The Impact of Bariatric Surgery on Obesity related Metabolic Traits with Specific Emphasis on Glucose, Insulin and Proinsulin

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Dissertation presented at Uppsala University to be publicly examined in Universitetshuset, sal IV, Universitetshuset Uppsala Universitet 740 10 Uppsala, Uppsala, Wednesday, February 10, 2010 at 09:30 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract
Johansson, H-E. 2010. The Impact of Bariatric Surgery on Obesity related Metabolic Traits with Specific Emphasis on Glucose, Insulin and Proinsulin. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 511. 78 pp. Uppsala. ISBN 978-91-554-7695-3.

Hyperproinsulinemia is associated with type 2 diabetes (T2DM) and obesity and is a predictor for future coronary heart disease. This thesis examines the effect of bariatric surgery on glucometabolic status including insulin and proinsulin responses after meal. Further we explored longitudinally the effects of bariatric surgery on glucose, insulin and proinsulin secretion as well as lipids, liver enzymes and magnesium concentrations.

We explored by a standardised meal test the postprandial dynamics of proinsulin and insulin and effects on glucose and lipids in patients treated with gastric bypass (RYGBP) surgery and in patients treated with bileopancreatic diversion with duodenal switch surgery (BPD-DS). Comparisons were made to morbidly obese patients and normal weight controls (NW). RYGBP surgery markedly lowers fasting and postprandial proinsulin concentrations although BMI was higher compared to NW-controls. BPD-DS surgery induces a large weight loss and normalises postprandial responses of glucose, proinsulin and insulin and markedly lowers triglycerides.

We evaluated non-diabetic morbidly obese patients who underwent bariatric surgery followed-up for up to four years after surgery. Long-term follow-up showed that RYGBP surgery is not only characterized by markedly and sustained lowered BMI but also lowered concentrations of proinsulin, insulin, ALT and increased HDL-C possibly via reduced hepatic insulin resistance.

We also examined how magnesium status is affected by bariatric surgery as magnesium has been shown to be inversely related to glucose and to insulin resistance. The serum magnesium concentrations increased by 6% after RYGBP and 10% after BPD-DS.

In summary, RYGBP and BPD-DS surgery results in marked weight loss, alterations in insulin and proinsulin dynamics, lowered fasting and postprandial proinsulin concentrations and improved glucometabolic and magnesium status.

Keywords: Proinsulin, insulin, glucose, obesity, bariatric surgery

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Overweight and obesity are now so common that they are replacing more traditional problems such as undernutrition and infectious diseases as the most significant causes of ill-health

(WHO, Tech Rep Ser. 2000; 894:i-xii, 1-253)

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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   **Johansson H-E**, Öhrvall M, Haenni A, Sundbom M, Edén-Engström B, Karsson F.A, Zethelius B.
Gastric bypass alters the dynamics and metabolic effects of insulin and proinsulin secretion.
Diabet Med 2007 Nov; 24(11):1213-20. PMID 17894830

II **Johansson H-E**, Haenni A, Karlsson F.A, Eden-Engström, Öhrvall M, Sundbom M, Zethelius B.
Bileopancreatic Diversion with Duodenal switch lowers Early and Late Phases of Glucose, Insulin and Proinsulin responses after Meal. Submitted.

III **Johansson H-E**, Haenni A, Öhrvall M, Sundbom M, Zethelius B.
Alterations in Proinsulin and Insulin Dynamics, HDL Cholesterol and ALT After Gastric Bypass Surgery. A 42-Monts Follow-up Study.
Obes Surg 2009 May; 19(5): 601-7. PMID 19229660.

IV **Johansson H-E**, Zethelius B Öhrvall M, Sundbom M, Haenni A.
Serum Magnesium Status After Gastric Bypass Surgery in Obesity.
Obes Surg 2009 Sep; 19(9):1250-5. PMID 18542850

Contents

Abbreviations

AGB	Adjustable Gastric Banding
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ARBs	Angiotensin II Receptor Blockers
AUC	Area Under the Curve
BPD-DS	Bilieo-Pancreatic Diversion with Duodenal Switch
BMI	Body Mass Index
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
FFA	Free Fatty Acids
F	Fasting
GLUT-4	Glucose Transporter 4
$HbA1_c$	Glycated Hemoglobine
HDL	High Density Lipoprotein
IGT	Impaired Glucose Tolerance
JIB	Jejuno-Ileal Bypass
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
MO	Morbidly Obese
MOC	Morbidly Obese Control
NAFLD	Non-alcoholic fatty liver disease
NBHW	National Board of Health and Welfare
NGT	Normal Glucose Tolerance
NW	Normal Weight
OGTT	Oral Glucose Tolerance Test
OSA	Obstructive Sleep Apnoea
P	Plasma
PAI-1	Plasminogen activator inhibitor-1
PCOS	Polycystic Ovarian Syndrome
RYGBP	Roux-en-Y Gastric By Pass
S	Serum
SD	Standard Deviation
SEM	Standard Error of the Mean

T2DM	Type 2 Diabetes Mellitus
TG	Triglycerides
VBG	Vertical-banded Gastroplasty

Introduction

Obesity, general introduction

Obesity has become a global epidemic and more than 400 million individuals worldwide are obese [1]. Obesity is not only a burden per se, but is also tightly connected to diabetes and cardiovascular diseases and many other diseases. Treatments for obesity include a range of therapies, such as dietary advice and physical activity, behaviour therapy, pharmacological therapy and bariatric surgery. The most pronounced weight loss is obtained by bariatric surgery as presented in a recent Cohrane review [2].

