Brain-gut-adipose interplay in the antidiabetic effects of gastric bypass surgery

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Abstract
Almby, K. 2024. Brain-gut-adipose interplay in the antidiabetic effects of gastric bypass surgery. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 2005. 65 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1996-4.

Gastric bypass surgery (GBP) leads not only to considerable and consistent weight loss but to a number of beneficial metabolic effects, often including a swift remission of type 2 diabetes (T2DM). Increases in the gut hormone GLP-1 are considered central to this effect, although several other mechanism are likely involved. One complication to GBP is post-bariatric hypoglycaemia (PBH), where the individual suffers from episodes of low blood sugar after meals. The mechanism behind this is incompletely understood.

Previous research has reported an attenuation of the counterregulatory response to hypoglycaemia in patients after GBP. Many hypoglycaemic episodes also appear to be asymptomatic. Together, this has led to the hypothesis that GBP and PBH may involve an adaptation to lower blood glucose levels, a lowered glycaemic set point. As much of hypoglycaemia counterregulation involves the central nervous system (CNS), such an adaptation would presumably involve neuroendocrine mechanism. Experimental treatment with GLP-1 receptor agonists (GLP-1RA) has been reported as successful against PBH, which is paradoxical as GLP-1RA stimulate insulin release.

The aim of this thesis is to further explore the metabolic changes after GBP that may influence glycaemic control. In Paper I, euglycaemic-hypoglycaemic clamps were used to assess whether infusion with GLP-1RA affects the counterregulatory response to hypoglycaemia after GBP. In Paper II, normoglycaemic-hypoglycaemic clamps were performed before and after GBP during simultaneous brain imaging with fMRI and FDG-PET techniques, cognitive testing and assessment of counterregulatory hormones. Paper III details the time course of metabolic changes after GBP in patients with previous T2DM with focus on adipose tissue, including gene expression, and possible anti-inflammatory effects. Paper IV approaches the same question as Paper I, this time in the setting of a standardized meal test. All papers include assessment of heart rate variability (HRV) as a potential reflection of autonomic nervous system (ANS) activity.

In Paper I, we do not find indications that GLP-1RA affects counterregulatory hormones, but that it may affect ANS activation during hypoglycaemia. In contrast, Paper IV reports higher cortisol levels with GLP1-RA after a meal, and indications of ANS effects, but no effect on post-prandial glucose levels. Results from Paper II support the hypothesis that GBP attenuates hormonal counterregulatory responses and affects how the CNS responds to hypoglycaemia. In Paper III we report sustained improvements in glucose uptake in adipocytes, potentially indications of decreased low-grade inflammation and signs of transient increases in parasympathetic activity.

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List of Papers

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

I. Almby, K.E.*, Abrahamsson, N.*, Lundqvist, M.H., Hammar, U., Thombare, K., Panagiotou A., Karlsson, F.A., Sundbom, M., Wiklund, U., Eriksson, J.W. (2018) Effects of GLP-1 on counterregulatory responses during hypoglycaemia after GBP surgery. *European Journal of Endocrinology*, 181(2):161–171

II. Almby, K.E.*, Lundqvist, M.H.*, Abrahamsson, N., Kvernby, S., Fahlström M., Pereira, M.J., Gingnell, M., Karlsson, F.A., Fanni, G., Sundbom M., Wiklund, U., Haller, S., Lubberink, M., Wikström, J., Eriksson, Jan W. (2021) Effects of Gastric Bypass Surgery on the Brain. Simultaneous Assessment of Glucose Uptake, Blood Flow, Neural Activity and Cognitive Function during Normo- and Hypoglycaemia. *Diabetes*, 70(6): 1265-1277

III. Almby, K.E.*, Katsogiannos, P.*, Pereira M. J., Karlsson F. A., Sundbom M., Wiklund, U., Kamble, P.G., Eriksson, J.W. (2021) Time course of metabolic, neuroendocrine and adipose effects during two years of follow-up after gastric bypass surgery in patients with T2D. *The Journal of Clinical Endocrinology & Metabolism*, 106(10): e4049-e4061

IV. Almby, K.E., Wiklund, U., Lundqvist, M.H., Pereira, M.J., Abrahamsson, N. Effects of acute GLP-1 receptor activation on the glycemic and neurohormonal responses to meal test after gastric bypass. *Submitted manuscript.*

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Contents

Abbreviations

ACTH	adrenocorticotropin
ADA	American Diabetes Association
ANS	autonomic nervous system
AUC	area under the curve
ARC	arcuate nucleus
ASL	arterial spin labeling
BMI	body mass index
BSA	body surface area
CBF	cerebral blood flow
CCK	cholecystokinin
cDNA	complementary DNA
CGM	continuous glucose monitoring
CNS	central nervous system
CRH	corticotropin releasing hormone
CSF	cerebrospinal fluid
DMV	dorsal motor nucleus of the vagus
DSST	digit symbol substitution test
EASD	European Association for the Study of Diabetes
ECG	electrocardiogram
EBW	excess body weight
EHSS	Edinburgh Hypoglycaemia Symptom Score
FDG	fluorodeoxyglucose
FFA	free fatty acid(s)
FFM	fat free mass

fMRI	functional magnetic resonance imaging
GIR	glucose infusion rate
GH	growth hormone
GHRH	growth hormone releasing hormone
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
HbA1c	glycated haemoglobin
HOMA-IR	homeostatic model for assessment of insulin resistance
HPA-axis	hypothalamus-pituitary-adrenal axis
HRV	heart rate variability
HSL	hormone-sensitive lipase
IQR	interquartile range
Ki	net influx rate constant (of ^{18}F-FDG in PET)
LH	lateral hypothalamic nucleus
MRGlu	estimated rate of glucose uptake (estimate from ^{18}F-FDG PET)
NTS	nucleus tractus solitarius
OECD	The Organization for Economic Cooperation and Development
OGTT	oral glucose tolerance test
PET	positron emission tomography
PHF	power of high frequency component (in spectral HRV analysis)
PLF	power of low frequency component (in spectral HRV analysis)
Ptot	total spectral power (of heart rate variability)
PVN	paraventricular nucleus of the hypothalamus
PYY	peptide YY (peptide tyrosine tyrosine)
RYGB	Roux-en-Y gastric bypass
SAT	subcutaneous adipose tissue
SGLT-2	sodium glucose transporter-2
SNS	sympathetic nervous system
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus

TICA	tensorial independent component analysis
TBW	total body weight
TMT	trail making test
VAT	visceral adipose tissue
WAT	white adipose tissue
VMH	ventromedial nucleus of the hypothalamus
WBC	white blood cell count
WHO	World Health Organization
WHR	waist/hip ratio

Gene symbols and their official full names

AKT1	AKT serine/threonine kinase 1
BMP4	bone morphogenetic protein 4
CEBPA	CCAAT/enhancer-binding protein alpha
CEBPB	CCAAT/enhancer-binding protein beta
CPT1B	carnitine palmitoyltransferase 1B
FABP4	fatty acid binding protein 4
FASN	fatty acid synthase
IRS1	insulin receptor substrate 1
PPARG	peroxisome proliferator activated receptor gamma
SLC2A4	solute carrier family 2 member 4 (GLUT4)

Introduction

Obesity (BMI $\geq$ 30 kg/m^2) is considered to be the fifth leading risk factor behind premature death in the world, ranking even higher in middle- and high income countries (1). Overweight, that is a BMI $\geq$25 kg/m^2, was estimated to affect 58% of the population in the OECD countries in 2019, with obesity estimated at 24% in 2016 (2). The corresponding numbers in Sweden were 52% and 16% in 2021(3).

Obesity infers an increased risk for several disease states including cardiovascular disease, several types of cancer, obstructive sleep apnoea syndrome, osteoarthritis and type 2 diabetes. It is estimated by the WHO to account for 2.8 million deaths annually (4).

The cause of the obesity "epidemic" is multifactorial and is considered to arise from a combination of modern environmental factors in unfortunate synergy with genetic factors promoting the conservation of energy that likely have offered evolutionary advantages in the past. Once weight has been gained, the human body is apt to maintain it. The heritability of obesity is substantial, with genetic effects estimated to account for around 70% of the variation in weight (5-7).

With its increasing prevalence, obesity has come to be the subject of much research during the last century. The aim has not only been to capture the risks that obesity presents to the individual and the costs to society coupled with such risks, but perhaps most importantly, to explore possible interventions.

A multitude of studies have explored the effects of different kinds of diets, commonly involving some form of calorie restriction on weight itself and on obesity related morbidity. Although an attempt to outline the results of such a mass of research would necessitate an oversimplification, it is fair to say that the majority of dietary interventions such as calorie restriction, while often providing impressive weight loss results in the shorter time frame, have usually proved difficult to maintain long term (8). It could thus be stated that while dieting might make you slimmer, you're unlikely to stay slim because of it. Likewise, interventions involving exercise, while showing beneficial health effects, often prove insufficient in attaining the substantial weight reductions hoped for (9), and generally do not provide sustained long term weight loss effects after the intervention is terminated.

Type 2 diabetes

Diabetes is defined by the WHO and the ADA as fasting plasma glucose values ≥ 7 mmol/l, a spontaneous glucose ≥ 11.1 mmol/l (or 2 hours after OGTT) or a Hba1c ≥ 48 mmol/mol, and should be confirmed by a second abnormal test in the absence of classical symptoms. Type 2 diabetes accounts for approximately 90% of cases. Unlike type 1 diabetes, it is not caused by autoimmune destruction of the beta-cells of the pancreas, but is characterized by the development of insulin resistance and an insulin secretion which thus becomes insufficient to normalize blood glucose. The majority of patients with type 2 diabetes are overweight and T2DM risk increases with age and a sedentary lifestyle. It is disease with a strong hereditary pattern and certain ethnic subgroups have an increased risk of T2DM than others (10).

Complications of diabetes include macrovascular (atherosclerosis and ischemic heart disease, heart failure, stroke) and microvascular diseases (retinopathy, neuropathy, nephropathy and kidney failure). Diabetes also infers increased risk for a number of other conditions, ranging from several forms of cancer and dementia to severe COVID and fractures. It increases the risk of non-alcoholic fatty liver disease and negatively affects the progression and outcome of this spectrum of conditions (11).

The available treatments for type 2 diabetes are numerous. Emphasis is put on lifestyle interventions, with a focus on weight loss, dietary changes and increased physical activity. Smoking cessation and limiting alcohol intake are also of importance for the risk of associated comorbidities (12).

A range of medications for glycaemic control are available, beginning with metformin, with important additions to the arsenal, GLP-1 analogues and SGLT-2 inhibitors, having been introduced in the last two decades. These later additions are moving up in priority as new evidence has been put forward demonstrating important risk reducing effects on cardiovascular complications and comorbidities. Older pharmaceuticals such as sulfonylurea and insulin are now usually chosen as later additions to an antidiabetic drug regime due to less beneficial effects on the risk of comorbidities and potential side-effects. Increasing emphasis is put on holistic care with customized treatment based on patients' individual glycaemic and weight goals, concomitant health conditions, risk of hypoglycaemia, side effects of and adherence to treatment et cetera (13) .

Bariatric surgery

Historic attempts at treating obesity have included drastic approaches. Attempts at more mechanical solutions, such as wiring the jaws together to restrict solid food intake (14) have proved less practical. Serendipitous observations of weight loss occurring in patients having undergone surgery against

peptic ulcers pointed the flashlight to the gastro-intestinal tract as an area of interest. The main 'giant leap' of obesity research in the 20th century thus came from surgeons, in the form of the development of the gastric bypass in the 1960's and -70's (15, 16). Several different surgical approaches were tried, but the Roux-en-Y gastric bypass (RYGB) introduced in the late 1970s soon became predominant (17). The name was inspired by techniques developed during the turn of the previous century by Swiss surgeon César Roux, whose technique involved creating a Y-shape (**Figure 1**) from the intestines (18).

During RYGB surgery the stomach is divided, leaving only a small 'pouch' to receive the food bolus. The jejunum is divided, and the distal portion of the jejunum is then anastomosed onto the gastric pouch, this portion is termed the alimentary limb. This means the food bolus reaches the cells of the jejunum almost immediately upon ingestion. The proximal portion, termed biliopancreatic limb, is reinserted in an anastomosis with more distal parts of jejunum. Thus, bile acids and pancreatic enzymes are redirected there.
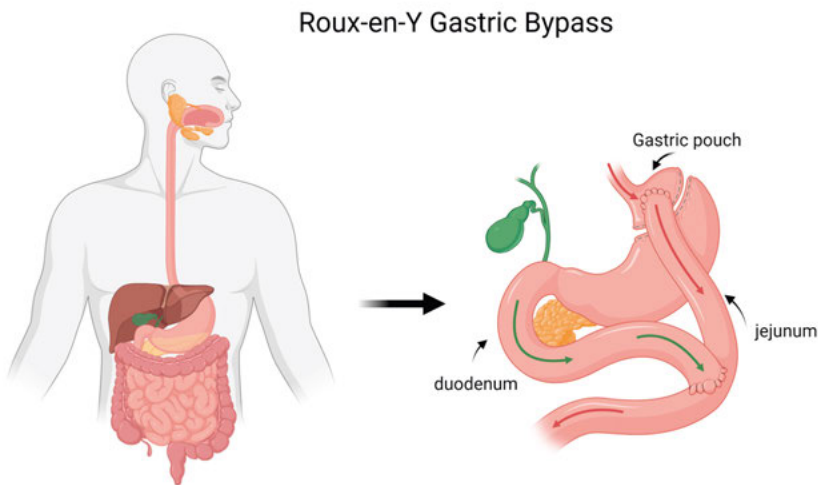

Figure 1. Overview of Roux-en-Y gastric bypass. Note the small gastric pouch and exclusion of the main stomach and duodenum from the passage of food. Created with BioRender.com.

