI kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop</td>
<td>20</td>
<td>54 (39)</td>
<td>5.4 (0.8)</td>
<td>12.0 (4.9)</td>
<td>2.9 (1.2)</td>
<td>44.6 (5.5)</td>
</tr>
<tr>
<td>D1</td>
<td>14</td>
<td>240 (179)</td>
<td>6.4 (1.8)</td>
<td>21.7 (2.8)</td>
<td>6.5 (5.3)</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>11</td>
<td>359 (275)</td>
<td>5.6 (1.3)</td>
<td>11.7 (5.7)</td>
<td>3.0 (1.9)</td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td>14</td>
<td>304 (166)**</td>
<td>4.7 (0.6)</td>
<td>6.4 (3.2)</td>
<td>1.3 (0.5)</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>20</td>
<td>155 (108)</td>
<td>4.8 (0.8)</td>
<td>7.4 (3.7)</td>
<td>1.6 (0.9)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>20</td>
<td>103 (92)**</td>
<td>5.0 (0.6)**</td>
<td>8.9 (4.4)*</td>
<td>2.0 (1.0)**</td>
<td>39.3 (5.4)</td>
</tr>
<tr>
<td>M6</td>
<td>20</td>
<td>100 (85)**</td>
<td>4.8 (0.5)**</td>
<td>7.3 (2.3)**</td>
<td>1.6 (0.6)**</td>
<td>34 (4.2)</td>
</tr>
<tr>
<td>M12</td>
<td>20</td>
<td>122 (138)*</td>
<td>4.8 (0.7)***</td>
<td>6.2 (2.8)***</td>
<td>1.4 (0.7)***</td>
<td>30.5 (4.5)</td>
</tr>
</tbody>
</table>
**Figure 4.** NT-ProBNP levels in 20 patients post GBP surgery. Thick line represents mean values, thin lines individual patients’ levels.

**Figure 5.** BMI and NT-ProBNP during the postoperative year, Means (sem). Changes are not correlated.
**Paper II**  
All five patients showed marked improvements regarding the hypoglycaemias during treatment with GLP-1-analogues. Liraglutide (Victoza®) was used as first-line treatment, but in two patients experiencing side-effects (nausea), treatment was changed to weekly Exenatide (Bydureon®). Exenatide abolished symptoms with fewer side-effects in these two patients. Figure 6 shows the CGMS-curves of 3 of the patients, 2 pre-treatment and 1 case before and after treatment. CGMS has not been performed on all patients pre and post treatment mainly because of the clinical challenge of persuading patients that feel well to perform investigations running over several days.

**A Case 3, pre-treatment.**

**B Case 4, pre-treatment.**

**C Case 5, pre-treatment.**

**D Case 5 on GLP-1-analogue treatment.**

**Figure 6.** 24-h continuous glucose measurements (CGMS) on three cases. Y-axes represent glucose (mmol/l), X-axes represent time.
Paper III  The DS-patients were slightly older, and comprised of more males compared to the GBP-group. BMI, blood pressure or heart rate at time of study did not differ (Table 6).

Table 6. Participants’ characteristics. N=15 per group. Medians (range), BP=Blood Pressure in mm HG, Pulse rate= beats/min.

<table>
<thead>
<tr>
<th></th>
<th>GBP (years)</th>
<th>DS (years)</th>
<th>Control (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46 (36-63)</td>
<td>42 (24-63)</td>
<td>53 (25-74)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>12/3</td>
<td>7/8</td>
<td>12/3</td>
</tr>
<tr>
<td>Years since surgery</td>
<td>1.5 (1.0-2.0)</td>
<td>2.0 (1.1-7)</td>
<td>n/a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 (26-38)</td>
<td>32 (26-38)</td>
<td>30 (28-35)</td>
</tr>
<tr>
<td>BMI loss (kg/m²)</td>
<td>13 (7-17)</td>
<td>23 (9-27)</td>
<td>n/a</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>115 (110-150)</td>
<td>120 (100-140)</td>
<td>125 (110-150)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>80 (65-100)</td>
<td>70 (60-90)</td>
<td>80 (75-95)</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>66 (60-81)</td>
<td>64 (56-76)</td>
<td>80 (63-76)</td>
</tr>
</tbody>
</table>

Glucose homeostasis

The two post-surgery groups exhibited quite different glucose curves, both as compared with each other and with controls. The GBP-group showed a high variability and the DS-group showed a low variability as compared with the controls’ normal variability. This was illustrated with both MAGE and CONGA variability measurements (Figure 7). Typical examples of glucose curves to illustrate the differences in variability can be seen in Figure 8.
Figure 7. Glucose variability scores, mean values (sem) per group. Statistical differences, p<0.05, are marked with * versus controls, and ** for GBP versus DS.