Obesity is associated with insulin resistance [3], fasting hyperinsulinemia [4] and fasting hyperproinsulinemia [5]. In addition to insulin resistance [6], hyperproinsulinemia, reflecting beta-cell strain, is associated with increased incidence of type 2 diabetes mellitus (T2DM) in men [7, 8] and women [9], with coronary heart disease (CHD) morbidity [10-12] and mortality in men [12]. Elevated proinsulin has been shown to predict conversion to T2DM independent of peripheral insulin resistance and early insulin response during glucose tolerance tests [7, 8]. Moderate weight loss due to lifestyle changes and pharmacological treatment is associated with improved insulin sensitivity and lowered hyperinsulinemia [13] and a reduced incidence of T2DM [14, 15].

Bariatric surgery ad modum Roux-en-Y Gastric bypass (RYGBP) is now a frequently used procedure for obesity treatment and has been shown not only to induce rapid weight loss, followed by long term weight stability, but also to reduce the risk of developing T2DM, cardiovascular diseases (CVD) and cancer [16-18]. The pronounced weight loss after RYGBP surgery in subjects with morbid obesity is also associated with a marked improvement in insulin sensitivity [19], lowered fasting hyperinsulinemia [20], lowered postprandial hyperinsulinemia [21] and a markedly reduced conversion to T2DM (over 10 years of follow-up) and to induce remission of T2DM [16, 22-24]. Regarding fasting and postprandial changes in proinsulin after RYGBP surgery in morbidly obese patients, there is only scant information.

Patients with insulin resistant conditions like T2DM have lower circulating magnesium concentrations than healthy subjects [25, 26]. Low serum magnesium concentration was shown to increase the risk of all-cause mortality when added to the conventional CVD risk factors and was significantly associated with all-cause mortality in T2DM patients [27]. A previous

weight-reducing surgical method, the jejunoileal by-pass (JIB) was associated with an increased risk of magnesium depletion [28]. Regarding RYGBP surgery in morbidly obese patients, there is only very limited information about changes, if any, in circulating magnesium concentrations [29].

Lipids play a major role in the pathogenesis of insulin resistance. Free fatty acids (FFA) are often increased in insulin resistant states and may contribute to increased hepatic glucose output, possibly by accumulation of triglycerides and FFA metabolites in the liver and muscle [30]. Dyslipidemia in insulin resistant states includes low high-density lipoprotein cholesterol, which is an independent risk factor for the development of CVD and death [31-33]. Obesity, insulin resistance, and dyslipidemia are major factors associated with non-alcoholic fatty liver disease (NAFLD) [34, 35]. Alanine aminotransferase (ALT) is a marker of NAFLD and liver fat content which also reflects the glucometabolic status. Serum ALT increases gradually before onset of T2DM and individuals with elevated levels have increased risk of CHD [36, 37].

BPD-DS is a more advanced bariatric surgery procedure than RYGBP with restrictive and malabsorptive components combined and with a restored emptying of the stomach [38]. Weight loss is more pronounced after BPD-DS surgery than after RYGBP and weight loss is initially rapid and ongoing for longer a time than for RYGBP. It is often 15-18 months after surgery before it stabilizes. Thus, we hypothesized that BPD-DS has the potential of normalizing postprandial insulin and proinsulin responses. To our knowledge, no previous study on proinsulin and insulin responses after a test meal in patients who underwent BPD-DS surgery has been published.

Obesity, definition and prevalence

Obesity is a public health disorder representing a burden for the individual, the health care system and the community. In Sweden the number of overweight individuals has doubled in two decades [39]. A body mass index of 25-30 kg/m^2 is classified as overweight, 30-35 kg/m^2 as grade 1 obesity, 35-40 kg/m^2 as grade 2 obesity and a BMI over 40 kg/m^2 as morbid obesity or grade 3 obesity. Public data from the National Bureau of Statistics report in Sweden in 2007 shows an obesity prevalence of 10% and a 50% prevalence of overweight in general [39]. The largest increase rate has been observed among young women, male workers and individuals living in non-urban areas [40].

Data from the US Centers for Disease Control and Prevention (www.cdc.gov) presented for 2007 showed that in only one state (Colorado) obesity had a prevalence of less than 20%. Thirty states had a prevalence equal to or greater than 25%; and three of these states (Alabama, Mississippi

and Tennessee) had a prevalence of obesity equal to or greater than 30% [41]. The trend in Sweden is similar to the US with a delay of about 15 years [42].

Body fat measurements

Body fat content can be measured by Dual Energy X-ray Absorptiometry (gold standard), Bod Pod, computer tomography or bioelectric impedance analysis. Acceptable fat content in men is approximately 18-25% and in women 25-31% [43]. Higher fat content is defined as obesity: 35% according to the WHO [43, 44]. For clinical praxis BMI is diagnostic, but the waist-to-hip ratio, the sagittal abdominal diameter or the waist circumference are useful for estimating intra-abdominal obesity. Recent data show possible benefits of using the sagittal abdominal diameter among women as a surrogate marker for insulin resistance instead of BMI [45].

Clinical manifestations

A large number of diseases are associated with obesity e.g. diabetes, hypertension, dyslipidemia, NAFLD, gout, sleep apnea, Pickwick syndrome, CHD, osteoarthritis, polycystic ovarian syndrome (PCOS), psychiatric disorders, musculoskeletal problems, incontinence, gallstones, complications during pregnancy and also some cancer manifestations, among them corpus uteri cancer, ovarian, breast, colon and prostate cancer.

Obesity is one of the strongest risk factors for the development of T2DM, mainly via impaired insulin sensitivity. An up to forty-fold increased risk for T2DM has been estimated when BMI $\geq$ 35 kg/m^2 is present [46]. Obesity is a stronger marker for chronic disease than smoking, alcohol consumption and economic status [47].