While the impressive clinical effects of the RYGB have made it the dominant bariatric procedure globally, other techniques have been introduced in the last decades, among them the duodenal switch and the sleeve gastrectomy (17). The latter has become more common in recent years, especially in the United States, and removes about 80% of the width of the stomach, leaving behind a tubular "sleeve". In Sweden, RYGB is again regaining popularity and constituted almost 60% of all bariatric surgery in 2022 (19).

The effects of bariatric surgery

RYGB has proved to be effective in reducing weight, with reductions of some 25-32% of total body weight (20) or some 75-80% of excess body weight (i.e. the weight that puts the individual at a BMI of above 25 kg/m^2), being reported (21). Beneficial changes to glucose homeostasis are observed post-operatively (21), interestingly generally before any major weight loss has occurred (22, 23). These effects reduce the need for glucose lowering medications in patients with T2DM, even allowing for complete cessation of antidiabetic drugs in about 77% of patients after two years (24). That a surgical procedure could cause "remission" of diabetes has naturally garnered much scientific interest (23).

While the RYGB procedure involves a restriction of gastric volume and thus a limited space in which to receive food, this fact is not believed to be of much importance for the beneficial clinical effects of RYGB (17). More importantly, a large portion of the effect of RYGB is thought to arise from a shift in the temporal dynamics of hormones produced in the GI tract in response to food. The intestinal rerouting of RYGB causes nutrients reach the enteroendocrine cells of the distal small intestines earlier and this is thought to contribute to a drastic increase in the secretion of peptides of importance for the regulation of both glucose homeostasis and food intake (25).

Most notably GLP-1, a peptide hormone produced in the intestinal L-cells, was isolated in 1987 (26). GLP-1 has been shown to contribute to satiety and slow gastric emptying as well as stimulate insulin secretion and inhibit glucagon secretion (17, 27). Infusion of GLP-1 can normalise glycaemia in patients with T2DM (28).

With the discovery of GLP-1 came the development of new pharmaceutical therapies in the form of synthetic GLP-1 analogues, with exenatide being the first to be approved, for the treatment of T2DM in 2005 (27). In later years, GLP-1 analogues have been approved for the treatment of obesity.

Secretion of GLP-1 is markedly stimulated post-prandially after RYGB and is thought to be a main contributing factor to the metabolic improvements after surgery (25). The exact mechanism by which the surgery induces this sharp rise in GLP-1 is incompletely understood, but the rapid transfer of food to more distal portions of the intestines, where GLP-1 secreting cells are more common (29), is thought to contribute (30). Moreover, hyperplasia of the jejunum with an expansion of the number of incretin-producing cells has been described (31).

Other peptide hormones have also been demonstrated to be affected by RYGB, among them GIP, PYY and CCK (17, 25). Their role in the beneficial effects of RYGB is less well delineated (25). Similarly, while changes in bile acid secretion, gut microbiota and gastrointestinal motility have also been observed, their individual contribution to the clinical effects remain somewhat elusive (32).

Post-bariatric hypoglycaemia

As with any surgical procedure there are possible side effects. Apart from the perioperative complications such as leaks, bleeding or infections (19), a new clinical entity has been described: post-bariatric hypoglycaemia. This usually does not occur immediately after surgery but after one year or more. The typical presentation is hypoglycaemia occurring 1.5-3 hours after meals (33). A temporal and quantitative mismatch between glucose uptake on the one hand and incretin and insulin secretion on the other is thought to partly lie behind this phenomenon, but the underlying mechanism is not completely understood (30, 33). The large increase in post-prandial GLP-1 levels is considered to be central to the development of hypoglycaemia, as experimental treatment with GLP-1 receptor antagonists reduce post-prandial hypoglycaemia (34).

The frequency of this complication varies greatly between studies, with number ranging between 0.1 and 75%, depending on the methods and definitions used (35-38). Studies with continuous glucose monitoring (CGM) by Abrahamsson et al have described hypoglycaemic values in around 50% of subjects, frequently going unnoticed by the patient (39, 40).

Hypoglycaemia and its counterregulation

Hypoglycaemia has been defined as "all episodes of an abnormally low plasma glucose concentration that expose the individual to potential harm" by one workgroup of the American Diabetes Association (41), but which specific glucose level should be considered "hypoglycaemic" is not universally agreed upon. Traditionally, glucose levels below 3.5 mmol/l have been defined as hypoglycaemia (42). Recently, a joint position statement by the ADA and its European counterpart EASD proposes a definition of glucose below 3.0 mmol/l as clinically significant biochemical hypoglycaemia, as this level does not occur during physiological conditions in healthy individuals (43).

Normal physiological fasting blood glucose values range between 4 and 6 mmol/l (33). When glucose drops below approximately 4.5 mmol/l, endogenous insulin secretion is supressed (33, 44), thus limiting glucose clearance from the blood through intracellular uptake. Insulin normally inhibits glycogenolysis in the liver and hepatic and renal gluconeogenesis, processes which thus increase when insulin levels drop.

There is considerable variation between studies and individuals as to what glucose levels activate other counterregulatory responses (**Figure 2**), with initial thresholds around 3.6-3.9 mmol/l (45). The polypeptide hormone glucagon is considered to be of most importance (33). It is released from the pancreatic alpha cells and rapidly acts on the liver to increase glycogenolysis and, to a lesser extent, gluconeogenesis (33, 45). This stimulatory effect of glucagon on glucose production lasts for approximately 90 minutes (33).

Glucagon also stimulates lipolysis and amino acid catabolism in the liver, which mobilizes gluconeogenic substrates (46). Whether it also acts to stimulate lipolysis in white adipose tissue is debated (46-48). Glucagon also stimulates ketone production in human hepatocytes in vitro (47).

At a similar glucose threshold of around 3.6-3.8 mmol/l levels the sympathetic nervous system is activated, which mainly involves the release of catecholamines epinephrine and noradrenaline from the adrenal medulla, and norepinephrine from sympathetic nerve endings. (33, 45). Adrenaline inhibits insulin release and stimulates glucagon secretion. Moreover, it directly and swiftly stimulates hepatic glycogenolysis and gluconeogenesis. Activation of sympathetic nerves to the liver suppress glycogen synthesis (49).

Adrenaline also acts to mobilize lactate and alanine (50), precursors used for de novo glucose production (33). In the liver, it downregulates lipid release and stimulates the production of ketone bodies (49). Simultaneously, adrenaline leads to a reduction of glycolysis in peripheral tissues, mainly skeletal muscle, and an increase in glycogen utilization, which further contributes to a net glucose increase in the blood (33).

Noradrenaline increases in the blood are described to be detected in response to glucose levels around 3.2 mmol/l (45). Its actions are similar to those of adrenaline. In the liver, it stimulates glucose production via glycogenolysis (33). Lipolytic effects of the catecholamines on adipose tissue increase the availability of as glycerol which may be used for gluconeogenesis (33, 51).

During hypoglycaemia, noradrenaline is both secreted from the adrenal medulla and from sympathetic nerve terminals innervating target organs. The neural SNS component is likely responsible for the autonomic symptoms (see below) perceived by the individual during hypoglycaemia (palpitations, tremor and anxiety), as these are not blunted in adrenalectomized patients. In contrast, the normal hemodynamic effects of hypoglycaemia (increased heart rate and lowered diastolic blood pressure) require intact adrenomedullary function and are thus likely an endocrine SNS effect (50).

Growth hormone, secreted by the pituitary, also increases in circulation at glucose levels around 3.6-3.8 mmol/l (33, 45). Although it transiently has a glucose-lowering effect (33), after some hours it raises blood glucose via stimulation of hepatic glucose production and inhibition of glucose utilization (52). GH also has a stimulatory effect on lipolysis (52). Results from animal studies suggest GH effects on glucose homeostasis may also be mediated via CNS effects (53).

Similarly, the steroid hormone cortisol takes 2 to 3 hours to have an effect (33, 54). It is released from the adrenal cortex in response to glucose levels around 3.5 mmol/l (45). Cortisol increases glucose through upregulation of hepatic gluconeogenic enzymes (33) and through increasing the availability of gluconeogenic precursors (55). The SNS seems to have a stimulatory effect on cortisol release, which is not mediated via an effect on ACTH.

These processes act in synergy, meaning their net effect together is larger than their individual effects on blood glucose when isolated. The magnitude of the counterregulatory response to hypoglycaemia is determined by how low the glucose levels rather than the speed of with which it develops (33). Interestingly, although the glycaemic thresholds for triggering the counterregulatory mechanisms are the same in both sexes, the hormonal responses are markedly lower in females than in males (56).
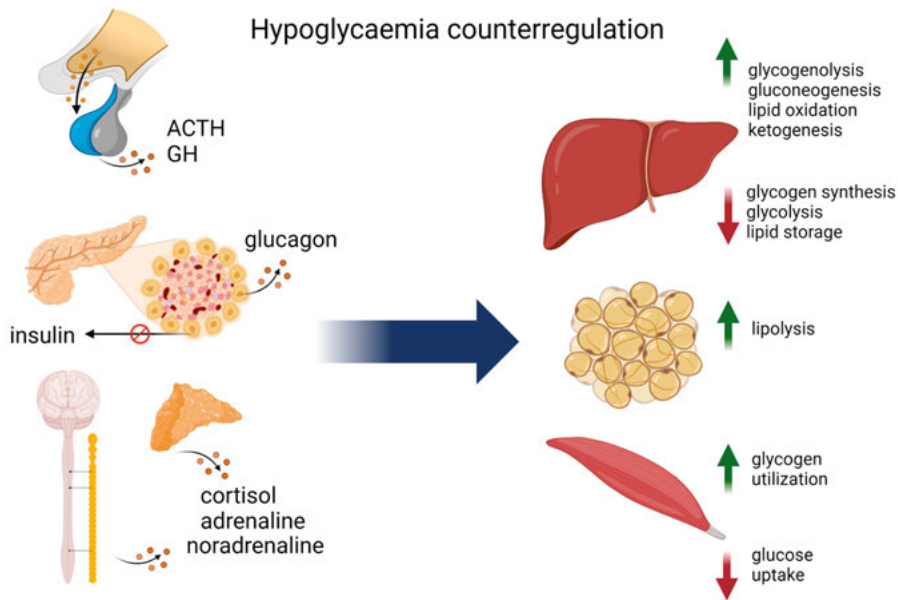

Figure 2. Counterregulatory mechanisms against hypoglycaemia involving signals from the hypothalamus, pituitary, pancreas, sympathetic nervous system and adrenal glands (left side) and their effects on the liver, adipose tissue and muscle (right side). Created with BioRender.com.

Symptoms of hypoglycaemia

Symptoms of hypoglycaemia occur somewhat later than the counterregulatory systems are activated, around a glucose level of 3.4 mmol/l (45). Early symptoms of hypoglycaemia are thought to arise from the aforementioned catecholaminergic activity, thus termed 'autonomic', and include palpitations, tremor and anxiety. Acetylcholine release from sympathetic nerves is also considered to contribute the autonomic symptoms, in the form of sweating, hunger and a tingling sensation (33).

So called neuroglycopenic symptoms also arise – i.e. symptoms from a brain that does not have access to the glucose it needs. These include fatigue,

cognitive difficulties and odd behaviour and at later stages loss of consciousness, seizures and death (57). Although traditionally attributed to lower glucose levels, around 2.8 (33), a recent meta-analysis of pooled hyperinsulinemic clamp data reported neuroglycopenic symptom debut around a glucose level of 3.4 mmol/l as well (45).

Hypoglycaemia unawareness

Generally, the glucose thresholds for hypoglycaemia counterregulation stated above are lower in patients with diabetes type 1 (45). This is true for both the release of hormones, and for the associated symptoms. This unawareness to hypoglycaemia has been extensively researched. It is defined as a failure to sense that blood glucose levels fall below the normal range. More specifically, it has been described as the absence of autonomic symptoms in response to hypoglycaemia (41, 58). This it is thought to be caused by frequent exposure to hypoglycaemia, usually due to too large or too frequent doses of exogenous insulin. It seems to stem from some form of neurological adaptation so that the counterregulatory mechanisms against hypoglycaemia are not activated as they normally would be. Specifically, a blunted sympathoadrenal response has been described in hypoglycaemia unawareness (58). Previous glucose levels below 3.9 mmol/l can lead to lower sympathoadrenal responses to subsequent hypoglycaemic episodes (41).

Moreover, exposure to a glucose clamped at 2.8 mmol/l led to a blunted response of glucagon and cortisol when the experiment was repeated the next day, as well as reduced hypoglycaemic symptom scores, also in subjects without diabetes (59). It has been postulated that the postprandial hypoglycaemia observed in patients after RYGB might arise from similar neuroendocrine adaptations (40).

Role of the CNS in hypoglycaemia counterregulation

Much of early research into glucose homeostasis has been centred on peripheral mechanisms, but in later decades most interest has been devoted to the role of the central nervous system (60). Glucagon release is thought to be mainly regulated locally via insulin effects, and it seems largely unaffected by experiments that interfere with sympathetic signalling (33). The other mentioned counterregulatory mechanisms protecting against hypoglycaemia involve the central nervous system at one or several levels. Moreover, the last decades of research into metabolic concepts like hunger and satiety have highlighted the importance of CNS regulation of metabolism in the macroscopic sense (6, 61), although much work still remains.