CGMS, 3 control subjects
Figure 8. Examples of typical CGM-curves, three per group. Note the high variability in the GBP-group in contrast to the low variability in the DS-group, controls’ variability in between.
Considering hypoglycaemias, both post-surgery groups exhibited a greater number of episodes and time with glucose <3.3 mmol/L compared to controls (that had no glucose-values <3.3 mmol/L at all, demonstrating the stability of the CGMS). The GBP-group had 42 min/24h (2.9 % of time) and the DS-group 85 min/24h (5.9 % of time) with glucose below 3.3 mmol/L. Time was halved for both groups when examining for glucose-values < 2.8 mmol/L (Table 7).

97% of hypoglycaemic episodes occurred in the postprandial state, i.e. fasting hypoglycaemia was very rare. Symptoms without measured hypoglycaemia were similarly very rare; only one GBP patient (three episodes during the 3 days of recording) reported this. Interestingly, most hypoglycaemic episodes were asymptomatic, a mere 20 % were symptomatic.

Regarding difference in frequency of meals between patients with and without hypoglycaemias, this was neither significant nor correlated to the number of hypoglycaemic episodes. The post-surgery groups consumed more meals/day than controls, which would be in accordance with post-surgery diet-instructions.

Table 7. Continuous glucose measurements results, hypoglycaemias, and meals/day. Means (SEM). n=15 in each group, Glucose in mmol/L. ΔGlucose = (fasting glucose - nadir (CGM) glucose). Glucose 3.3 mmol/L = 60 mg/dL, 2.8 mmol/L = 50 mg/dL. Statistical differences, p<0.05, are marked with * versus controls, and # for GBP versus DS. “Hypoglycaemic episodes with symptoms” gives the percent of the hypoglycaemic episodes that were accompanied with symptoms.

<table>
<thead>
<tr>
<th></th>
<th>GBP</th>
<th>DS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average glucose</td>
<td>5.9 (0.3) #</td>
<td>4.8 (0.2) *</td>
<td>6.2 (0.3)</td>
</tr>
<tr>
<td>Peak glucose</td>
<td>10.5 (0.5) #</td>
<td>7.1 (0.3) *</td>
<td>9.1 (0.5)</td>
</tr>
<tr>
<td>Minimum glucose</td>
<td>3.3 (0.3) *</td>
<td>3.1 (0.2) *</td>
<td>4.1 (0.1)</td>
</tr>
<tr>
<td>ΔGlucose</td>
<td>2.0 (0.3)</td>
<td>1.5 (0.2)</td>
<td>1.8 (0.2)</td>
</tr>
<tr>
<td>Minutes/day &lt;3.3</td>
<td>42 (15) *</td>
<td>85 (46) *</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Minutes/day &lt;2.8</td>
<td>21 (11) *</td>
<td>39 (31) *</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N of postprandial hyp-</td>
<td>0.6 (0.2) *</td>
<td>1.0 (0.3) *</td>
<td>0</td>
</tr>
<tr>
<td>glycaemias/24h</td>
<td>22%*</td>
<td>20%*</td>
<td>0</td>
</tr>
<tr>
<td>Hypoglycaemic episodes</td>
<td>7.5 (0.5) *</td>
<td>7.3 (0.5) *</td>
<td>6.0 (0.3)</td>
</tr>
<tr>
<td>with symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean glucose and HbA1c levels were lower in the post-surgery groups than in controls, and lowest in the DS-group. GH levels were higher in the DS-group compared to the controls (Table 8). No differences regarding laboratory parameters between subjects who subsequently presented with and without hypoglycaemic episodes were found, except for fasting glucose in post-
GBP subjects with and without hypoglycaemia (glucose 4.5 mmol/l (81 mg/dl) and 5.2 mmol/l (93.6 mg/dl) respectively), p 0.03.

Table 8. Metabolic parameters, inflammatory markers, and counter-regulatory hormones measured at baseline prior to the start of CGM examination. Means (SEM), n=15. Statistical differences, p<0.05, are marked with * versus controls, and # for GBP versus DS.