Treatments for obesity

Non surgical treatment of obesity

Basic treatment for obesity includes lifestyle changes, dietary advice, physical activity and behavior changing strategies. Regarding pharmacological treatment two substances are available: Orlistat which partly blocks the lipase enzyme and leads to a reduced uptake of fat in the intestine and Sibutramine, whose metabolites suppress reuptake of noradrenalin, serotonin and also to some extent dopamine, increase the feeling of satiety and reduce food intake. A third substance, the cannabinoid receptor II blocker (Rimonabant®) was withdrawn from the market in the autumn of 2008 due to psychiatric adverse effects. New drugs are under development. Tesofensine is a new experimental drug for weight loss. It is a triple monamine reuptake in-

hibitor, shown to have weight loss properties beyond that of existing weight loss medications with minimal cardiovascular side effects. This drug is now under consideration for phase III clinical trials [48].

Bariatric surgery

The concept of surgical treatment of obesity was developed from observations of weight loss in patients who had their stomach or small intestines partially removed. Different schools of bariatric surgery developed with different types of bariatric surgery procedures mainly categorized as malabsorptive, restrictive or combined.

The first obesity procedure, a partial small bowel resection, was performed in Gothenburg, Sweden in 1952, resulting in good weight loss [49]. Then in 1953 intestinal bypass surgery was developed by Richard L Varco to induce weight loss [50]. It was an end-to-end jejunoileostomy, a purely malabsorptive procedure. The technique was later modified by Payne and Sherman by the abandonment of anastomosis to the colon, which was common at that time, and restoration of bowel continuity proximal to the ileocecal valve by an end-to-side jejunoileostomy [51, 52]. Jejunoileal bypasses were performed during the 1960s and 1970s. Due to malabsorptive and postoperative complications the procedure bileopancreatic diversion was later introduced: a procedure which involves a partial gastrectomy and the creation of a short common intestinal channel. This technique has been later modified and combined with duodenal switch, BPD-DS [53-56]. Gastric banding is a restrictive procedure to decrease the size of the stomach by fundus-duplication or by an inflatable band without any reduction or transposition. What is claimed to be the first claimed gastric bypass operation representing a combined malabsorptive and restrictive procedure was performed in 1966 by Mason an Ito (RYGBP) [57]. It is today a very common and well tolerated procedure which is considered superior to gastric banding [2, 58].

Indications for bariatric surgery

According to national Swedish guidelines for obesity surgery (NBHW) based on the 1991 National Institute of Health conference and the 2004 American Society of Bariatric Surgery conference consensus the indications are: BMI $\geq$ 40 kg/m^2 or $\geq$35 kg/m^2 with co-morbidity to obesity such as diabetes or hypertension, age18-60 years (approximately), mental stability, understanding of the principles and consequences of bariatric surgery, obesity over a long time period and previous unsuccessful conservative treatments.

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Contraindications

The following conditions may imply contraindications: MI within 6 months, CHD, instable angina pectoris, chronic obstructive pulmonary disease, impaired pulmonary vital capacity, severe tromboembolic disease, earlier several complicated abdominal surgery procedures, severe mental retardation, and severe portal hypertension. Other conditions to be taken into consideration are psychotic diseases, split personality, affective disorders, drug and alcohol abuse and binge eating disorders.

Dietary advice after bariatric surgery

After RYGBP surgery patients are advised to eat an amount of food equivalent to 1-2 deciliters every 2-3 hour during the day. The intake of vegetables is reduced to insure sufficient energy intake and protein rich foods are prioritized. The recommended a fluid intake for patients is 1½-2 liters per day in-between meals. Following RYGBP-surgery alcohol uptake is faster, resulting in higher serum levels of alcohol than in controls and it is possible that there is a slightly higher risk of alcohol abuse or dependence spontaneously developinng after bariatric surgery [59]. Mineral and vitamin deficiencies may occur and serum levels should be evaluated repeatedly. Vitamin B-12 deficiency is common and long term deficiency can cause peripheral neuropathy [60, 61]. Other common deficiencies are folate, calcium, vitamin-D, ferritin and zinc [29]. More unusual deficiencies are B-1 (Wernicke encephalopathy)[62] and copper (neurodegenerative symptoms)[63]. Thus, supplementary cobalamin (vitamin B-12) and over the counter multivitamin tablets are recommended for long term use after surgery.

Bariatric surgery procedures

In principal four different procedures are used (Figures 1A-D).

Gastric banding

A non-elastic band equipped with an inflatable silicon balloon on the gastric side is placed around the stomach creating the small proximal reservoir. The balloon is connected to a subcutaneous injection port, and adding or removing fluid postoperatively changes the inner diameter of the stoma (Figure 1A).

Sleeve Gastrectomy

The greater curvature of the stomach is resected, leaving the pylorus region intact resulting in a so called sleeve gastrectomy (Figure 1B). This procedure is less commonly used in Sweden.

Roux-en-Y-gastric bypass, (RYGBP)

RYGBP excludes the passage of food from the stomach and duodenum [64, 65]. The flaccid part of the lesser omentum and the first gastric vessel on the lesser curvature is divided, just below the fat pad, to create a small gastric pouch (2 by 3 cm). The pouch is then totally separated from the main stomach, which is left in the abdomen. The small bowel is divided 30 cm distal to the ligament of Treitz and the aboral end is connected to the small gastric pouch. This jejunal limb, the so-called Roux limb, is made 70 cm long and placed behind the excluded stomach and transverse colon. The small bowel continuity is maintained by an entero-enterostomy between the Roux limb and the prevoiusly divided proximal jejunum. This creates the Y-shaped junction where ingested food passes, via the Roux-limb, and gastric acid and bile is mixed (Figure 1C).