Peripheral glucose-sensing occurs at several sites, such as the portal vein and intestines, and hypoglycaemia triggers signals from these sites, that reach the brain via sympathetic afferents (62). These relay to the NTS in the brain stem which coordinates ANS responses via sympathetic and vagal efferents. Sympathetic efferent from the NTS travel the spinal cord before reaching end organs. Stimulation of sympathetic nerves innervating the pancreas inhibits insulin release and increase glucagon release (63). Part of the counterregulatory response to hypoglycaemia is abolished in transsectional damage to the spinal cord, proving this communication with the brainstem is essential for complete hypoglycaemia counterregulation (33).

The NTS also projects to hypothalamic regions including the ARC, LH, PVN and VMH nuclei, centra known to be involved in feeding regulation and metabolic homeostasis (61, 62). Hypoglycaemia results in an activation of these centra as well as the DMV, (which regulates parasympathetic efferents). These nuclei (NTS, DMV, VMH, LH, ARC, PVN) also have their own glucose-sensing neurons whose activity is either excited or inhibited by local glucose levels (64). Glucose-sensing neurons in the VMH are considered responsible for the initiation of the autonomic counterregulatory responses (33).

GH release is controlled via the GHRH-secreting neurons situated in the ARC. Studies on transgenic mice show that these increase their firing in response to hypoglycaemia (65), likely explaining the downstream increases in circulating GH levels observed during hypoglycaemia.

ACTH and downstream cortisol release is stimulated by CRH-secreting neurons originating in the PVN of the hypothalamus (61). The hypothalamus' position adjacent to the third ventricle means it comes in contact with circulating levels of glucose in the CSF. The glucose levels of CSF is not representative of the glucose levels in the systemic blood circulation and brain areas behind the blood-brain barrier normally about have one fourth of the glucose concentration of blood. The median eminence, a ventromedial area of the hypothalamus, however, has a permeable blood-brain barrier consisting of fenestrated capillaries (66). This region is thus exposed to glucose levels that are close to those of the circulation (64). There is evidence of bidirectional communication between the median eminence and the VMH and ARC (61, 66).

The VHM and ARC have both been implicated in CNS modulation of insulin secretion, as lesions in and stimulation of these areas lead to changes in circulating insulin levels (64).

Taken together, it seems that there are independent and to some extent redundant parallel systems for glucose sensing and activation of hypoglycaemia counterregulation in the brain and in the periphery (62). The hypothalamus is of particular interest in this regard.

Heart rate variability as reflection of ANS

The activation of the sympathetic nervous system in response to hypoglycaemia is a stress response which also effects the cardiovascular system. Adrenergic stimulation leads to increases in heart rate and a widened arterial pulse pressure (42). Hypoglycaemia during insulin treatment, and associated fluxes in plasma potassium, have also been associated with prolonged QT-intervals and risk of arrhythmia, including bradycardic episodes, particularly at night. This could be explained by sympathetic activity leading to an increased counteracting parasympathetic tone, with vagal effects decreasing heart rate (67).

The effects of hypoglycaemia on heart rate can be studied with heart rate variability analysis. HRV is based on calculations of ECG-recordings, specifically the duration of the R-R intervals and the fluctuations in beat-to-beat heart rate. These are affected, among other things, by respiratory rate. Such variation is considered to a large extent to mirror the effects of the autonomic nervous system (68) and has been used to evaluate parasympathetic and sympathetic activity in research settings.

HRV can be described and studied in different ways, one of which is spectral analysis of frequency domains. This has been a preferred method to portray ANS effects on HRV, particularly the relative proportion of HRV frequency components to one another. The total spectral power of HRV and the power of high frequency (PHF) domains are considered to mirror parasympathetic activity, whereas sympathetic tone is reflected in the relative ratio of low frequency to high frequency power (PLF/PHF) (68).

Reduced variability has been linked to poorer prognoses for some diseases (69). An increased nocturnal dominance of sympathetic indices has been observed in patients with diabetes, which could account for non-dipping of blood pressure at night. Lundqvist et al. reported that overweight individuals did not have the same reduction of parasympathetic tone (as reflected by PHF) during hypoglycaemia as their lean counterparts (70). Weight loss has been associated with improved global HRV and parasympathetic indices (68).

Treatment of post-bariatric hypoglycaemia

There is no consensus on how to manage hypoglycaemia after RYGB. Dietary advice is considered a cornerstone, with instructions to ingest less simple and more complex carbohydrates, in order to reduce glucose and insulin peaks. Moreover, patients are instructed to restrict (but not completely avoid) carbohydrate intake in favour of increased protein and healthy fat intake (30, 33).

Several experimental treatments have been tried. Acarbose slows and decreases the uptake of glucose from the intestines, and is effective in limiting the spike in postprandial glucose, but often has intolerable GI-side effects.

Diazoxide blunts insulin release, but is often not well tolerated due to numerous side effects. Somatostatin analogues, which inhibits the release of both GLP-1 and insulin alleviate post bariatric hypoglycaemia but is costly and requires injections, and also inhibits other endocrine axes. Attempts have also been made with calcium channel blockers, DPPIV-inhibitors and SGLT-inhibitors, with varying results (30, 71, 72).

There have been case reports of attenuated hypoglycaemic tendencies (reflected by CGM) as well as alleviated symptoms with treatment with GLP-1 analogues in patients with post-bariatric hypoglycaemia (73, 74). This is intriguing, as GLP-1 analogues stimulate insulin release and are used clinically to lower glucose in patients with diabetes.

Adipose tissue function in relation to obesity

It is by now well established that adipose tissue does not function as merely a passive depot of excess energy in the body, but as an endocrine organ influencing energy homeostasis. Adipose tissue consists of fat cells, adipocytes, as well as surrounding stroma, including immune cells, fibroblasts, adipocyte precursor cells and collagen networks (6). Adipocytes store excess energy in the form triglycerides in lipid droplets, stores which can be mobilized through lipolysis and released into the blood stream as free fatty acids and glycerol (6, 75).

Adipocytes can increase in size (hypertrophy) and number (hyperplasia) when needed, i.e. in conditions of net excess energy(6). The adipocyte hypertrophy observed in severe obesity has been associated with metabolic disease and predicts development of T2DM (76), presumably because a threshold has been reached where the cells no longer can harbour the excess lipids (77), leading to deposits in ectopic sites (75).

Adipose tissue has been implicated as causative in the dysmetabolic changes observed in obesity, particularly intraabdominal fat deposits, so called visceral fat. Clinically, measurements such as waist circumference and waist/hip ratio are often used as reflections of abdominal obesity (78). There is a strong association between VAT and the risk for type 2 diabetes and cardiovascular disease (75). The association between subcutaneous adipose tissue and metabolic dysfunction is not as strong (75, 78). How much the reduction of either depot contributes to the beneficial effects of bariatric surgery on the associated comorbidities is under investigation, as well as any contributing mechanism.

Research on patients who have undergone gastric bypass surgery has naturally showed reduced adiposity in general (up to a 50% reduction of total body fat within one year) as well as reduced visceral adiposity (75). Studies have shown reductions in adipocyte size in WAT after RYGB (75), and that this is

associated with improvements in insulin sensitivity (79) and diabetes remission (77).

Basal lipolysis occurs without stimulation, but can be increased by catecholamines and inhibited by insulin. The rate of basal lipolysis has been reported to be increased in obesity (80) which can be explained by a resistance to the inhibitory effects on insulin. This causes an elevation of FFAs and glycerol in the circulation. There are reports of improved inhibitory effects of insulin after RYGB on lipolysis during hyperinsulinemic clamps (81). Similarly, there seems to be a resistance to the stimulatory effects of catecholamines on lipolysis in the obese state (80).

Glucose is taken up by adipocytes to be either stored as triglycerides or metabolized by the cell. This occurs at both basal rates and is stimulated by insulin. Studies have shown a blunted response to insulin stimulation on glucose uptake in obese compared to lean individuals (82).

Adipose tissue secretes various proteins, adipokines, to the circulation which function as signals of the metabolic state of the tissue. This signalling is often impaired in obese states (75). Full adipocytes release the hormone leptin as a signal that systemic metabolic demands are met, which has been thought to signal satiety. However, while leptin levels rise in obesity, it seems that the CNS sensitivity to this satiety signal becomes impaired and leptin has not proved to be an effective treatment for obesity (6, 83). The secretion of the adipokine adiponectin, which has beneficial effects on insulin sensitivity and glucose and fat metabolism, is decreased in obesity and T2DM (6, 84).

Some adipokines have proinflammatory properties, including tumor necrosis factor-α, interleukin-1β and -6 and interferon-γ. The adipose tissue expansion in obesity has been considered to have a pro-inflammatory effect that is thought to contribute to the development of adipocyte insulin resistance. As the effects of insulin on adipocytes is anti-lipolytic, resistance to this signals leads to increased circulating levels of fatty acids, which in turn contribute to hyperglycaemia (6).

Aims

The aim of the following papers has been to further explore the metabolic effects of bariatric surgery with a focus on changes to glucose homeostasis and potential underlying neuroendocrine mechanisms.

Paper I

The aim was to investigate whether an infusion with exenatide would lead to a different counterregulatory response and lessen symptoms of hypoglycaemia in subjects who had undergone RYGB, compared to saline.

Paper II

The study was performed to investigate whether subjects after RYGB had different hormonal responses, hemodynamics, cerebral blood flow, cerebral glucose uptake, regional brain activation patterns, cognitive function and symptoms during hypoglycaemia than before surgery.

Paper III

This study aimed to capture changes to body composition, glycaemic control, metabolic status, subcutaneous adipose tissue as well as autonomic nervous system effects on heart rate as they occur over time in patients with T2DM undergoing RYGB. The hope was that the chronology of changes might shed light on the causal relationship between the different adaptations that occur after surgery.

Paper IV

The aim was to determine if exenatide affected the hormonal response to a standardized meal given after RYGB in a way that might explain its alleviating effects on post-bariatric hypoglycaemia.

Study design and participants

Paper I

The first paper is an experimental study of the effects of exogenous GLP-1 (synthetic analogue exenatide) versus saline on counterregulatory mechanisms against hypoglycaemia in patients after RYGB. In the study 13 non-diabetic participants were enrolled approximately 12 months after their RYGB. The study included two visits with euglycaemic-hypoglycaemic clamps and subjects were randomized to either concomitant infusion of exenatide or saline (placebo). This was then repeated for the second visit with the other type of infusion, in a crossover design. 12 subjects (6M/6F) completed both visits. During clamps, subjects underwent repeated blood sampling, heart rate variability recordings and assessment according to the Edinburgh Hypoglycaemia Symptom Score.

Paper II

The study behind Paper II was based on normoglycaemic-hypoglycaemic clamp studies with simultaneous neuroimaging in patients before and after RYGB. Eleven non-diabetic participants (3M/8F) completed the study. For inclusion, subjects had to be aged 18-60 years with a BMI between 35 and 45 kg/m^2 and planning to undergo RYGB surgery. The study included one preoperative and one postoperative visit during which subjects underwent a clamps during simultaneous blood sampling, neuroimaging with f-MRI and FDG-PET, HRV recording, EHSS assessment and cognitive testing.

Paper III

The third paper is based on a prospective clinical study of patients with T2DM investigated before RYGB and up to two years after surgery. In this study subjects between 18 to 60 years of age with T2DM and a BMI of 30-45 kg/m2 were enrolled. The subjects must have been diagnosed with T2DM for less

than 10 years and be treated with oral or injectable antidiabetic drugs excluding insulin. Subjects were randomized to either undergo RYGB (n=13) or to continue with (nonsurgical) standard care diabetes treatment (n=6).

Participants came for study visits before surgery and at 4, 24 and 104 weeks after. Blood samples, biometric measurements and adipose tissue sample were also obtained at the day of surgery, that is, after a 4 week low calorie diet. During visits, subjects underwent blood sampling, HRV recordings, subcutaneous adipose tissue biopsies, an arginine challenge followed by oral glucose tolerance tests.

At the 104-week follow-up, on which Paper III is based, all subjects in the control group had opted to also undergo bariatric surgery, thereby withdrawing from the study. One subject became pregnant before the last visit. Thus, Paper III details the twelve subjects (3M/9F) of the initial RYGB group. Separate data from 22 healthy volunteers were added to use as a point of reference for results of the adipose tissue analyses two years post RYGB.

Paper IV

The fourth paper details an experiment to assess counterregulatory hormones, this time in a meal setting, with simultaneous infusion of either exenatide or saline. Ten study participants (1M/9F) without previous or current diabetes, 18-60 years of age, were recruited approximately one year after their RYGB. They came for two separate visits where they undertook a meal test i.e. a standardized liquid meal with concomitant infusion of either exenatide or saline as a control condition. The experiment was then repeated for the other condition after 1-2 weeks. During the meal-test, repeated blood tests and ECG recordings for HRV were performed.

Table 1. Participant characteristics in Papers I-IV.