<table>
<thead>
<tr>
<th></th>
<th>GBP</th>
<th>DS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>3.6 (1.6)</td>
<td>1.3 (0.4)</td>
<td>2.8 (0.7)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39.1 (0.6)</td>
<td>38.0 (1.4)</td>
<td>38.5 (0.7)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.2 (0.2)#</td>
<td>3.6 (0.1)*</td>
<td>5.3 (0.2)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 (0.07)#</td>
<td>1.1 (0.07)*</td>
<td>1.5 (0.08)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.2 (0.2)#</td>
<td>1.9 (0.14)*</td>
<td>3.0 (0.4)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 (0.2)</td>
<td>1.0 (0.1)</td>
<td>1.6 (0.3)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.3 (0.2)</td>
<td>4.6 (0.1)</td>
<td>5.9 (0.2)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>95 #*</td>
<td>83 *</td>
<td>106</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>36 (0.9)</td>
<td>29 (1.3)</td>
<td>38 (1.3)</td>
</tr>
<tr>
<td>HbA1c (NGSP%)</td>
<td>5.4 #</td>
<td>4.8 *</td>
<td>5.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.2 (0.5)#</td>
<td>1.0 (0.2)*</td>
<td>3.2 (0.6)</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>11.1 (2.6)#</td>
<td>5.0 (0.8)*</td>
<td>12.3 (2.2)</td>
</tr>
<tr>
<td>fP-Glukagon (ng/L)</td>
<td>66.9 (6.1)</td>
<td>77.5 (5.7)</td>
<td>77.5 (6.8)</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>348 (28.2)</td>
<td>295 (31.8)</td>
<td>362 (25)</td>
</tr>
<tr>
<td>Growth hormone (µg/L)</td>
<td>1.9 (0.6)</td>
<td>3.1 (0.9)*</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>3-Betahydroxybutyrate (mmol/L)</td>
<td>0.06 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
<td>0.48 (0.06)</td>
<td>0.43 (0.04)*</td>
<td>0.55 (0.03)</td>
</tr>
<tr>
<td>P-Leptin (µg/L)</td>
<td>14.5 (2.1)*</td>
<td>8.7 (2.1)*</td>
<td>28.9 (3.6)</td>
</tr>
<tr>
<td>P-Adiponectin (mg/L)</td>
<td>13.5 (1.4)</td>
<td>20.1 (3.8)*</td>
<td>11.4 (1.5)</td>
</tr>
</tbody>
</table>

Paper IV 12 patients (8 women, 4 men), non-diabetic obese subjects with mean age 43 years, mean pre-surgery BMI 41 kg/m², with significantly
lower post-surgery BMI, 30 kg/m² Table 9. Weight at first clamp examination was 116.5 kg and did not differ from weight at day of surgery, 112.2 kg. Body fat percent and body surface decreased pre- vs post-surgery.

Table 9. Anthropometric measures, Means (SD). Body surface according to (DuBois 1916). BP = Blood Pressure in mm Hg, Heart rate in beats/minute.

<table>
<thead>
<tr>
<th></th>
<th>Pre-surgery</th>
<th>Post-Surgery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.1 (10.8)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 (0.09)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>116.5 (15.7)</td>
<td>86.4 (16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>40.6 (3.1)</td>
<td>30.1 (4.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>43.3 (7.4)</td>
<td>36.0 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Surface (m²)</td>
<td>2.25 (0.2)</td>
<td>1.96 (0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>126 (12)</td>
<td>121 (12)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>81(9)</td>
<td>75(5)</td>
<td>ns</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>65(8)</td>
<td>56(10)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fasting metabolic parameters indicated improved glucose metabolism post-surgery with lower glucose, 5.9 mmol/L (106 mg/dL) and 5.4 mmol/L (97 mg/dL), HbA1c 37.4 mmol/mol (5.6%) vs 32.8 mmol/mol (5.2%), fasting insulin 22.3 vs 8.1 mU/L, and HOMA-IR 6.32 vs 1.96 respectively. In line, lipids were decreased; cholesterol 4.7 vs 3.8 mmol/L, LDL-cholesterol 3.1 vs 2.3 mmol/L and triglycerides 1.7 vs 1.1 mmol/L. And finally, renal parameters were improved with lowered Creatinine; 75.8 to 66.7 umol/L, and increase in eGFR from 82.2 to 88.1 mL/min/1.73.

Edinburgh hypoglycaemia symptom score

Patients graded symptoms according to the Edinburgh Hypoglycaemia Score and final score was 24.0 (SD 6.9) before surgery and 18.5 (SD 5.2) after surgery. The increase in total added symptom score from beginning to end of clamp (Δ) was in the pre-surgery group 10.7 compared to 5.2 in the post-surgery group, rendering a significant difference in Δ-increase, p 0.02 (Figure 9).
Figure 9. Difference of increase in symptom score during clamp, p=0.02.

Hypoglycaemic clamp

Mean plasma glucose levels and glucose infusion rate per lean body mass during the clamps are depicted in Figure 10. Note that the amount of glucose required for every time period was significantly higher during the post-surgery clamp and that during the hypoglycaemic phase of the clamp, glucose-infusion had to be raised considerably during the post-surgery examination. Insulin levels plateaued at steady levels, differing with about 25% lower level post-surgery, reflecting the increased insulin sensitivity and clearance. C-peptide levels were downregulated as an expected response to the supra-physiological insulin levels.

The systolic blood pressure change from initial level to peak level ($\Delta$) was significant both pre and post-surgery. Further there was an increase in heart rate in both groups, from 65 to 75 and 56 to 69 beats/minute post-surgery, respectively.