Bileopanceatic diversion with duodenal switch, (BPD-DS)

The greater curvature of the stomach is resected leaving the pylorus region intact. The duodenum is divided 3 cm distal to the pylorus. The small bowel is divided 250 cm proximal to the ileo-cecal valve and anastomosed to the divided duodenum (and stomach) proximal to the entry of the common bile duct. The bilio-pancreatic limb (from the duodenum) is anastomosed 100 cm proximal to the ileocecal valve, creating a 100 cm common limb for absorption. Digestion with juices from the upper gastrointestinal tract can only occur in this most distal part of ileum (Figure1D).
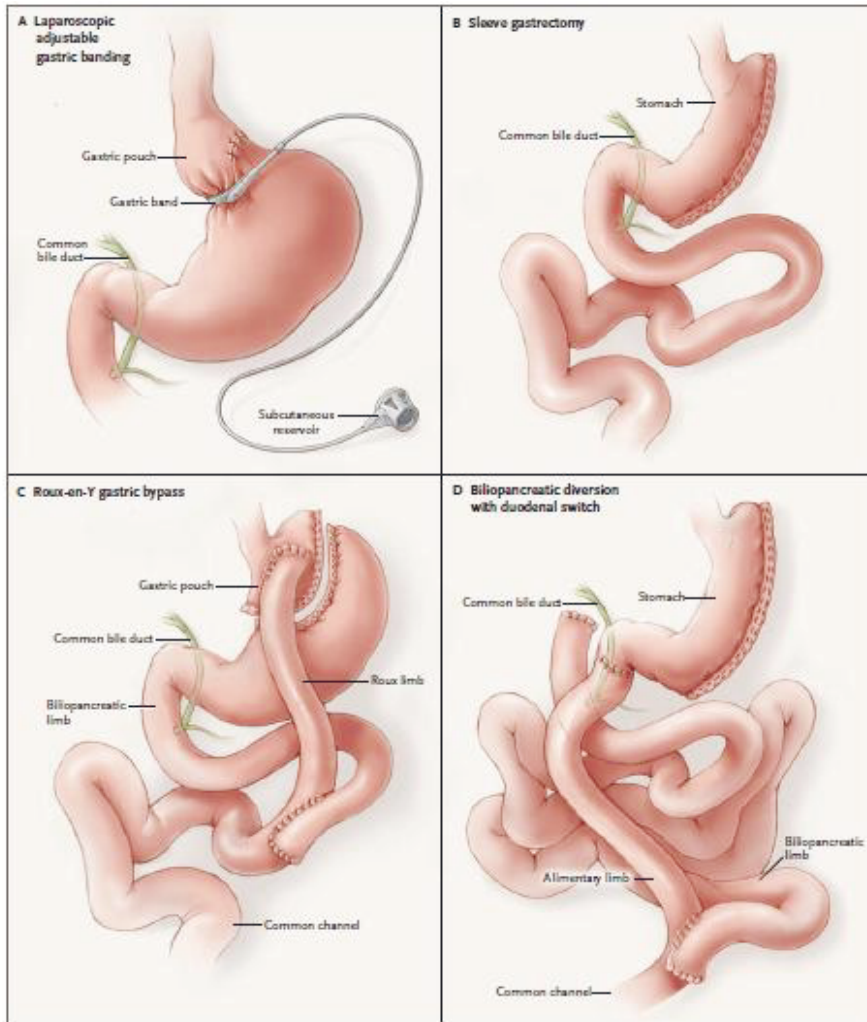
Figure 1 A-D. Common Surgical Procedures for Weight Loss.

Restrictive operations for the treatment of morbid obesity and its coexisting conditions, popular today particularly because of laparoscopic surgical approaches, include adjustable gastric banding (Panel A) and vertical (sleeve) gastrectomy (Panel B). Roux-en-Y gastric bypass (Panel C), a procedure that combines restriction and malabsorption, is considered by many to be the gold standard because of its high level of effectiveness and its durability. More extreme malabsorption accompanies biliopancreatic diversion procedures, commonly performed with a duodenal switch (Panel D), in which a short, distal, common-channel length of small intestine severely limits caloric absorption. This procedure also includes a sleeve gastrectomy.

Effects of bariatric surgery

Weight loss

In the SOS study weight was reduced by 25% after 10 years in the RYGBP group, by 16% in the VBG treated subjects and by 13% in gastric banding group whereas control patients did not loose weight significantly. The weight loss pattern over 15 years following different bariatric surgery procedures is shown in Figure 2. RYGBP is clearly superior to VBG and gastric banding for long term weight stability. Unfortunately the SOS study does not include patients who have been treated using the BPD-DS technique.
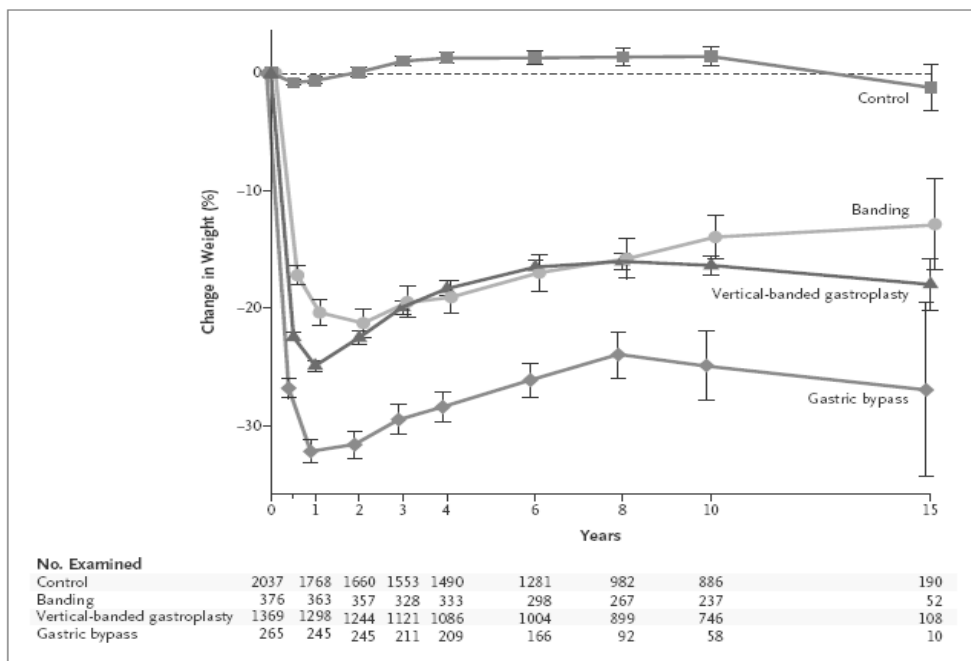
Figure 2. Mean Percent Weight Change during a 15 Year Period in the Control group and the Surgery Groups.