Participant characteristics	Paper I		Paper II		Paper III		Paper IV	
	Preop	Postop (1 year)	Preop	Postop (4 months)	Preop	Postop (2 years)	Preop	Postop (1 year)
Sex (male/female)	6M/6F		3M/8F		3M/10F	3M/9F	1M/9F	
Age (years)	41 (7)		35 (8)		51 (10)		41 (15)	
Weight (kg)	119.0 (15.9)	83.3 (11.4)	117.7 (14.7)	87.5 (12.8)	99.8 (13.7)	76.9 (10.1)	108.0 (29.0)	81.0 (35.8)
BMI (kg/m²)	39.8 (3.7)	28.1 (3.6)	40.2 (3.6)	29.9 (4.0)	36.8 (3.9)	28.6 (3.5)	41.3 (4.8)	30.9 (9.0)
Fat percentage (%)		28.6 (8.6)	45.7 (6.2)	34.0 (10.2)	42.8 (7.5)	33.7 (9.3)		37 (11)
Waist circumference (cm)			119 (10)	96 (10)	114.5 (6.2)	95.8 (8.8)		95 (19)
Hba1c (mmol/mol)	38.2 (6.8)	33.5 (2.1)	34.1 (1.9)	31.7 (2.5)	54.9 (12.2)	41.4 (5.2)		33.5(2.9)
Glucose (mmol/L)	6.1 (0.9)	5.7 (0.6)	6.0 (0.5)	5.3 (0.5)	8.3 (1.9)	6.5 (1.1)		5.2 (0.2)
Cholesterol (mmol/L)	5.3 (0.8)	4.1 (0.5)			4.7 (0.9)	4.7 (1.0)		3.6 (0.7)
HDL (mmol/L)	1.1 (0.4)	1.3 (0.3)	1.3 (1.1)	1.0 (0.2)	1.0 (0.1)	1.1 (0.1)		1.4 (0.3)
LDL (mmol/L)	3.6 (1.0)	2.5 (0.7)	3.0 (0.9)	2.1 (0.6)	2.9 (0.8)	3.1 (1.1)		2.0 (0.8)
Triglycerides (mmol/L)	2.0 (1.0)	0.9 (0.2)	1.4 (0.8)	0.9 (0.3)	2.2 (0.9)	1.2 (0.4)		0.9 (0.2)

Data are means (SD). Glucose and triglycerides are fasting values.

Methods

Roux-en-Y gastric bypass

All participants that were included in the Paper I-IV studies underwent laparoscopic RYGB at the Department of Surgery, Uppsala University Hospital. According to clinical routine, patients undertake a 4-week low caloric diet (800-1100 kcal) prior to surgery to reduce the risk of intraoperative complications. The Roux-limb which attaches to the gastric pouch is approximately 100 cm and the biliopancreatic limb is approx. 50 cm. The anastomoses are created with staples and sealed with sutures.

Euglycaemic hypoglycaemic clamp

For Paper I subjects underwent two euglycaemic-hypoglycaemic clamps. These are performed with a simultaneous fixed infusion of insulin (56 mU/BSA/minute) and an infusion of 20% glucose at a variable infusion rate. This enables the study staff to govern the blood glucose level through blood samples every five minutes for glucose analysis. The clamp was initiated with a 60-minute period of euglycaemia (glucose 5.0 mmol/l) after which the level was lowered stepwise to 4.0, 3.2 and 2.7 mmol/l (see **Figure 3**). During the clamp, arterialized blood was drawn at minutes 0, 60, 90, 120, 135, 150, 160 and 195 for hormone and metabolite analysis. To study the effects of GLP-1 receptor activation, an infusion with either exenatide (0.066 pmol/kg/min) or 0.9% saline (10 ml/h) as a control was started at minute 0 for the first clamp.

When the clamp was repeated for each individual after 1-4 weeks, the other type of infusion was given, their order being randomized.
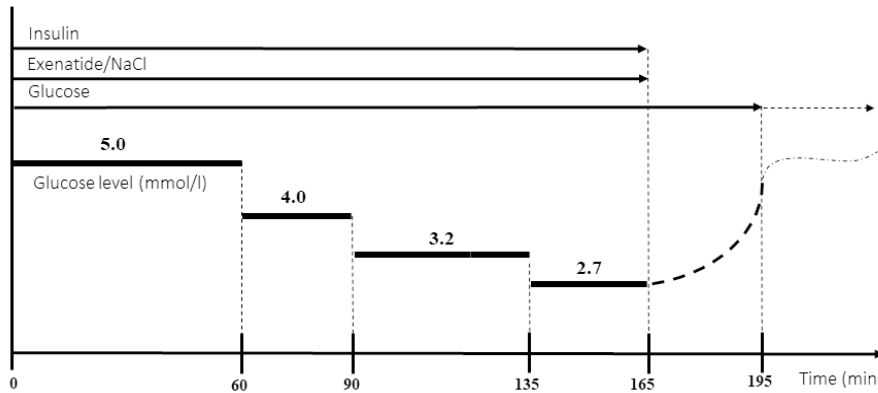
Figure 3. Schematic of design of stepwise euglycaemic, hypoglycaemic clamp with exenatide or saline (as placebo).

Normoglycaemic hypoglycaemic clamp

The clamp procedure for Paper II is of similar design as the previous paper's, but the first period consists of a normoglycaemic phase, where the subject's spontaneous baseline glycaemia is maintained, using a variable glucose infusion and an infusion of insulin at 80 mU/BSA/min. This phase also includes baseline HRV registration, assessment according to EHSS as well as baseline cognitive tests (detailed below). Still in the normoglycaemic phase, the subject is placed in the PET-fMRI scanner and undergoes a series of imaging procedures as visualized in **Figure 4** and detailed below. After this, the subject's glucose is lowered to 2.7 mmol/l and resting state fMRI imaging (detailed below) is performed during this glucose lowering phase. Having reached hypoglycaemic conditions, blood sampling is performed as during normoglycaemia, as well as imaging. When imaging is completed, the subject exits the scanner to perform HRV, EHSS and cognitive tasks while still in the hypoglycaemic state. After this, the insulin infusion is stopped and the subject's blood glucose level is allowed to recover. Venous blood sampling continues throughout the clamp.
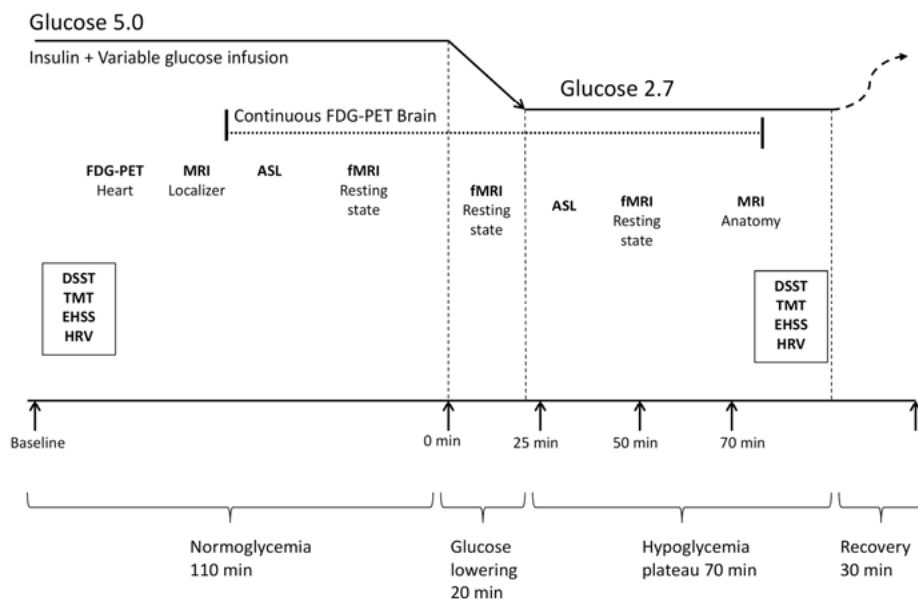
Figure 4. Schematic of normoglycaemic hypoglycaemic clamp design with simultaneous imaging

Meal test

The meal tests performed for Paper IV were initiated after fasting baseline venous sampling and baseline resting HRV recordings. Then, the participants were instructed to drink a 300 kcal standardized liquid meal in 10 minutes. After this, an infusion of either exenatide (0.066 pmol/kg/min) or 0.9% saline (10 ml/h) was initiated, while HRV recordings and venous blood sampling continued. Samples for glucose, c-peptide, insulin and paracetamol were drawn at minutes 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 195 and for GH, cortisol, ACTH, catecholamines, glucagon, GLP-1 and GIP at minutes 0, 60, 90, 120, 180 and 195. After 1-2 weeks, the meal test was repeated for the other type of infusion, the order of which was randomized.

Edinburgh Hypoglycaemic Symptom Scale

The Edinburgh Hypoglycaemic Symptom Scale was used in Papers I and II to assess symptoms of hypoglycaemia. EHSS is a self-assessment instrument where subjects grade each of 11 symptoms from a scale of 1 to 7, with 7 being the most severe. The symptoms have been partitioned into sections with shaking, sweating, palpitations and hunger representing autonomic symptoms, drowsiness, confusion, speech difficulty, odd behaviour and incoordination

considered to represent neuroglycopenic symptoms while headache and nausea are categorized as "general malaise" (85).

Cognitive tests

To assess effects of hypoglycaemia on cognitive functions for Paper II, the instruments Digit Symbol Substitution Test and Trail Making Test were used at normo- and then hypoglycaemic phases during the clamp. The DSST is represented in **Figure 5**. The subject is given a pen and paper with nine different symbols paired with the digits 1 through 9 at the top. Underneath is a sequence of digits and the subject is instructed to fill in as many of the corresponding symbols as he/she can during 60 seconds. The number of correctly filled in symbols has been noted to inversely correlate with several neurological/psychiatric diseases and is thus taken as a measure of cognitive function, although it is not entirely known exactly what it measures (86).

Figure 5. Example of DSST-test used for Paper II. Reprint from Patel and Kurdi (87)

TMT assesses "visual search, scanning, speed of processing, mental flexibility, and executive functions" (88). The subject is given a pen and paper with the numbers 1-13 and letters A-L printed upon it. The subjects is asked to trace the figures in sequence, alternating between numbers and letters. The time it takes to complete the exercise is measured.

Neuroimaging – FDG-PET

Neuroimaging during clamps for Paper II was performed on an integrated 3 Tesla MRI and PET-scanner (Signa, GE Healthcare, Waukesha, WI, USA)

(89). FDG-PET uses a radiolabeled glucose analogue (^{18}F-fluorodeoxyglucose) tracer with PET-scanning to visualize glucose uptake into cells, thereby creating an image of the metabolic activity of tissues (90).

Neuroimaging – fMRI and ASL

Functional-MRI is a method of studying changes in local blood flow as a proxy for the pattern of activation of different areas of the brain during functional tasks or in response to a particular stimulus. The method is based on the fact that increased focal neuronal activity creates an increased metabolic demand in the active tissue, which creates a transient decrease in the oxygenation of the blood in this area. This is met with a compensatory increase of oxygenated blood to the area, which creates a change in the signal that can be detected with magnetic resonance imaging (91).

Resting state fMRI depicts the activity of a brain in resting state, that is, while the individual is not occupied with a task or particular stimulus (92). Tensorial Independent Component Analysis (TICA) is a method used to analyse MRI-data in order to decompose the signal into components, which can allow for discrimination between spatial, temporal and subject-dependent variations in the signal. The method can be used to investigate patterns of activation of different brain regions, essentially creating a map of connections between areas (93). This can help identify networks of importance in the brain's response to the assessed condition.

Arterial spin labeling is an application of MRI technology to depict perfusion without the use of contrast agents. Typically, an area of the carotid arteries is subjected to a radiofrequency pulse, which causes the protons of the arterial blood water to rotate ("spin") by 180 degrees. As this blood reaches the brain, the spin labeled blood creates a contrast with non-labeled tissues when magnetic resonance imaging is performed. By comparing the signal pattern to that of a control image, the cerebral blood flow is thus visualized (94).

Biochemistry

Routine blood analyses for Paper I-IV were performed by the Uppsala University Hospital Department of Clinical Chemistry.

Plasma-glucose was determined with Architect (Abbott) for Papers I-III, but had changed to Cobas c 503 (Roche) for Paper IV, which was also used for paracetamol. Insulin, c-peptide, cortisol were quantified with Cobas e 602/801, ACTH and GH with Immulite 2000XPi (Siemens Healthcare Global), and IGF-1 with Liaison XL (DiaSorin).

Real time glucose values on which the glucose infusion was titrated during clamps for Paper I and II was analysed using a handheld glucometer (Contour Glucose Meter (Bayer Healthcare).

Some hormone and metabolite analyses were performed by the laboratory at Clinical Diabetes and Metabolism using immunoassays: glucagon (#10-1271-01; Mercodia), GIP (#EZHGIP54K; Merck Millipore) and active GLP-1 (V-PLEX kit, Meso Scale Diagnostics). For Paper IV, U-PLEX Metabolic Group 1 (#K151ACL-1, Meso Scale Diagnostics) was used for GIP and active GLP-1 instead.

Quantification of plasma adrenalin and noradrenalin was performed at Karolinska University Hospital Laboratory for Paper I using Dionex UltiMate 3000 automated system (Thermo Fisher Scientific) and DECADE II Electrochemical Detector (Antec Scientific). For Paper IV, catecholamines were analysed at the laboratory at Clinical Diabetes and Metabolism using 3-CAT Research ELISA (#BA E-5600R; LDN).

The Free Fatty Acid Fluorometric Assay Kit (Cayman Chemical) was used to measure FFA, and glycerol was measured with Free Glycerol reagent (Sigma-Aldrich).