Lipolysis markers FFA and glycerol were analysed at 0, 135, and 165 minutes of clamp (Table 11). They exhibit a similar pattern, with equal fasting levels at start of clamp, and then significantly higher levels at the pre-surgery examination during hypoglycaemic phase of clamp.
Table 10. Parameters during hypoglycaemic clamp examination. Means (SD), Symptom Score refer to the Edinburgh hypoglycaemia score, Δ (delta) denote rise from beginning to peak symptom score during clamp, BP = Blood Pressure in mm Hg, HR – Heart Rate, M-Value during euglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Symptom Score</td>
<td>10.7(6.4)</td>
<td>5.2(4.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic BP -Peak</td>
<td>132 (13)</td>
<td>125 (12)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Δ Systolic BP</td>
<td>6 (9)</td>
<td>4(6)</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic BP - Peak</td>
<td>82(9)</td>
<td>78(5)</td>
<td>ns</td>
</tr>
<tr>
<td>Δ Diastolic BP</td>
<td>1(6)</td>
<td>3(6)</td>
<td>ns</td>
</tr>
<tr>
<td>HR Peak(Beats/min)</td>
<td>75(9)</td>
<td>69(10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Δ HR (Beats/min)</td>
<td>10(9)</td>
<td>13(5)</td>
<td>ns</td>
</tr>
<tr>
<td>M-Value (mg/kg LBM/min)</td>
<td>2.36(1.22)</td>
<td>4.19(1.36)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Figure 10. Means (SD) A: Glucose-levels during the different plateaus of the clamp, pre- and post-surgery. B: Insulin levels during the different plateaus of the clamp, pre- and post-surgery. C: C-peptide levels during the different plateaus of the clamp, pre- and post-surgery. D: Glucose Infusion Rate per Lean Body Mass during clamp, pre- and post-surgery, for time-periods noted. All time-points differ significantly. Note the marked increased demand for glucose during hypoglycemia during the post-surgery examination.
Counter-regulatory hormones during clamp

Counter-regulatory hormones are depicted in Figure 11 and table 11. Excepting Cortisol who responded at 135 min both pre and post-surgery, all hormones responded earlier during pre-surgery clamp with a significant increase at 120 min compared to fasting levels, while hormones responded significantly at 135 minutes post-surgery.

Glucagon levels were markedly decreased post-surgery, both at all time-points and as measured with area under curve during hypoglycaemia; AUC\textsubscript{hypo}, (90-165 min) p< 0.001.

Cortisol levels were similar during normoglycemic phase of clamps, with the cortisol response being stronger pre-surgery than post-surgery from 120 minutes and onward.

Catecholamines epinephrine and norepinephrine followed the same pattern as other counter-regulatory hormones in that their responses were lower at post-surgery clamp.

The pattern of GH-secretion was more complex. Levels were similar up to 120 minutes, from where the levels during pre-surgery clamp increased significantly with the levels during post-surgery clamp responding significantly at 135 minutes. However, when reaching 150 minutes, the GH response is markedly higher in the post-surgery group, reaching a 3-fold higher level at 165 min in the post vs the pre-surgery group, 5.9 vs 15.2 ug/L.

Incretin Hormones during clamp

Both before and after surgery, GLP-1 levels increased significantly during hypoglycaemia, but at a later time post-surgery; at 150 minutes compared to 135 minutes. The AUC\textsubscript{hypo} was larger before surgery, corresponding to the more attenuated curve and later response after surgery.