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Diabetes control and improvement

As previously mentioned, morbid obesity is often associated with T2DM as well as impaired glucose tolerance. Studies have shown prevalences up to 1/3 of both [66]. The mechanisms behind these associations are not clear. Probably it is a long-term process over 10-15 years, including increasing insulin resistance, raised fasting glucose, impaired glucose tolerance, hyper-insulinemia, and, finally, failure in beta-cell function and development of T2DM (Figure 3).

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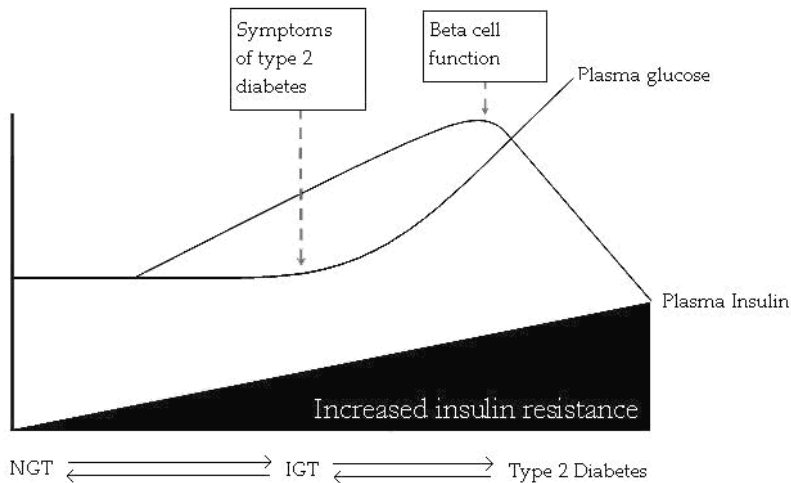
Figure 3. Insulin resistance and beta-cell dysfunction leads to typ 2-diabetes. Schematic description of the development from normal glucose tolerance (NGT) via impaired glucose tolerance (IGT), a state of insulin resistance and hyperinsulinemia to, finally, beta-cell failure and T2DM.

Diabetes Diagnostic Criteria

Increased fasting plasma glucose is due to insulin resistance in the liver, with increased hepatic glucose output. Normal fasting plasma glucose values are < 6.1 mmol/L, 6.1-6.9 mmol/L are considered as a prediabetic state (impaired fasting glucose), and ≥ 7.0 mmol/L is classified as diabetes.

Increased postprandial plasma glucose levels are mainly associated with impaired beta-cell function. To evaluate this, a 75 gram oral glucose tolerance test can be preformed. A two hour value under 7.8 mmol/L is considered normal, 7.8-11.0 mmol/L is classified as IGT and ≥ 11.1 mmol/L is equal to diabetes.

Several studies have shown remarkable improvements in glucometabolic control in T2DM patients or obese patients with IGT, with normalization of

glucose tolerance up to 80-90% after RYGBP [67, 68]. Porie et al, have evaluated 352 obese patients with T2DM or IGT eight years after they underwent RYGBP. They reported a normalisation of plasma glucose in T2DM of 83% and furthermore a normalisation of plasma glucose in IGT of 99% [68]. A report on 15 normal weight T2DM patients with insulin treatment who underwent RYGBP surgery (bypassing a segment of proximal intestine) documented that one year after surgery, no weight loss was observed, but 13 of 15 no longer needed insulin therapy and mean HbA1c was improved from 8.9 to 7.9 % [69]. In a prospective, non-interventional study on weight stable individuals, having impaired fasting glucose or IGT, the reversal to normal glucose tolerance was predicted by low fasting and 2 h insulin, homeostasis model assessment of insulin resistance and of pancreatic beta-cell function, body mass index and waist circumference. The factors predicting the reversal to normal glucose tolerance were correlated with low insulin resistance and lower insulin secretion possibly indicating a lower pancreatic work load [70].

Coronary heart disease

In 2007 Sjostrom et al reported bariatric surgery for severe obesity to be associated with long-term weight loss and decreased overall mortality. In this follow-up study over 15 years MI was almost twice as common in controls as compared to the surgery group [17]. The connection between obesity and CHD is complex, several components are involved e.g. lipids, glucometabolic variables, liver steatosis in NAFLD and hypertension. CHD is predicted by proinsulin independent of conventional risk factors in population-based studies but very little is known on the proinsulin-CHD relation in obesity.