Heart rate variability

HRV analysis for Papers I-IV is based on quantifying the spectral power ("signal strength") of separate frequency components of the readings. It is presented as total power (PTOT), power of high frequency (PHF), power of low frequency (PLF) and PLF/PHF ratio. PHF (0.15-0.50Hz) is considered to reflect sympathetic tone while PLF (0.04-0.15Hz) is thought to reflect both sympathetic and parasympathetic tone. The ratio of PLF/PHF was used to mirror the relative balance between sympathetic and parasympathetic activity. HRV recordings were performed for the duration of the clamp for paper I. For Paper II, HRV was recorded for six minutes at the start of normoglycaemia and at the end of hypoglycaemia. For Paper III, HRV was recorded for six minutes in the resting condition, before other study procedures. For Paper IV, HRV was recorded at baseline and then throughout the experiment.

Adipocyte tissue biopsy

Adipocyte tissue biopsies were performed for Paper III at the baseline visit and at 4-, 24- and 104-week visits. The patient is placed in the supine position, and local anaesthetic Xylocain is injected subcutaneously in the lower two quadrants of the abdomen after which a biopsy needle is used to aspirate ap-

proximately 5-10 grams of subcutaneous fat. The tissue is immediately divided and either snap frozen in liquid nitrogen or incubated straight away at the Clinical Diabetes and Metabolism laboratory.

Adipocyte tissue analyses

Adipose tissue from biopsies was analysed at the Clinical Diabetes and Metabolism laboratory for paper III. Collagenase is used to separate the tissue into adipocytes. The rate of glucose uptake in the cells (basal as well as stimulated by insulin) is assessed using radiolabelled ^{14}C-D glucose. To assess the basal and stimulated/inhibited lipolysis (using isoproterenol or insulin+ isoproterenol) in the cells, the release of glycerol is measured. The volume/size of adipocytes was estimated through measurements made with standardized light microscopy. These values were compared to that of samples from a control population that had not undergone RYGB.

Adipose tissue gene expression was assessed for a number of genes known to have a role in metabolic processes as well as inflammation. Total RNA was harvested from the tissue using RNeasy lipid tissue mini kit (Qiagen) and measured. It was then multiplied with reverse transcriptase using High Capacity cDNA Reverse- Transcriptase Kits (Applied Biosystems). The expression is then analysed with the TaqMan real-time PCR assay (Thermo Fisher) and quantified with QuantStudio 3 sequence detection system (Applied Biosystems).

Results

Paper I

Twelve subjects completed both clamps. Glucose levels, glucose infusion rate, insulin, free fatty acid and glycerol levels were similar for both conditions. C-peptide levels were significantly higher during clamps with exenatide compared to saline (**Figure 6**). We observed no statistically significant difference in the secretion of counterregulatory hormones glucagon, cortisol, GH, adrenalin or noradrenalin when comparing exenatide to saline. GIP levels were generally lower with exenatide, significantly so at minute 120 and 150, when compared to saline.

While the hypoglycaemic phase of the clamps induced an increase of hypoglycaemic symptoms in most subjects, there were no significant differences in symptoms of hypoglycaemia between exenatide and saline.

When receiving exenatide, subjects had a higher heart rate, than when receiving saline, which was significant at minutes 60, 90, 120 and 150, while systolic and diastolic blood pressure was largely similar between both conditions. The heart rate variability recordings showed that the power of the low frequency component was lower during exenatide compared to saline during clamps, which was significant during the hypoglycaemic phase. The power of the high frequency component was higher for exenatide than for saline, with a significant difference only during euglycaemia. When comparing the PLF to PHF ratio between conditions, there was a significant difference during both euglycaemia and hypoglycaemia, with exenatide showing consistently lower PLF/PHF (**Figure 7**).
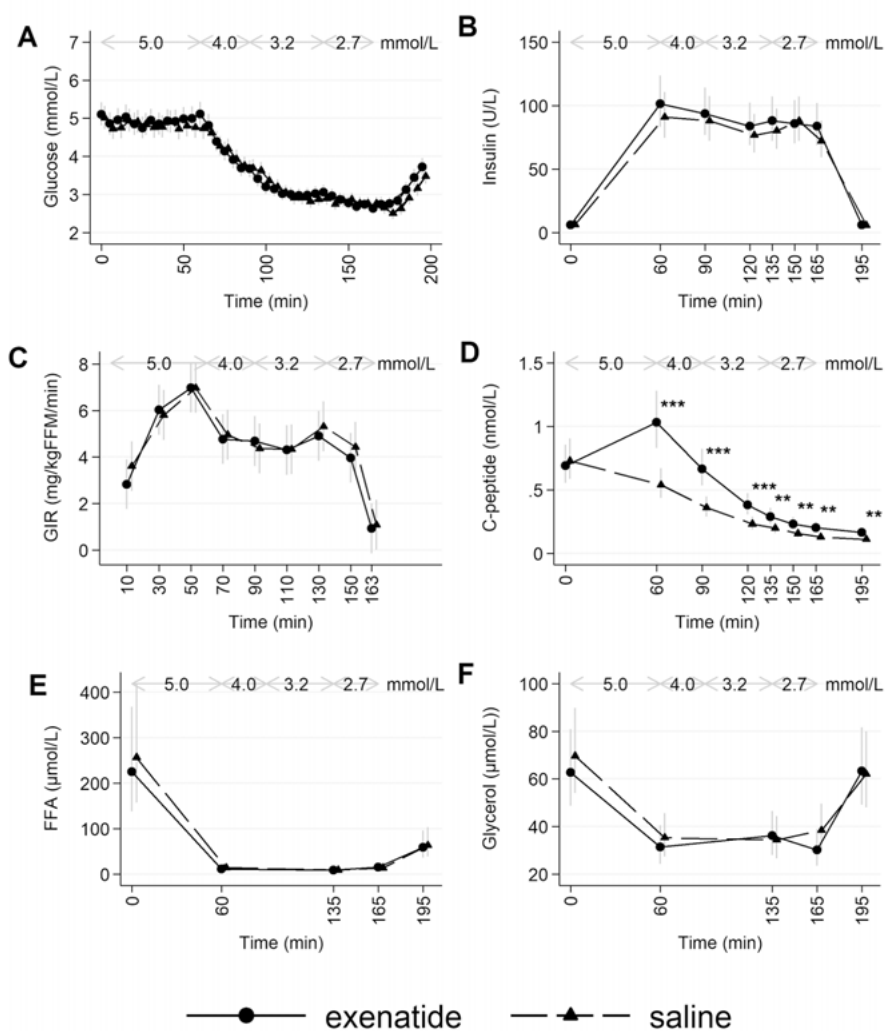
Figure 6. Levels of glucose (A), insulin (B), C-peptide (D), FFA (E) and glycerol (F) and glucose infusion rate (C) during hypoglycaemic clamps with concomitant infusion of exenatide or saline. Data are geometric mean and 95% confidence interval. *P < 0.05, **P < 0.01 and ***P < 0.001 for exenatide vs saline.
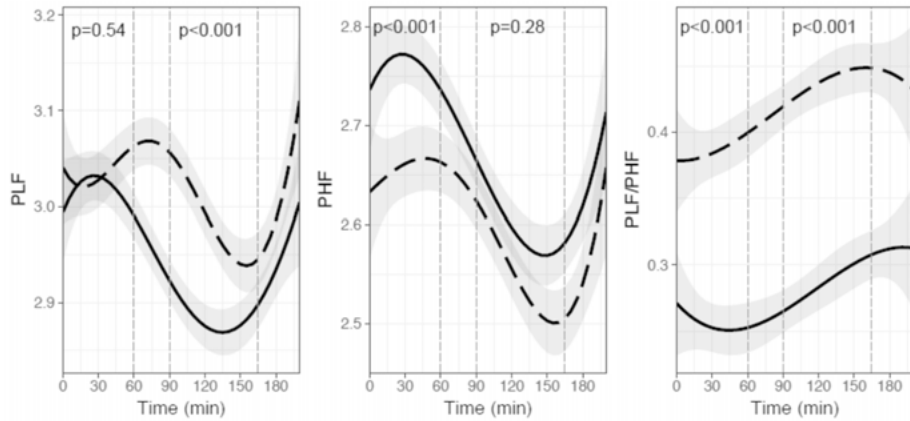
Figure 7. Heart rate variability during euglycaemic, hypoglycaemic clamps after RYGB with exenatide (solid lines) and saline (dashed lines) infusion. PLF, power of low frequency component; PHF, power of high frequency component; PLF/PHF; ratio. Values are ms^2 (log) and the shaded areas indicate S.E.M. P values refer to differences between exenatide and saline during normoglycaemia (0–60 min) and during hypoglycaemia (90–165 min), respectively.

Paper II

Eleven subjects completed both clamps, with approximately four months between visits. Their clinical characteristics and changes after surgery were representative for a population undergoing RYGB. The clamps were successful in achieving hypoglycaemia with no significant difference in glucose values in the normo- and hypoglycaemic phase between pre- and postoperative visits. During the recovery phase, preoperative subjects rose faster in glucose levels. Glucose infusion rates were similar between visits. Subjects experienced a rise in counterregulatory hormones glucagon, ACTH, cortisol, GH and a drop in c-peptide levels during hypoglycaemia. The rise in glucagon was significantly attenuated after surgery **(Figure 8)**. ACTH and cortisol levels were slightly lower after surgery, although not significantly so. GH levels during hypoglycaemia were significantly increased at the postoperative visit.
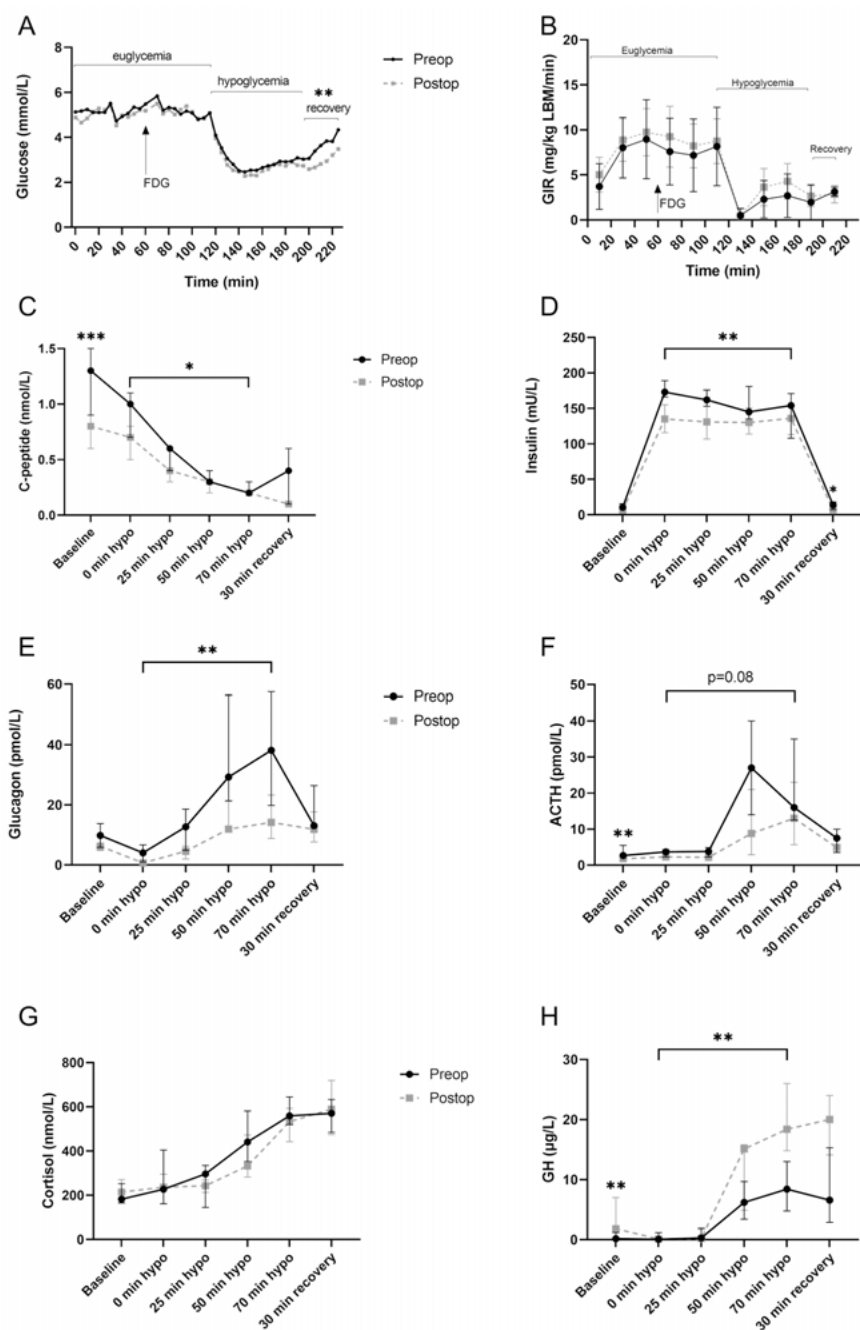
Figure 8. A–H: Glucose levels, glucose infusion rate (GIR), and hormone levels during normoglycaemic and hypoglycaemic clamp. Data are median± IQR (all but panel A) or mean ± SD (A). *P < 0.05, **P < 0.01, ***P < 0.001. P values refer to differences between before and after RYGB, during the hypoglycaemic period (brackets, AUCs) or fasting levels (baseline). P values within brackets from Wilcoxon signed rank tests of deltaAUC (for all panels except D, where total AUC is shown).