Likewise, the levels of GIP increased during the hypoglycaemic part of clamps both before and after surgery. Levels started to differ significantly at 120 min where levels increased at pre-surgery clamp, whereas during the post-surgery clamp levels increased later and to lower levels.
Figure 11 Means (SEM) Counterregulatory Hormones, Incretin Hormones, and Heart Rate Variability during hypoglycemic clamp pre- and post-surgery. * denotes significant difference. Target glucose level during clamp in top. AUC$_{\text{hypo}}$ = Area under curve for hypoglycemic period (90-165 min) diagrams inserted. A, Glucagon. B, Cortisol. C, Epinephrine. D, Norepinephrine. E, Growth Hormone. F, Heart Rate Variability analysis; P$_{\text{LF}}$/P$_{\text{HF}}$ (Power Low Frequency / Power High Frequency). Note a marked reduction of the response during post-surgery clamp reflecting a downregulated sympathetic nervous response. G, GLP-1. H, GIP.
Table 11. Counter regulatory Hormones and lipolytic markers during hypoglycaemic clamp. Means (SD). AUC – Area Under Curve. Δ - Difference between initial and Peak level.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Surgery</th>
<th>Post-Surgery</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon (pmol/L) - Δ</td>
<td>30.4(19.2)</td>
<td>15.2(10.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucagon AUC&lt;sub&gt;hypo&lt;/sub&gt; (pmol/L*min)</td>
<td>2132(859)</td>
<td>1077(483)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cortisol (nmol/L)- Δ</td>
<td>391.9(195.2)</td>
<td>300.2(178.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cortisol AUC&lt;sub&gt;hypo&lt;/sub&gt; (nmol/L*min)</td>
<td>28936(6744)</td>
<td>21248(5609)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GH (ug/L)- Δ</td>
<td>7.0(4.2)</td>
<td>14.1(8.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GH (ug/L*min) AUC&lt;sub&gt;hypo&lt;/sub&gt;</td>
<td>270.8(122.0)</td>
<td>436.1(280.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Epinephrine (nmol/L)- Δ</td>
<td>2.8(1.2)</td>
<td>2.2(1.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Epinephrine AUC&lt;sub&gt;hypo&lt;/sub&gt; (nmol/L*min)</td>
<td>130.3(62.4)</td>
<td>88.7(35.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)- Δ</td>
<td>1.3(0.8)</td>
<td>0.9(0.6)</td>
<td>0.13</td>
</tr>
<tr>
<td>Norepinephrine AUC&lt;sub&gt;hypo&lt;/sub&gt; (nmol/L*min)</td>
<td>145.9(49.1)</td>
<td>112.8(42.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GLP-1-(pM) Δ</td>
<td>28.6(25.2)</td>
<td>17.5(15.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>GLP-1 AUC&lt;sub&gt;hypo&lt;/sub&gt; (pM*min)</td>
<td>2621(2162)</td>
<td>1245(1194)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GIP (pg/mL) Δ</td>
<td>42.2(43.6)</td>
<td>14.2(10.6)</td>
<td>0.049</td>
</tr>
<tr>
<td>GIP AUC&lt;sub&gt;hypo&lt;/sub&gt; (pg/mL*min)</td>
<td>4691(2437)</td>
<td>2905(984)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Free Fatty Acids AUC&lt;sub&gt;hypo&lt;/sub&gt; (uM*min)</td>
<td>1715(826)</td>
<td>800(793)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycerol AUC&lt;sub&gt;hypo&lt;/sub&gt; (uM*min)</td>
<td>1374(458)</td>
<td>814(471)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Heart Rate Variability Analysis

The response of the autonomic nervous system was evaluated using heart rate variability (HRV) analysis in 6 patients who had complete recordings. When comparing HRV during post-surgery and pre-surgery, there were significant increases in HRV-indices P<sub>TOT</sub> (total variability measure) and components (P<sub>LF</sub>, P<sub>HF</sub>) during both normoglycaemia and hypoglycaemia. P<sub>LF</sub>/P<sub>HF</sub> ratio, a measure of sympathetic/parasympathetic balance, was reduced during hypoglycaemia post-surgery. Moreover, P<sub>LF</sub>/P<sub>HF</sub> showed less increase from the normoglycaemic part of the clamp to hypoglycaemic part post-surgery as compared to the response during the pre-surgery recording (Fig 11). R-R interval was longer post-surgery, corresponding to the decrease in heart rate observed.
Discussion

Paper I
The NT-ProBNP levels during the first postoperative week exhibit an immediate increase, with a 6-fold rise peak on day 2. As comparison regarding quick changes in the natriuretic peptide system; in normal weight subjects BNP increased only 3-fold after major thoracic surgery and was in that study correlated to weight increase and increase in fluid balance (Cagini 2011).

NT-ProBNP then increased 2-fold at the one month control and finally it was 125 % of pre-surgical level at the one year control. This is contrary to studies describing the changes after diet-induced weight loss, when levels fall (Minami 2000). Earlier, our group has observed that patients do not alter their NT-ProBNP-levels after four weeks of low calorie diet, but do so significantly four weeks after GBP-surgery, in spite of similar weight loss (7-8kg) (Kullberg 2011). Patients after gastric band surgery had one year post-surgery, with a weight loss of 19.5 kg, similarly decreased 40 % in NT-ProBNP (Hanusch-Enserer 2003).

In line with the present study, Changchien et al have reported that NT-ProBNP increased from 51 to 156 pg/mL with BMI decreasing from 46 to 31 kg/m² in patients examined one year after GBP surgery, regrettably the fall-out was 65% in that study (Changchien 2011).

These findings suggest that the response in the natriuretic system, which usually is reduced in obesity (Minami 2004), is altered by GBP surgery. This alteration supposedly comes quickly considering the rapid and exaggerated NT-ProBNP increase during the first postoperative week, and would then not be driven by weight change but rather by some neuronal/hormonal change brought about by the surgical relocation of the intestinal system.