Proinsulin and insulin, secretion and metabolism

Proinsulin plays pathophysiological and predictive roles in diabetes. In prediabetic subjects disproportionately increased proinsulin concentration, an indicator of defective insulin secretion, is associated with conversion to diabetes over a short time period [71] and over long periods of time [8]. Proinsulin is synthesized in the pancreatic beta-cells as a polypeptide of 86 amino acids [72]. The gene for preproinsulin is located on chromosome 11. Synthesis, induced by glucose, starts with the formation of preproinsulin by the ribosomes in the endoplasmatic reticulum. Preproinsulin is cleaved by a protease to proinsulin. Proinsulin is packed into vesicles in the Golgi apparatus [73-75] and in the granula it is subsequently cleaved enzymatically, by proinsulin convertases 1 and 2 and carboxypeptidase E, releasing insulin into the circulation along with a residual fragment called connecting peptide (C-peptide) and a fraction of uncleaved proinsulin [76]. As these processes are

not perfect, partially converted split products, such as 32-33 split proinsulin, can also be found in the plasma. Hyperproinsulinemia might represent insufficient insulin secretion capacity [77]. An increased proportion of proinsulin in the granula may reflect a slower than normal rate of conversion from proinsulin to insulin, i.e. beta-cell dysfunction [78]. In insulin resistant states a higher proportion of proinsulin may be due to short transition times from synthesis to release at secretion. These aspects are not possible to separate from each other in clinical studies.

Proinsulin binds to the insulin receptor and, like insulin, induces cellular glucose uptake via GLUT-4. Proinsulin has relatively low biological activity (approximately 5-10% of insulin´s potency), and is the major storage form in the granula. Normally, only small amounts of proinsulin enter the circulation, 3% of the amount of insulin. The hepatic clearance of proinsulin is only about 25% that of insulin clearance. The half-life of proinsulin is 115 minutes, compared to 5 minutes for insulin, and concentrations of proinsulin in the fasting state are approximately 10%-15% of insulin concentrations in individuals with normal glucose tolerance [79]. The liver and kidneys are the principal sites for insulin and proinsulin clearance. In obese patients with hyperinsulinemia and high levels of free fatty acids insulin hepatic clearance is impaired [80] whereas plasma proinsulin clearance does not differ between individuals with or without T2DM [81]. The metabolic clearance of split proinsulin products is higher than that of intact proinsulin and glucose lowering ability is greater for split products than for intact proinsulin [82]. Insulin can also be cleared also by muscle-cells, adipocytes, gastrointestinal cells, fibroblasts, monocytes and lymphocytes which contain insulin receptors and internalization and regulation mechanism for insulin metabolism [80]. The excretion of proinsulin products and insulin can also be measured in urine and relatively higher proportions of proinsulin are noticed also in the rare conditions of pancreatic islet cell tumors. Clearance of proinsulin and insulin is similarly constant over a wide range of serum concentrations and the urinary proinsulin represents a constant fraction of the filtered load. The twenty four hour excretion of these hormones is a useful indicator of mean daily serum level [83].

Elevated proinsulin levels have been found to be a risk factor for the development of T2DM and also an independent risk factor for CHD in both diabetic and non-diabetic populations. Earlier attempts in clinical trials to treat T2DM patients with proinsulin have resulted in increased incidence of CVD [79]. Proinsulin is also associated with PAI-1 activity, insulin resistance and elevated triglycerides, mechanisms that are possibly involved in coronary events[84]. Increased proinsulin concentrations may also be detected in patients with chronic renal failure, cirrhosis, or hyperthyroidism.
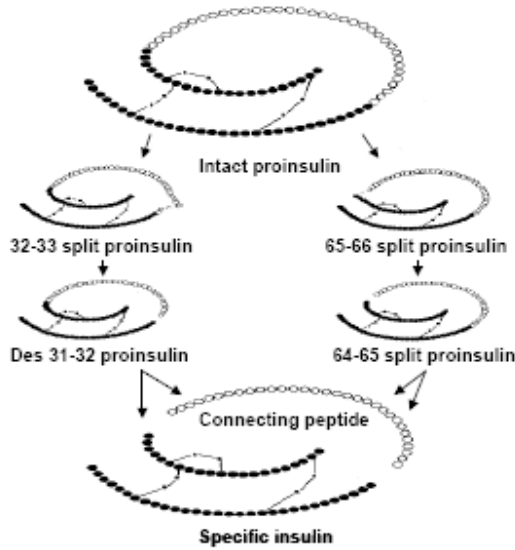
Figure 4. Proinsulin to insulin processing.

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Insulin resistance

From the earliest days of the use of insulin in the treatment of T2DM there have been reports of cases in which larger doses were required than the clinical condition of the patient seemed to warrant [85, 86]. The concept that insulin resistance may be an underlying cause of T2DM was advanced by Prof. Wilhelm Falta and published in Vienna in 1931 [87], and confirmed during 1936-39 by Sir Harold Percival Himsworth´s work [88-90]. The Swede Eskil Kylin also pointed out in 1920s that hypertension is often found in patients with T2DM and described the metabolic syndrome in detail ("Das Hypertoni-Hyperglykemie und Hyperurikemie syndrome") [91].

Insulin resistance is the condition in which normal amounts of insulin are inadequate for producing a normal glucose uptake in fat, muscle and liver cells. Insulin inhibits the lipolysis and increases the storage of FFAs as triglycerides in adipose tissue whereas catecholamines have the opposite effects Insulin resistance in fat cells results in increased hydrolysis of stored triglycerides. The increased mobilization of stored lipids in these cells elevates free fatty acids in the circulation. High levels of circulating FFAs could

directly harm insulin production in the pancreas, i.e. lipotoxicity. Insulin resistance in muscle cells reduces glucose uptake and the local storage of glucose as glycogen whereas insulin resistance in liver cells also reduces storage of glycogen and increases hepatic glucose output. Both phenomena cause elevated blood glucose levels.

In conditions with constantly high insulin levels, a down regulation at the receptor level may occur inducing a worsening of glucose tolerance. A competitive situation is also found in the muscle between glucose and FFAs, Randels cycle, when high levels of FFAs are available these are used as fuel and relatively less glucose is utilized resulting in increased blood glucose levels [92].