Cognitive test performance for both DSST and TMT was negatively affected by hypoglycaemia during the preoperative visits. At the postoperative visits, there was no difference in performance for DSST between normo- and hypoglycaemia and for TMT the subjects improved their score between normo- and hypoglycaemia. During hypoglycaemia, subjects scored significantly better after surgery than before, for both DSST and TMT. Symptoms of hypoglycaemia according to EHSS was not significantly different between visits.

Imaging with FDG-PET during clamps showed a global four-fold increase in ^{18}F-FDG-glucose influx (Ki) to the brain during hypoglycaemia (**Figure 9),** both before and after surgery. In the hypothalamic area, however, Ki was reduced during hypoglycaemia, both before and after surgery. There was a slight reduction in glucose influx and uptake (MRGlu) in the brain during normoglycaemia after RYGB.
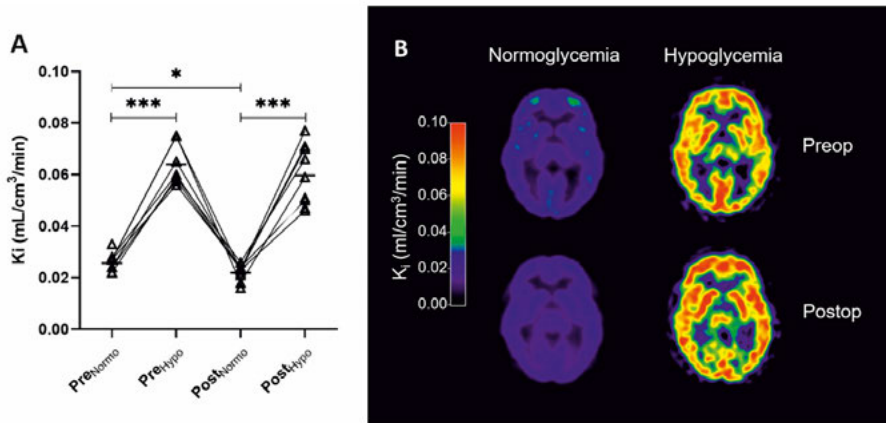
Figure 9. Cerebral glucose influx during normo- and hypoglycaemia, before (Pre) and after (Post) RYGB. A: Whole-brain Ki values, *P < 0.05, ***P < 0.001 for post- vs. presurgery (paired t tests). B: Ki PET images reflecting a typical patient, before and after surgery during normo- and hypoglycaemia.

Arterial spin labelling analysis revealed that subjects had a higher global cerebral blood flow during normoglycaemia after surgery, as well as higher CBF for all investigated brain regions. There were no differences before vs after surgery in the effects of hypoglycaemia on CBF. There were positive correlations between the blood flow on ASL and better results on cognitive tests after surgery.

TICA-analysis of functional MR-imaging during resting state identified an individual component – i.e. a network of connections – which accounted for most of the variability of the data. This component involved the hypothalamus, bilateral thalamus and frontal cortical regions in the right hemisphere. It

was differentially activated during the glucose lowering phase/early hypogly-caemia when comparing pre- to post-surgery, with significantly higher activation after surgery (**Figure 10.**)
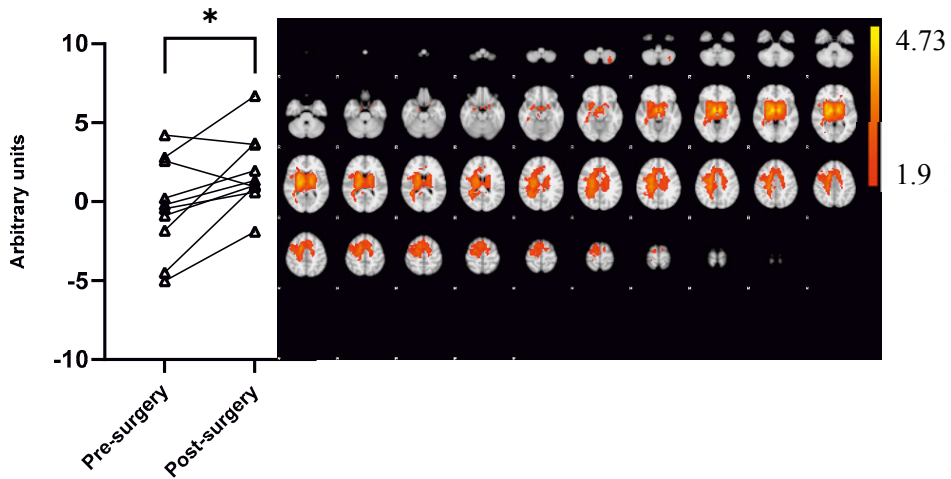

Figure 10. Individual component (connectivity network) including bilateral thalamus and hypothalamus identified during fMRI as differentially activated before vs after surgery. Colours represent T-values. Left panel: Individual data of the above activity during the initial phase of hypoglycaemia *P < 0.05.

Paper III

Thirteen patients with T2DM were followed for the duration of the study, and twelve of these completed all visits. The group had lost 22.5% of their weight after six months (24 weeks) and remained at approximately the same weight at the two year (104 week) visit. Likewise, BMI, waist and hip circumference decreased after surgery and remained largely stable between six months and two years. The fasting glucose levels and Hba1c were reduced after surgery and remained reduced at the 104-week visit relative to baseline, despite a reduction in antidiabetic treatment. At preoperative baseline, all subjects were treated with antidiabetic agents. This was reduced to one, two and four patients at the 4-week, 24-week and 104-week visits, respectively. At all visits after surgery metabolic indices Matsuda and HOMA-IR were both significantly improved relative to baseline values, whereas the insulinogenic index was unaffected.

The release of free fatty acids during OGTT was significantly changed at 24 weeks and 104 weeks relative to baseline, with reduced levels. Glycerol was also significantly different at 104 weeks, with a reduction of approximately 50% from baseline. White blood cell count was lower at post-operative visit, significantly so at 4 weeks and 24 weeks. C-reactive protein levels were

lower at all post-operative visits, which reached significance at 24 and 104 weeks. (**Figure 11**)
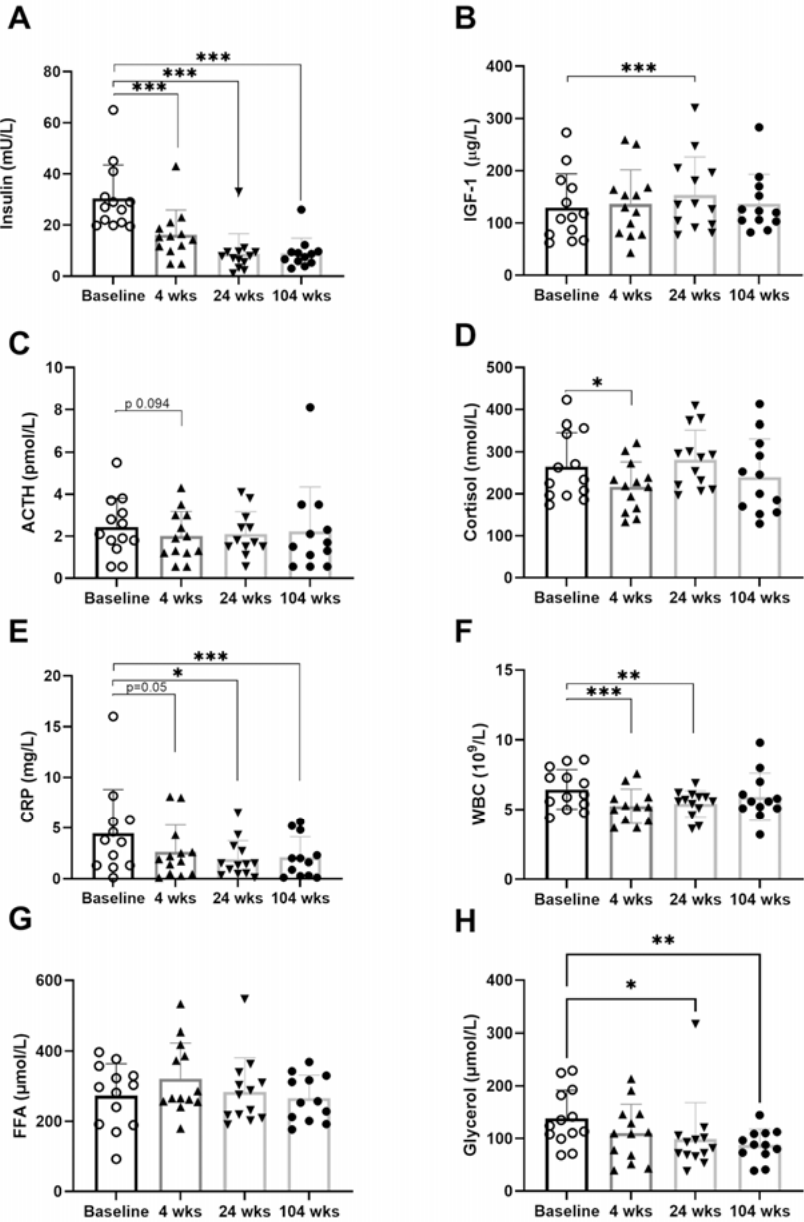

Figure 11. Hormonal, inflammatory and lipolysis measures. Fasting morning levels of (A) insulin, (B) IGF-1, (C) ACTH, (D) cortisol, (E) C-reactive protein (CRP), (F) white blood cell count (WBC), (G) free fatty acids (FFA) and (H) glycerol at preoperative baseline and at 4, 24, and 104 weeks (wks) after RYGB. Data are means ± SD. P-values from pairwise comparison with baseline using mixed effects model, corrected for false detection rate. *P < 0.05, ***P < 0.01, ***P < 0.001.

Two years after surgery, patients had reduced their total body fat by about 21%. Adipose tissue analyses revealed that the volume of subcutaneous adipocytes was reduced by approximately 23% during the duration of the stud. At the 104-week visit subjects had a mean adipocyte volume which was 30% less than that of a group of age-, sex- and BMI-matched, non-operated controls. At 104 weeks the basal and insulin stimulated glucose uptake of cells was increased relative to baseline, reaching similar uptake levels as the control group. There was no significant effect on lipolysis rate with surgery.

Adipose tissue gene expression was affected for a number of genes. At the 104 weeks visit, expression of the genes for adipokines adiponectin and leptin has increased approximately threefold whereas the expression of the gene for resistin, a peptide hormone linked to insulin resistance (95) was reduced by approximately one third two years after surgery.

Most analysed genes of relevance for adipogenesis (*BMP4*, *CEBPA*, *CEBPB*, and *FASN*) had an increased expression at 104 weeks, the exception being *FABP4* and *PPARG* which were significantly increased at 24 weeks but not at 104 weeks. Expression of the gene for transcription factor E2F1, which promotes adipogenesis (96), showed a reduced expression after surgery. Genes involved in mitochondrial function (*CPT1B*) and glucose transport (*SLC2A4, IRS1, AKT1*) showed increased expression at 24 and 104 weeks. Genes coding for pro-inflammatory cytokines interleukin 6 and interleukin 18 decreased in expression after surgery.

Heart rate variability recording showed increases in PTOT, PLF and PHF at 4 and 24 weeks, but the difference was no longer significant at 104 weeks, compared to baseline. The PLF/PHF ratio was reduced, significantly so at 24 weeks.

Paper IV

10 individuals completed both meal tests. C-peptide and insulin levels were higher with exenatide than with saline, when comparing areas under the curve, as is expected. Cortisol AUC was higher during exenatide (**Figure 12**). This did not correspond to higher glucose AUC with exenatide. We observed no significant differences for other measured hormones.

HRV analysis revealed a significant difference in parameters RR, Ptot, PHF and PLF between saline and exenatide which became apparent around 1 hour after the liquid meal. The RR-interval was reduced with exentide, which is an effect of increased heart rate. Ptot, PHF and PLF were lower with exenatide, which could indicate reduced parasympathetic tone.
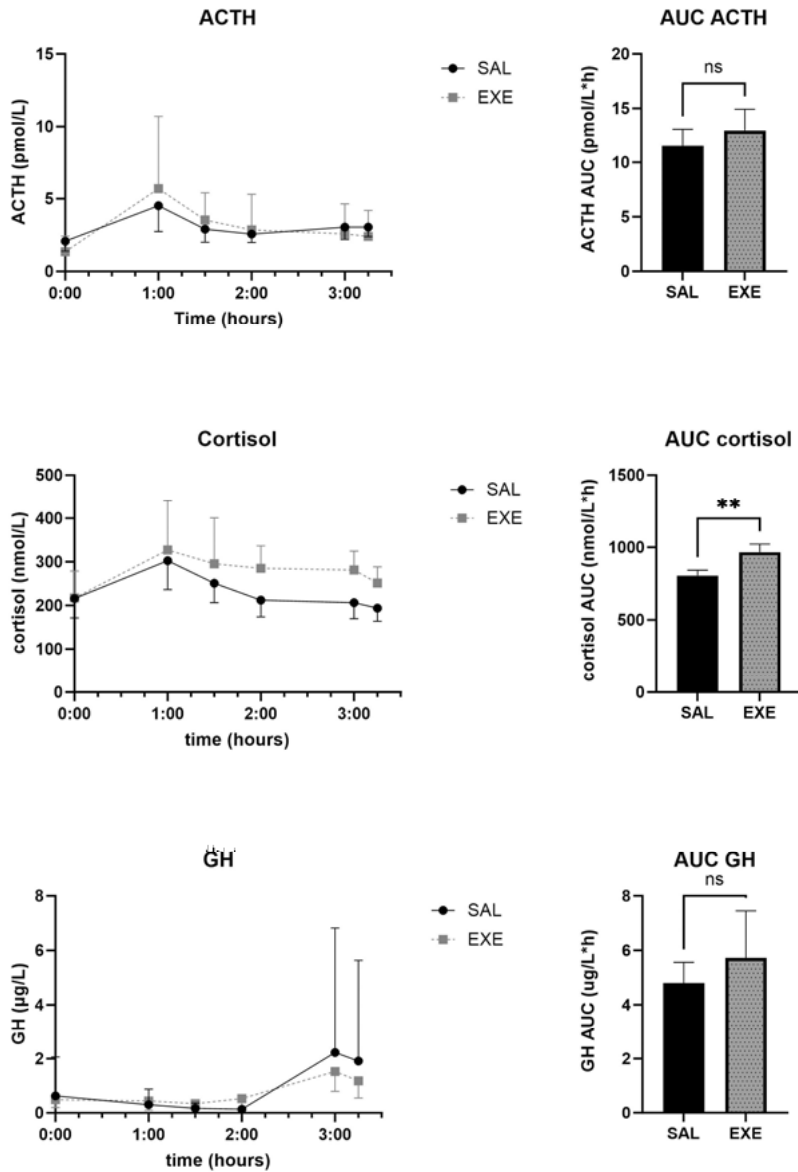
Figure 12. Cortisol, ACTH and GH levels during meal test in 10 patients approx. 1 year after RYGB during saline or exenatide infusion. Values are geometric means + 95% confidence intervals. AUC = Mean total area under the curve calculated with trapezoidal rule + SEM. NS = non-significant, **p<0.01.