A gut-heart axis has been proposed following the find that GLP-1-receptors exist on the (mouse) cardiac atria and stimulation of these increases ANP-production with following natriuresis and lowered blood pressure (Kim 2013). These findings were however not reproduced in men; when testing GLP-1-infusion for 2 hours natriuresis did increase significantly, but no increase in ANP or BNP levels could be detected (Skov 2014). However, the latter study was not designed to evaluate the hypothesis of a gut-heart axis, and there are questions by the authors themselves as to if the samples were
handled correctly. If a lipid accumulated human heart has decreased ability to synthesise BNP in analogy with a mouse heart as reported (Bartels 2010) is not known. If so, it could possibly be a part of the explanation for the chronic change but not explain the immediate change in NP response pattern post GBP surgery.

Gandolfini et al. has since the present study’s publication investigated 34 patients before and after GBP biochemically and with heart-ultrasonography, and report an improvement (i.e. decrease) in heart rate, left ventricular mass and E/A-ratio one year after surgery. Authors could in addition detect a 71 % increase in NT-ProBNP that was correlated with an improvement in heart rate variability and an 18-fold increase in postprandial GLP-1 that was correlated with an improvement in blood pressure (Gandolfini 2015).

**Paper II-IV**

In these three papers postprandial hypoglycaemias are investigated with regards to prevalence, counter-regulatory mechanisms, and treatment.

**In Paper II,** a clinical report of 5 cases, we describe the promising effect of off-label treatment with GLP-1 analogues to reduce the problems with postprandial hypoglycaemias post GBP. The mechanism behind the effect of the GLP-1-analogue to counter hypoglycaemia is not entirely clear but can be speculated upon; both GLP-1 and GIP have been reported to stimulate glucagon release from α-cells in rat (Ding 1997). There is further the question of receptor promiscuity, since Liraglutide and Exenatide are not entirely exclusive to the GLP-1-receptor. Binding to either or both of the GIP or GLP-2 receptor on the α-cells may occur, resulting in glucagon-release from α-cells during hypoglycaemia (Christensen 2014, Meier 2006). Degn et al. report of an increased glucagon response during hypoglycaemic clamp in subjects treated with Exenatide compared with placebo (Degn 2004). Likewise, a faster and initially higher glucagon response is reported for the GLP-1-analogue Albiglutide, when tested during hypoglycaemic clamp, even though the total AUC of glucagon was equal between treated and placebo-groups (Hompesch 2015).

In addition, there are reports of a 38 % increased glucagon release during hypoglycaemia in subjects treated with DPPIV-inhibitors (inhibiting the degradation of endogenous GLP-1) (Ahren 2009). This would correspond to a mechanism where a supra-physiological concentration of GLP-1 at meals, especially after high carbohydrate meals, stimulates a supernormal insulin release that overshoots the body’s need and thus causes a hypoglycaemia. The endogenous GLP-1 peak is rapidly degraded, but the long-acting exogenous GLP-1 analogues or DPPIV-inhibitor treat-
ment might then act to strengthen the postprandial glucagon response to hypoglycaemia.

**In paper III** Three groups of subjects; 15 patients post-GBP, 15 patients post-DS, and 15 non-diabetic obese controls were examined with CGMS during 3 days of normal activity. Based on MAGE and CONGA variability comparisons the post-GBP group demonstrates a high variability compared to the DS-group and controls. The DS-group exhibited a stable glucose curve with little variability. Further, both groups exhibited a significant amount of hypoglycaemic episodes compared to controls. The majority of episodes, 80%, were asymptomatic.

The different glucose-curve variability following these two surgeries is probably explained in the different location where glucose is first presented and absorbed in the small intestine; i.e. in the proximal jejunum in the GBP patients and in the distal ileum in the DS patient. Saccharide degradation by the pancreatic amylase and the intestinal disaccharidases and oligosaccharidases (which occur in declining concentrations down the small intestine) is greatly diminished and thereby absorption is decreased. This is especially evident for the DS subjects with their low non-variable glucose curves.

It thus seems that the post-bariatric surgery patient is at a quite high risk of developing asymptomatic episodes of hypoglycaemia, when using the glucose limits defined for healthy people. The effect of these asymptomatic hypoglycaemic episodes deserves further studies. Recently, a study describing the effect on CGMS-curves during low carbohydrate diet, mixed meal test, and ordinary diet demonstrated that the glucose variability was reduced during low carbohydrate diet compared with ordinary diet. During mixed meal test, glucose values in the hypoglycaemic range was in fact overestimated by about 1.1 mmol/L compared with plasma values, thereby indicating that low values might be even more common than earlier reported and what was found in the present study. CGMS-device Medtronic IPRO-2 was used in both this study and the present study paper III (Nielsen 2016).