Subsequently most T2DM and glucose intolerant patients are insulin resistant and the beta-cells in the pancreas fail to release enough insulin to normalize blood glucose. Further, in obese insulin-resistant individuals the ability to increase lactate release is impaired in adipose tissue and skeletal muscle and a defective insulin regulation and local blood flow is also involved [93]. However, individuals with normal fasting glucose concentrations can be found who have insulin resistance, but they are compensating for the insulin resistance with pronounced hyperinsulinemia. Insulin resistance is associated with visceral adiposity, hypertension, hyperglycemia and dyslipidemia involving elevated triglycerides and decreased HDL cholesterol levels. Furthermore, insulin resistance is also often associated with a hypercoagulable state (impaired fibrinolysis) and increased inflammatory cytokine levels. Insulin-mediated sodium retention may also participate in the development of hypertension in obese subjects. Positive correlations have also been found between insulin resistance and increased concentrations of parathyroid hormone and calcium, which may be associated with the increased cardiovascular morbidity and mortality seen in primary hyperparathyroidism [94].

Magnesium

Magnesium is a cofactor in more than 350 known enzymatic steps, for example in glucose metabolism but it is also involved in DNA synthesis, RNA synthesis, muscular function and nerve signalling. Magnesium deficiency may impair glucose metabolism. Lowered circulating serum magnesium concentrations are often found in patients with T2DM and hyperglycemia may induce hypomagnesemia. The serum magnesium concentrations have been shown to be inversely related to circulating glucose concentrations and to insulin resistance in diabetic patients as well as in non-diabetic patients [95-98]. Huerta et al have reported lower serum magnesium concentrations in obese non-diabetic children and further observed that lowered circulating magnesium concentrations were associated with increased insulin concentra-

tions and impaired insulin sensitivity [99]. Furthermore, a low intracellular magnesium concentration, often found in T2DM patients, may result in a defective tyrosine-kinase activity at the insulin receptor level and exaggerated intracellular calcium concentration. This may also impair insulin action and decrease insulin sensitivity. Oral supplementation with magnesium has been shown to increase insulin sensitivity, measured by the euglycaemic insulin clamp technique, but not to improve HbA1c [100]. One meta-analysis of randomized double-blind controlled trials has shown that oral magnesium supplementation for 4-16 weeks may be effective in reducing plasma fasting glucose levels and raising HDL cholesterol in patients with type 2 diabetes, although long-term benefits and safety remain to be determined [101].

Recently Håglin et al [27] reported a significant increase of prediction of all- cause mortality when electrolytes, especially magnesium, were added to traditional risk factors in a population with diabetic patients. The knowledge on how circulating magnesium concentrations are affected by RYGBP surgery in relation to improved glucometabolic status is limited.

Aims of the thesis

The overall aim of the thesis is to study metabolic effects of bariatric surgery induced weight loss in morbidly obese patients cross-sectionally as well as longitudinally on proinsulin and insulin responses, circulating magnesium concentrations, liver enzymes and serum lipids.

Specific aims were:

Study I

To investigate postprandial proinsulin and insulin responses, effects on glucose, FFA and TGs in previously morbidly obese subjects with RYGBP-induced weight loss, in comparison with subjects with morbid obesity and normal weight controls.

Study II

To examine postprandial proinsulin and insulin responses, effects on glucose, FFA and TGs in previously morbidly obese patients who underwent bileopancreatic diversion with duodenal switch, in comparison with normal weight controls. Further, to evaluate long term effects on glucose, lipids, HbA_{1c}, ALT, creatinine, uric acid and magnesium.

Study III

To determine long term effects, over several years after RYGBP surgery on proinsulin and insulin status, further aims were to evaluate effect on ALT as a marker for NAFLD and serum lipids.

Study IV

To study alterations in serum magnesium concentrations and glucometabolic variables one year after RYGBP surgery in morbidly obese patients as compared to a matched control group of morbidly obese patients.

Patients and Methods

Participants

Patients were recruited from the outpatient clinic for obesity care, University Hospital, Uppsala, Sweden, where pre-evaluations before and long term check-up visits of patients after surgery was performed. Control subjects were locally recruited. All subjects were Caucasians, free from established diabetes and not on pharmacological treatment for hypertension.

Patients in study I

Three groups of participants were compared:

-Ten surgically treated patients with BMI 34.8 kg/m^2 (SD 6.2) and considered weight stable after weight loss due to RYGBP surgery, which had been performed one to five years (average 4.1 years) before study. The mean pre-surgery, BMI was 45.3 kg/m^2 (SD 7.6). Absolute weight loss was on the average 31.8 kilo (16-47 kilo). The average drop in BMI after surgery was 10.5 kg/ m^2 (23%).

-Ten surgically untreated morbidly obese (MO) patients, with BMI 44.0 kg/m^2 (SD 3.1). These patients had refused offered surgery or were on waiting list for surgery after pre-evaluations. Mean BMI matched with pre-surgery BMI in the group of subjects treated with RYGBP surgery.

-Twelve healthy normal weight (NW) controls with BMI 23.2 kg/ m^2 (SD 2.4). The three groups studied were matched for sex and for age cf. Table 1.

Patients in study II

Ten MO patients who had undergone BPD-DS surgery (five men, five women), all Caucasians, free from established diabetes and not on pharmacological treatment for diabetes, were recruited from the Outpatient Clinic of Obesity Care, Uppsala University Hospital, Uppsala, Sweden.

These patients were recruited for a standardized test meal study separated from ordinary follow-ups. Data from the test meal in the BPD-DS treated group was compared to data obtained from normal weight controls (six men, six women). The surgically treated patients were considered weight stable after weight loss due to BPD-DS surgery, with body mass index (BMI) 29.0 ± 5.2 kg/m^2 (mean±SD), who had undergone surgery 26 months (median, 18-44

months range) before the present study. The mean presurgery BMI was 53.5 $\pm$ 3.8 kg/m^2. Age and gender distribution were similar in both groups but NW-controls weighted 20% less than the BPD-DS group, BMI 23.2$\pm$ 2.4 kg/m^2. Basal characteristics of the patients are presented in Table 2.