Discussion

Paper I

Previous work has reported increases in both GIP and GLP-1 in response to hypoglycaemia during clamps (40). This is an interesting observation for hormones primarily known for their insulinogenic, i.e. glucose lowering, effects in the post-prandial setting. A rise in GLP-1 and GIP in response to hypoglycaemia suggests they may play a physiological role in hypoglycaemic counter-regulation. At the same time, comparisons of patients before and after RYGB revealed that patients had lower levels of GLP-1 and GIP during hypoglycaemia after surgery. It has been suggested that decreased glucagon secretion might be the mechanism through which lower levels of GLP-1 and GIP might predispose towards hypoglycaemia. The aim of Paper I was to explore whether the described attenuated GLP-1 secretion during hypoglycaemia could explain the tendency towards postprandial hypoglycaemia in patients after RYGB.

To address this hypothesis we wanted to determine whether infused exogenous GLP-1 might enhance counterregulatory hormones or neuroendocrine effects or affect the severity of hypoglycaemic symptoms. This theory was suggested by the fact that patients with recurrent hypoglycaemia after RYGB reported fewer hypoglycaemic episodes after initiating treatment with GLP-1 analogues for other reasons, and have indeed been shown to have more stable glucose levels after initiating GLP-1 treatment (73).

Results from Paper I suggest that hypoglycaemia was indeed achieved during the clamps, as is evident from the elevation of counterregulatory hormones. We however found no signs of an acute effect of GLP-1 analogue exenatide infusion on the hormonal counterregulatory response. We did note a decrease in the incretin GIP. This may be due to the structural similarity of GLP-1 to GIP, with exenatide potentially binding to GIPR and causing negative feedback effects.

The effects of exenatide vs saline on heart rate variability parameters point towards modulatory effects on the autonomic nervous system in the form of a relative increase in parasympathetic over sympathetic tone. This is particularly interesting as hypoglycaemic counter-regulation is mainly attributed to increases in sympathetic rather than parasympathetic activity. Further studies are needed to assess whether the observed changes to HRV parameters can be

linked to the alleviating effects of exogenous GLP-1 in patients with hypoglycaemia after RYGB.

It is possible that potential alleviating effects of GLP-1 analogues on the tendency towards hypoglycaemia could be attributed mechanisms involving for example changes to eating habits, effects on the dynamics of insulin secretion or changes to gastrointestinal motility. It could also be that such benefits might require more chronic exposure to GLP-1 analogues. These questions are beyond the scope of the current work but should be of interest for further studies.

Paper II

What role the CNS plays in glucose homeostasis under physiological conditions is incompletely known (60). The aim of this study was to explore whether the changes that are thought to occur in patients with T1DM hypoglycaemic unawareness could be seen in patients after RYGB and explain the common tendency towards (often asymptomatic) hypoglycaemia. With the combination of several modes of investigation, we hoped to be able to approach the question from different perspectives.

This study supports previous findings that the hormonal responses to hypoglycaemia are blunted after RYGB for glucagon, which supports previous findings by Abrahamsson et al. and others (40). We also see indications, albeit non-significant, of lower HPA axis responses after surgery, as reported previously (40, 97). We found an increase in GH in response to hypoglycaemia after surgery, also in line with previous results (40, 98). Interestingly, recent work by Lundqvist et al. (70) has shown an enhanced ACTH response to hypoglycaemia in subjects with overweight/obesity, as well as a tendency to higher cortisol and a lower GH during hypoglycaemia. Both obesity and insulin resistance was associated with higher ACTH and cortisol responses.

The observed changes to counterregulatory systems could thus be considered a normalization of endocrine and neuroendocrine responses to hypoglycaemia dysregulated by obesity and insulin resistance. The potentially attenuated HPA–axis response in this work and others (40) could be considered as support for a neuroendocrine adaptation to lower glucose levels mediated via the CNS. Heart rate was decreased after surgery, both during normo- and hypoglycaemia, which also could point towards changes to ANS activity, as has been suggested by previous publications (40) and the results from Paper III. In this study, however, we found no changes to heart rate variability parameters to support this, which could be due to the limited size of our cohort or due to the fact that HRV recordings needed to be performed after the end of imaging, i.e. at the very end of the hypoglycaemic phase. It is likely that any differential activation of the autonomic response would occur in the earliest

stages of glucose lowering/hypoglycaemia, and that effects of ANS activation on HRV might not be detectable at a later stage.

ASL imaging revealed distinct increases to global as well as regional cerebral blood flow after RYGB, which was not attributable to a significant increase in cardiac output or cardiac index. At the same time, cerebral glucose influx and uptake was decreased during normoglycaemia after RYGB. This discrepancy between blood flow changes and glucose utilization is intriguing.

Moreover, we observed indications of improved results from cognitive tests after surgery, which was significant during hypoglycaemia. Evidence of cognitive improvements after RYGB have been presented previously in several studies (99). The correlation between CBF in some regions during hypoglycaemia and improved test results could points towards cognitive benefits of RYGB. That the improvements are apparent during hypoglycaemia may support the concept of a general adaptation to lower glucose levels.

Resting state functional-MR imaging identified increased activity after surgery in a network including the hypothalamus and thalamus during early hypoglycaemia. Interestingly, studies on patient with T1DM and hypoglycaemic unawareness have found attenuated responses to hypoglycaemia involving the same regions, as well as the amygdala and striatum (100-103). The hypothalamus is of particular interest, as neurons responsive to changes in extracellular glucose levels have been identified here (64). The hypothalamus could feasibly be the link between CNS adaptations and changes to neuroendocrine signalling and endocrine responses to hypoglycaemia.

Paper III

The aim of this study was to capture the temporal changes that occur after RYGB in a cohort of patients with T2DM. Of interest was also whether a group with somewhat lower BMI than is the average for bariatric patients would have clinical benefits of the same magnitude. This study demonstrates substantial improvements after RYGB in most clinically relevant variables examined, which are largely preserved over time. Our results point to early improvements in insulin sensitivity and therefore reduced needs for antidiabetic treatment as soon as 4 weeks after surgery (104). These changes precede most of the observed weight loss, in concurrence with earlier studies (105).

We also report early, transient decreases to cortisol and ACTH at 4 weeks post-RYGB, although the latter did not reach significance. Hyperactivity of the HPA axis has been suggested to be present in subjects with obesity and visceral adiposity, but data has been inconsistent between studies (106, 107). After surgery we also observed reduced levels of CRP and a lower WBC. This is of interest in the light of the observed effects on adipose tissue gene expression, more specifically the reduced expression of genes for adipokines and

interleukins 6 and 18. Taken together, these results could be interpreted as signs of reduced inflammatory activity.

Paper IV

The purpose of Paper IV was to determine whether GLP-1 analogue exenatide compared to saline was associated with a change in any of the hormones known to be involved in hypoglycaemia counterregulation in a standardized meal test on subjects who had undergone bariatric surgery, as had been suggested by previous case reports (73). Our main finding was an increase in cortisol levels during exenatide infusion. This was, however, not associated with a more beneficial glucose curve after the meal test with exenatide. As cortisol takes several hours to have an effect on glycaemia, it is still conceivable that treatment with exenatide could protect against later hypoglycaemia development, if it consistently affects cortisol in this manner. There are only a few studies reporting on the effects of GLP-1 analogues on the cortisol axis, and our findings suggest this warrants further exploration.

An attempt to summarize the results from these papers, along with conclusions from previous work by Abrahamsson et al. (40) and others has been illustrated in **Figure 13.** This image proposes a hypothetical framework for understanding the glucose lowering effects of RYGB, with particular focus on post-bariatric hypoglycaemia and potential changes to counterregulatory mechanisms.

It is conceivable that the beneficial effects of RYGB on glucose control and the phenomenon of post-bariatric hypoglycaemia are phenomena on the same spectrum, and do not involve separate mechanisms, but rather differences in timing and magnitude of involved nutrients and hormones. It should be noted that the effects of RYGB on glucose control are overwhelmingly positive, and that for the majority of patients, the procedure offers distinct health benefits. This is true for both aspects such as patient satisfaction (108) and quality of life (109) as well as for end points such as remission of type 2 diabetes (24), risk of cardiovascular events (110) and all-cause mortality (111).
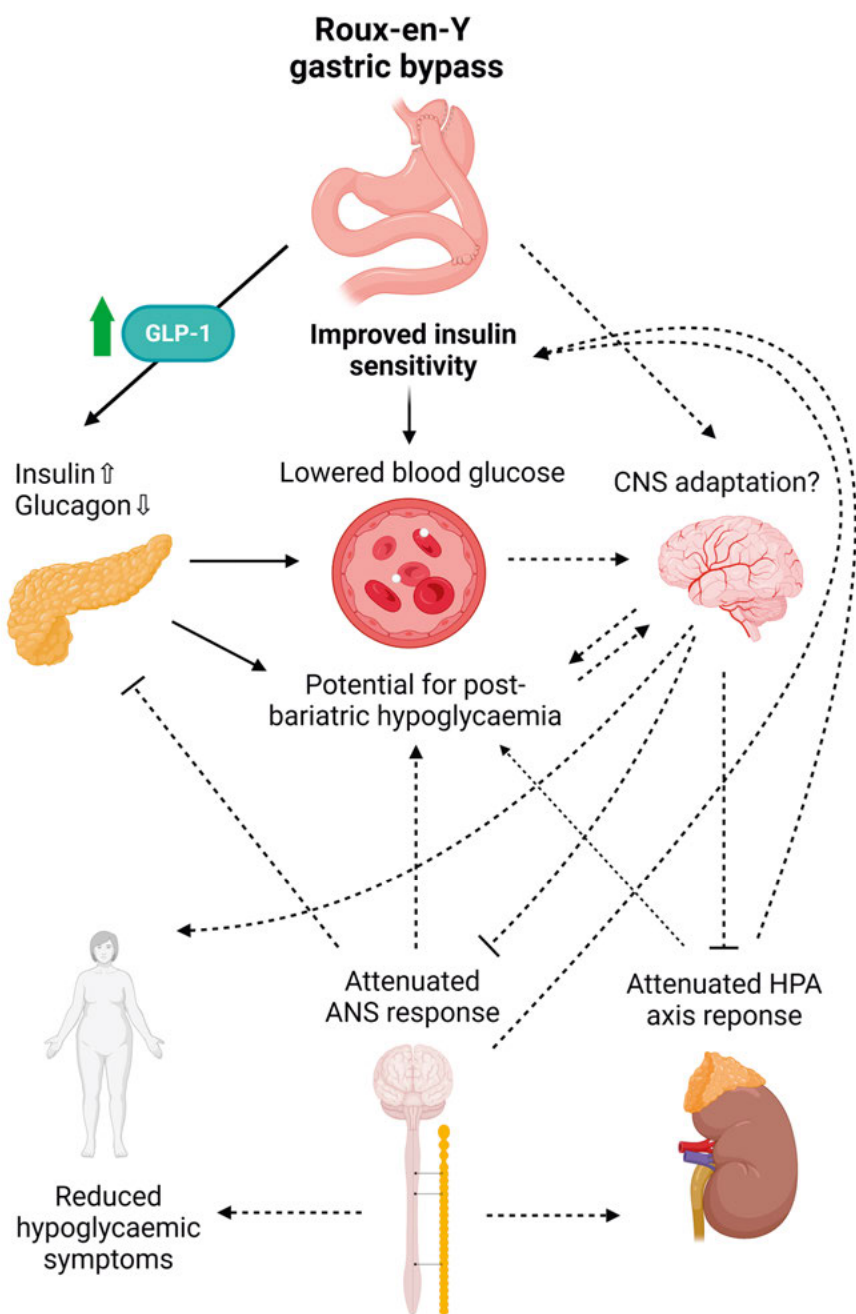
Figure 13. Hypothetical schematic overview of glucose lowering effects of RYGB and mechanisms behind and effects of post-bariatric hypoglycaemia. Solid lines = established effect. Dashed lines = hypothetical effect. Arrows = leads to, T-shaped lines = inhibition. CNS = central nervous system. ANS = autonomic nervous system. HPA axis = hypothalamic-pituitary-adrenal axis. Created with BioRender.com.