**Paper IV** This study shows that the symptoms, counter-regulatory, incretin, lipolytic, and sympathetic nervous responses to hypoglycaemia post-GBP-surgery are markedly downregulated. Considering the global downregulation of the counter-regulatory system, this alteration would most probably be connected to a brain adaptation to lower glucose levels post-GBP-surgery. Interestingly, the results demonstrate significantly increased levels of GLP-1 and GIP in response to hypoglycaemia, implicating a thus far unknown role for the incretins, at least GLP-1, in the hypoglycaemic response.
The counter-regulatory glucagon response to hypoglycaemia is mediated via several mechanisms as low glucose, low levels of insulin, epinephrine (Farngren 2014), autonomic nervous input from both parasympathetic and sympathetic fibres (Havel 1993), GIP (Christensen 2011), and GLP-2 (Meier 2006). Autonomic input plays a major role, when blocking the autonomous input, glucagon-release was decreased by 75% in hypoglycaemia (Christensen 2011). Recently, Lund et al reported the existence of physiological levels of glucagon in total pancreatectomised patients, indicating a secretion of glucagon from other cells than α-cells. This secretion would most probably come from L-cells since both glucagon and incretins GLP-1/2 are derived from pro-glucagon (Lund 2015). An effect that might perhaps be related to the increase in postprandial glucagon secretion reported in the post-GBP-patient (Salehi 2014).

The cortisol and catecholamine responses are also markedly attenuated during clamp. Regarding the GH-response this hormone exhibits a more complex such; similarly with other counter-regulatory hormones it exhibits an earlier response during pre-surgery than post-surgery examination. The response during post-surgery clamp is conversely to the other hormones significantly higher though. This would probably represent a partial normalization of the GH-response, connected with the known downregulation of GH in obesity (Eden Engstrom 2006), following the participants’ loss of weight since surgery (mean 10 BMI-units). Response of GH is in fact still probably attenuated post-surgery if comparing to the expected response seen in healthy subjects, e.g. Mitrakou et al report a mean rise in GH to 25 ng/mL in response to hypoglycaemia (Mitrakou 1991), compared with the rise to 15 ng/mL in the present study from a similar initial level.

To our knowledge, incretin response to hypoglycaemia has not previously been reported in humans. Pre-surgery, GLP-1 doubled during the hypoglycaemic phase. This twofold increase is of the same order as the GLP-1 response to hyperglycaemia after a mixed meal test in healthy controls (Dirksen 2013). The basal fasting level of GLP-1 was lower post-surgery as was the total response to hypoglycaemia. This is in sharp contrast to the marked increase in GLP-1-level typically seen during a meal-stimulated hyperglycaemic phase post-surgery (Holdstock 2008). GLP-1 stimulates insulin secretion primarily via parasympathetic innervation (Balkan 2000), and its effect on glucagon secretion is primarily via nervous circuits (Gotoh 2013). Innervation of L-cells could possibly involve GLP-1 in the hypoglycaemic response (Hansen 2004). In addition, GLP-1 has been reported to stimulate glucagon exocytosis in rat α-cells (Ding 1997), and human α-cells have been shown to express GLP-1-receptors (de Marinis 2010).
GIP levels showed a marked difference between clamps, much like GLP-1, where it increases during hypoglycaemia in pre-surgery clamp but exhibit a more flat response curve during post-surgery clamp. When infusing GIP in physiological doses during hypoglycaemia, glucagon increased 1.5 times more than in controls, showing the marked effect of GIP on glucagon response in hypoglycaemia (Christensen 2011). It has further been shown that treatment with the DPPIV-inhibitor Vildagliptin can increase the glucagon response to hypoglycaemia significantly, and the authors interpret this as an effect mainly of GIP (Malmgren 2015).

The rate of lipolysis was downregulated post-surgery, as evaluated with FFA and Glycerol, probably reflecting the higher insulin sensitivity coupled with the decreased sympathetic stimulation.

The efferent autonomic nervous response to hypoglycaemia was evaluated using HRV in the present study, and it shows a decreased and later response in the efferent sympathetic nervous system as measured with Power analysis ($P_{HF}/P_{LF}$).

It seems likely that brain adaptation to a lowered glucose level would ensure the brain’s need for glucose, and simultaneously downregulate the central nervous response to lowered glucose levels. Such resetting is known to occur as mentioned above also in insulin treated diabetics, pregnant diabetic women, and in normal subjects following exercise, illustrating the brain’s capability to increase its glucose uptake under several different physiological settings. Experimentally, this adaptation process seems to be quick; the threshold for cognitive impairment during 4 consecutive days of hypoglycaemic clamp was on first day 3.6mmol/L, and decreased to 2.5mmol/L on day 4 (Boyle 1994).