Strengths and limitations

The papers included in this thesis detail a comprehensive attempt at trying to understand the effects of gastric bypass surgery on glucose homeostasis, in particular in regards to hypoglycaemia. As such, they complement each other, and can be used together to draw conclusions on the concept of post-bariatric hypoglycaemia. Paper III stands out in that it is the only study on patients with type 2 diabetes before surgery, and does not cover the question of hypoglycaemia counterregulation. It does however have the advantage of longitudinal follow-up, which adds another aspect to the study of the effects of Roux en-Y gastric bypass on glucose homeostasis.

One limitation when approaching the question of post-bariatric hypoglycaemia in Paper I, II and IV, is the fact that our subjects had not previously reported those symptoms, and had no diagnosis of post-prandial hypoglycaemia but were taken from the general RYGB operated population. Due to the relative rarity of patients with this condition clinically established, such a cohort was not available to us. It would also have been interesting to screen the included study subjects for occurrence of post-prandial hypoglycaemia before clamps/meal-tests. It is possible that if we had selected only subjects with reported symptoms of post-prandial hypoglycaemia and with verified hypoglycaemia during CGM-monitoring or after OGTT we would have seen different results on counterregulatory hormones and the effect of exenatide.

The initial case series detailing successful treatment with GLP-1 analogues for post-bariatric hypoglycaemia (73), which inspired the experimental protocol for Papers I and IV, actually used the GLP-1 analogue liraglutide, and not exenatide. It is possible that there are differences between GLP-1 analogues in this regard and that such could explain our results.

The clamp protocols for Papers I, II and IV, particularly when performed during simultaneous imaging, are laborious for staff and participants alike, which limited the number of participants and experiments possible to perform. All together, the studies include fewer patients than would be ideal and it is possible that some potentially significant results could have been unearthed if we had larger study populations.

Another concern is that the hyperglycaemic clamp method used for paper I and II can only partly mimic the hypoglycaemic condition as it occurs in real life in patients with post-prandial hypoglycaemia. Although post-prandial hypoglycaemia has been attributed to higher peak insulin levels following meals

(30) a sustained hyperinsulinemia like in this study would not occur during postprandial hypoglycaemic conditions as hypoglycaemia itself inhibits insulin release.

The meal test used in Paper IV cannot be considered entirely comparable to an actual meal. Although it has the advantage of being standardized in terms of volume and caloric content, it is liquid, which means it is ingested faster and likely also passes to distal portions of the gastrointestinal tract faster than a meal of consisting of a variety of textures. Moreover, 300 kcal is relatively little energy and it is likely that post-bariatric patients do have larger meals, especially those times that post-prandial hypoglycaemia is triggered.

Heart rate variability analysis, although commonly used in a number of different fields of research, does offer a lot of challenges as there is no established gold standard on what method of analysis to use and how the different parameters should be interpreted. Variations in methodology between studies make results difficult to compare.

In our studies of exenatide, the known stimulatory effect of GLP-1 analogues on heart rate likely effects HRV parameters, and it is difficult to determine how much should be attributed to specific effects on ANS activity. The hypothetic possibility of direct chronotropic effects of GLP-1 receptor activation on the heart is a possible confounder to the interpretation of our results. The results from animal studies, however, suggest that GLP-1 analogues act mainly via indirect effects on the heart mediated via beta-adrenergic receptors and thus imply sympathetic nervous system involvement (112).

Conclusions

The four papers presented here approach the complex question of metabolic changes after Roux-en-Y gastric bypass from a number of different aspects. As such, to summarize the potential mechanisms involved into one overarching hypothesis is challenging. We present further evidence of an attenuated counterregulatory response to hypoglycaemia after RYGB. Overall, this does not seem to be significantly affected by acute administration of the GLP-1 analogue exenatide during a hypoglycaemic clamp. When given in conjunction with a meal test, the same GLP-1 analogue does seem to be associated with a rise in cortisol levels. Although cortisol is known to counteract hypoglycaemia, we saw no indication of higher glucose levels after the meal with exenatide.

Due to differences in method, not all results can be compared between studies, which limits the interpretation of, for example, HRV data. Taken together with previous work from our group, there are indications that RYGB affects the ANS activity towards an increase of parasympathetic relative to sympathetic tone. This seems to be true also during hypoglycaemia (40), which could influence part of the phenomenon of post-bariatric hypoglycaemia. Infusion of exenatide during hypoglycaemia was associated with changes to HRV which could indicate attenuating effects of the GLP-1 analogue on sympathetic tone, relative to the parasympathetic, similar in direction to the effects seen with surgery.

We demonstrate marked increases in cerebral blood flow after RYGB surgery, however this does not seem to be affected by hypoglycaemia. Still, rapid lowering of glucose elicits an increase in activity in a functional network of brain regions involving the thalamus and hypothalamus, which is not the case before surgery. Preoperatively, glucose uptake is decreased during hypoglycaemia in the hypothalamic area and this effect of hypoglycaemia on glucose uptake is less pronounced after surgery. Detrimental effects of hypoglycaemia on cognitive performance seem blunted after surgery. Taken together, these results suggest that there may be post-surgical adaptations occurring in the CNS with regards to hypoglycaemia that could potentially explain part of the attenuated counterregulation as well as the fact that PBH often is asymptomatic. Whether the early, subtle effects of RYGB on HRV, markers of inflammation and the ACTH-cortisol axis could influence such adaptive CNS effects would be of interest to investigate further.

Future perspectives

The true prevalence of post-bariatric hypoglycaemia is likely underestimated and little is known about the long-term implications of recurring and sometimes severe hypoglycaemic episodes in patients without insulin treatment. Undoubtedly, it causes discomfort and inconvenience to those afflicted. To elucidate if and why GLP-1 analogue treatment protects against post-bariatric hypoglycaemia thus remains a relevant research topic as the post-bariatric population continues to grow.

To affirm the role of GLP-1 analogues in the treatment of post-bariatric hypoglycaemia, larger studies are needed. Thus far, evidence is limited to a handful of case reports. Ideally, such studies should not only include more participants and repeated CGM before and after intervention with GLP-1 analogues, but should be randomized and controlled. Blinding would require sham-injections, which may or may not be feasible. Ideally, different GLP-1 analogues should also be compared.

Moreover, to more completely understand the potential protective mechanism against post-bariatric hypoglycaemia from this drug class, study participants should be asked to record any effects on dietary choices, meal frequency and size. Such data of course has its own limitations.

At a later stage, GLP-1 analogue treatment for post bariatric hypoglycaemia studies with longer-follow up would be ideal. If such studies are conducted, they should include measurements of HPA-axis activity, as this seems to be underexplored.

The full spectrum of mechanisms behind the beneficial effects of RYGB on glucose homeostasis has yet to be revealed. Several other gut hormones, such as GIP, oxyntomodulin and PYY, are effected by surgery and the importance and dynamics of their function on weight and glucose control are less well described compared to that of GLP-1. Moreover, (afferent and efferent) ANS in mediating or modulating the effects of surgery is intriguing and would be of interest to explore more in detail. How weight control and glucose homeostasis is affected by the central nervous system is still very much in the dark and to shed light on this topic offers the potential to discover new targets for the pharmaceutical treatment of obesity and type 2 diabetes, making it a worthy research focus.

Svensk sammanfattning

Övervikt och obesitas är viktiga riskfaktorer bakom sjuklighet världen över. Gastric bypass-kirurgi utgör ett effektivt verktyg för att behandla obesitas och ger mestadels bestående resultat över tid. Metoden har även visat sig ge mycket gynnsamma effekter hos patienter med typ 2 diabetes, och kan ofta leda till att sjukdomen helt går i regress. Hur detta går till är av stort intresse för det medicinska samfundet.

I denna avhandling behandlas frågor som rör hur gastric bypass påverkar sockeromsättningen hos personer med och utan typ 2 diabetes, och hur detta yttrar sig över tid. Frågan belyses från ett antal olika aspekter, med fokus på samspelet mellan det hormonella systemet, centrala nervsystemet och fettvävnaden.

I det första, andra och fjärde delarbetet är ämnet specifikt inriktat på vad som uppträder vid låga blodsockervärden hos personer som har genomgått gastric bypass-kirurgi. En av komplikationerna till kirurgin är att patienten kan få en tendens att utveckla lågt blodsocker (hypoglykemi) efter måltid. Vad detta beror på är ofullständigt utrett, likaså hur det bör åtgärdas. Tidigare fallrapporter har demonstrerat att behandling med läkemedel baserat på hormonet GLP-1 (GLP-1 analoger) kan avhjälpa tendensen att utveckla lågt blodsocker hos dessa patienter. Detta är paradoxalt eftersom kroppseget GLP-1 stimulerar till insulinfrisättning, vilket i regel sänker blodsockret. En central fråga för denna avhandling har varit med vilken mekanism som GLP-1 analoger kan ge denna skyddande effekt mot lågt blodsocker.

En hypotes har varit att GLP-1 analogbehandling på något vis skulle kunna påverka det system som vanligtvis skyddar kroppen mot låga blodsocker. Detta, så kallade motregulatoriska system, inkluderar aktivering av det autonoma nervsystemet som verkar via adrenalin och noradrenalin, både i nervändar och i blodomloppet. Motreglering mot lågt blodsocker består också i frisättning av hormonerna glukagon, tillväxthormon (GH) och kortisol i cirkulationen.

I delarbete ett studerar vi detta system hos tolv personer som genomgått gastric bypass kirurgi. Försökspersonerna försätts i ett tillstånd av hypoglykemi genom ett så kallad "clamp"-experiment (ungefär 'tång') där intravenös tillförsel av insulin används för att sänka sockret, och takten på detta styrs av ett justerbart dropp med sockerlösning. Samtidigt studeras det hormonella

svaret genom blodprover som upprepas över tid. Detta genomförs vid två tillfällen per person, ett med intravenös tillförsel av GLP-1 analogen exenatid, och ett med koksaltlösning, som utgör ett kontrollförhållande. Vi fann vi inga tecken på att exenatid ökade det motregulatoriska hormonsvaret under en hypoglykemisk clamp.

Clamp-metoden används även i delarbete två. Studien jämför det motregulatoriska svaret mot hypoglykemi hos elva personer före och efter deras gastric bypass-kirurgi. Samtidigt som studiedeltagarnas blodsocker fixeras och sänks studerades deras hjärna med en kombinerad magnetkameraundersökning och så kallad PET-undersökning. På så vis undersöktes det centrala nervsystemets sockerupptag och blodflöde under både normalt blodsocker och under hypoglykemi. Deltagarna fick också genomföra kognitiva tester under normalt och lågt blodsocker, och skatta sina symptom på hypoglykemi.

Vi fann tecken på att det motregulatoriska svaret på hypoglykemi är lägre efter kirurgin, vilket framförallt syntes på hormonet glukagon. Hjärnans genomblödning under normala blodsockernivåer var högre efter operationen, samtidigt som glukosupptaget i hjärnan var lägre. Under hypoglykemi ökade sockerupptaget i hela hjärnan högre, vilket ej föreföll påverkas av kirurgin. Dock minskade glukosupptaget i hypothalamusområdet under hypoglykemi, ett område där hjärnans kontroll av kroppens energiomsättning och blodsockerregleringen tros vara lokaliserad. Denna minskning av glukosupptag under hypoglykemi i hypothalamus var mindre uttalad efter kirurgin, vilket kan stödja tesen att det sker en form av tillvänjning till lägre glukosnivåer i det centrala nervsystemet efter gastric bypass-kirurgi. Deltagarnas resultat på kognitiva tester under hypoglykemi förbättrades också efter operationen.

I delarbete tre beskrivs 13 patienter med typ 2 diabetes som genomgått överviktskirurgi med gastric bypass. De följdes från innan operationen till två år efter, för att studera olika aspekter av deras ämnesomsättning och relaterade hälsofaktorer. Bland annat undersöktes deltagarnas fettcellers ämnesomsättning och genuttrycket i deras fettvävnad, samt hur detta förändrades över tid. Deras fettvävs egenskaper efter två år jämfördes också med en kontrollgrupp bestående av 22 personer med motsvarande vikt, ålder och kön som ej genomgått kirurgi. Vi fann en tidig och övergående sänkning av kortisol efter operationen, likaså en liten minskning i antalet vita blodkroppar och inflammationsmarkören CRP, vilket skulle kunna tyda på gynnsamma antiinflammatoriska effekter av kirurgin. Uttrycket av inflammationsdrivande gener (IL-6, IL-18) minskade 6 månader efter kirurgin. Fettcellernas glukosupptag hade förbättrats två år efter kirurgi, vilket avspeglades i fettvävens genuttryck.

Delarbete fyra berör samma frågeställning som det första delarbetet. Här har vi studerat effekten av exenatid jämfört med koksalt på de motregulatoriska hormonerna hos tio patienter som genomgått gastric bypass kirurgi samtidigt som de fick genomgå ett så kallat standardiserat måltidstest. Vi observerade högre kortisolnivåer med exenatid under måltidsbelastningen, men inte någon skillnad i glukosnivåer.

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