One important component of brain adaptation to a lowered glucose balance might be glucokinase activity, especially in the hypothalamus. Glucokinase activity in the arcuate nucleus regulates energy homeostasis; increased activity drives food intake (especially glucose) and weight increase, and vice versa (Hussain 2015). Similarly, in ventromedial hypothalamic nucleus, glucokinase activity regulate the counter-regulatory response, more specifically increased activity attenuates hormonal counter-regulatory response to insulin induced hypoglycaemia (Levin 2008). In line, it is reported that after insulin induced hypoglycaemia, glucokinase in ventromedial hypothalamus is increased and this increased responsiveness leads to blunted counter-regulation to hypoglycaemia via the adrenomedulla (other hormones not measured) (Kang 2008). Moreover, the gene expression for glucokinase, GLUT-2, and GLP-1-receptor in hypothalamus is increased in food-restricted rats (Zhou 2003), pointing to a similarity in the regulation of gluicosensing in counter-regulation and food intake controlling areas of hypo-
thalamus. Possibly, altered glucokinase activity driven by a lowered glucose level post-surgery could contribute to both a lowered counter-regulation and lowered food intake drive, aiding in weight loss and weight maintenance. These areas deserve further studies.
Conclusions

**Paper I**  
Gastric Bypass surgery immediately alters the balance of NT-ProBNP secretion to a higher level that is consistent over time. This adaptation was not correlated to weight loss, and would be important to consider when investigating post-GBP patients for heart failure.

**Paper II**  
GLP-1-analogue treatment was useful in the treatment of postprandial hypoglycaemias post-GBP-surgery. Considering the side-effects with other proposed treatments, GLP-1-analogue treatment should be considered early in treatment if diet-regiments fail.

**Paper III**  
Postprandial hypoglycaemias post-GBP and post-DS surgery were more common than previously acknowledged; occurring in about 50% of GBP-patients and 75% of DS-patients. These episodes were most often asymptomatic, probably because of adaptation to the lowered glucose values. Further, the GBP-subjects had very variable glucose curves, which might explain sensation of hypoglycaemia even in the absence of detectable such.

**Paper IV**  
Symptomatic, counter-regulatory hormonal, incretin, lipolytic, and sympathetic nervous responses to hypoglycaemia were markedly down-regulated post-GBP-surgery. Given the general down-regulation, this indicates that the post-GBP patient has adapted to a lowered glucose level centrally, possibly by increased central nervous glucose sensing and glucose uptake.

Artikel I   I denna studie undersöktes hur GBP operationen påverkar nivåerna av hjärtsviktshormonet NT-ProBNP på 20 patienter under ett år. Nivåerna förändras dramatiskt direkt efter operation, och kvarstår förhöjda under ett år från operation. Dessa förändringar är inte relaterade till förändring i vikt, utan påverkas troligen av de hormonella faktorer som ändras omedelbart efter operation. Detta faktum är viktigt att beakta när patienter som genomgått GBP bedöms för hjärtsvikt eller annan hjärtåkomma.

Artikel II   Baserat på GLP-1-analogers kända glukosstabiliserande effekt provades denna behandling på fem patienter med uttalade besvär av postprandiella hypoglykemier (anfall av lågt blodsocker efter måltid) där övrig behandling ej hjälp. I studier finns beskrivet att svaret av det glukos-höjande mothurmonet glukagon stigit mer hos patienter som behandlats med GLP-1-analog jämfört med placebo under hypoglykemi. Behandling med GLP-1-analoger fungerade väldigt väl hos dessa patienter, och effekten beskrivs med hjälp av kontinuerliga glukos mätningar.
Artikel III  För att utröna hur vanligt det är med hypoglykemier efter överviktskirurgi studerades 15 patienter efter GBP, 15 efter DS, och 15 obesa kontroller med kontinuerlig glukosmätning under 3 dygn, samtidigt som födointag och symptom registrerades. Resultatet visade att hypoglykemier är väldigt vanligt förekommande, GBP-patienter spenderar ca 40 minuter per dag med glukos under 3,3mmol/L, och DS-patienter c:a 80 minuter. Totalt sett drabbades 50 % av GBP-patienterna och 75 % av DS-patienterna av hypoglykemier. 80% av dessa var dock asymptomatiska, vilket indikerar en väldigt hög grad av nedreglering av symptom på hypoglykemi. Vidare kunde ses en väldigt hög variabilitet, dvs svängande glukos, i GBP-gruppen, medan DS-gruppen upprisade en väldigt stabil glukos-kurva.

Artikel IV  På basen av hur vanligt det i Artikel III visat sig vara med hypoglykemier undersökes här motsvarat på hypoglykemi genom att undersöka 12 patienter före och efter GBP med en hypoglykem clamp (en undersökning där blodsockret sänks under kontrollerade former med hjälp av insulindropp). Resultatet visade att motsvaret är generellt sänkt, såväl symptom, moothermoner (glukagon, cortisol, adrenalin, noradrenalin, och GH), inkretinhormoner (GLP-1 och GIP), och lipolys (glycerol och fria fettsyror), som svaret i det sympatiska nervsystemet. Detta pekar på att patienter efter GBP troligen har en nedreglering i centrala nervsystemet av svaret på hypoglykemi, möjligens beroende av ett ökat intag av glukos till glukos-avkännande nervceller i hypotalamus.
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