Table 1. *Clinical characteristics in morbidly obese subjects, obese subjects after RYGBP surgery and normal weight control subjects in the fasting state of the study*

	Morbidly obese (A)	RYGBP treated (B)	Control group (C)	Group A vs. B P-value	Group B vs. C P-value	Group A vs. C P-value
Sex (women/men)	5/5	5/5	6/6	—	----	—
Age (years)	41.6 (6.8)	44.7 (5.3)	41.1 (7.5)	0.272	0.218	0.872
Body mass index (kg/m^2)	44.0 (3.1)	34.8 (6.2)	23.2 (2.4)	0.005	< 0.001	< 0.001
Weight (kg)	133.8 (20.2)	106.1 (28.9)	70.9 (12.5)	0.023	0.001	< 0.001
Height (cm)	174.1 (12.1)	173.5 (13.6)	174.2 (9.6)	0.918	0.882	0.974
f-P-glucose (mmol/l)	5.3 (0.6)	5.2 (0.7)	4.8 (0.6)	0.721	0.225	0.098
HbA$_{1c}$ (%)	4.5 (0.3)	4.5 (0.2)	4.2 (0.2)	0.800	0.018	0.018
f-P-proinsulin (pmol/l)	19.4 (18.1)	7.0 (3.5)	5.9 (7.4)	0.047	0.680	0.028
f-P-insulin (pmol/l)	56.4 (28.2)	24.6 (13.8)	19.8 (13.2)	0.005	0.408	0.001
PIR	0.34	0.28	0.30	0.74	0.33	0.26
f-P-FFA (mmol/l)	0.96 (0.32)	0.74 (0.13)	0.78 (0.32)	0.052	0.716	0.182
f-P-TG (mmol/l)	1.97 (1.00)	1.37 (0.70)	0.91 (0.65)	0.137	0.128	0.007

Data given are arithmetic mean ±SD.
f, Fasting; P, plasma; PIR, proinsulin to insulin ratio; FFA, free fatty acids; TG, triglycerides; RYGBP, roux-en-Y gastric bypass.

Table 2. *Clinical characteristics in the fasting state of the meal test for morbidly obese patients, median 26 months (range 18-44), after BPD-DS surgery and for normal weight controls*

	BPD-DS patients	NW-controls	P for difference
Gender (women/men)	5/5	6/6	-
Age (years)	39.1 (6.5)	41.1 (7.5)	0.501
BMI (kg/m^2)	29.0 (5.2)	23.2 (2.4)	0.002
Weight (kg)	87.6 (22.5)	70.9 (12.5)	0.039
Height (cm)	173.0 (12.1)	174.2 (9.6)	0.778
f-P-glucose (mmol/l)	4.2 (0.3)	4.8 (0.6)	0.007
HbA$_{1c}$ (%)	3.9 (0.5)	4.2 (0.2)	0.800
f-P-proinsulin (pmol/l)	2.6 (1.0)	5.9 (7.4)	0.179
f-P-insulin (pmol/l)	15.2 (4.7)	19.8 (13.2)	0.005
f-P-FFA (mmol/l)	0.53 (0.22)	0.78 (0.32)	0.054
f-P-TG (mmol/l)	0.78 (0.30)	0.91 (0.65)	0.560

Data given are arithemic mean ±SD
f, fasting; P, plasma; FFA, free fatty acids; TG, triglycerides; BPD-DS, bileopancreatic diversion with duodenal switch; NW, normal weight
The standardised testmeal was separated in time from ordinary follow-ups.

Patients in study III and IV

Twenty-one patients, (three men, 18 women) were investigated before and one year after RYGBP surgery. Data from the RYGBP group was compared to that of a control group, recruited from the waiting list for RYGBP surgery, consisting of twenty-one obese patients, five men and 16 women, all free from established diabetes and not on pharmacological treatment for hypertension. The control group was recruited to match BMI, fasting glucose and proinsulin, in relation to the corresponding baseline values in the RYGBP group. Baseline characteristics of the patients are shown in Table 3. In paper III the 21 patients who had undergone RYGBP surgery was   included in a longitudinal long-term follow-up with investigations before (baseline), after 12 months (1st follow-up) and finally 42 months, range 36-50 months (2nd follow-up) of proinsulin, glucometabolic status, lipids, ALT and uric acid.

Table 3. *Clinical characteristics at baseline for patients before RYGBP surgery and morbidly obese controls*

	RYGBP surgery group	Morbidly obese controls	p-value for difference
Sex (women/men)	18/3	16/5	0.429
Age (years)	45.7 (9.7)	38.7 (7.5)	0.013
BMI (kg/m^2)	42.3 (5.2)	44.3 (5.1)	0.211
Weight (kg)	120.0 (16.4)	124.0 (17.3)	0.444
Height (cm)	168.4 (6.2)	167.2 (8.5)	0.614
ASD (cm)	28.9 (2.1)	30.0 (2.4)	0.239
f-P-glucose (mmol/l)	4.9 (0.6)	5.1 (0.6)	0.197
f-S-proinsulin (pmol/l)	13.5 (7.6)	15.1 (9.5)	0.612
f-S-insulin (pmol/l)	83.4 (56.4)	103.2 (37.8)	0.209
Proinsulin/insulin ratio	0.16 (0.12)	0.15 (0.08)	0.117
S-ALT(μkatal/l)	0.62 (0.25)	0.57 (0.48)	0.691
f-S-total cholesterol (mmol/l)	5.1 (1.0)	5.1 (0.83)	0.806
f-S-LDL-C (mmol/l)	3.4 (1.0)	3.23 (0.82)	0.560
f-S-HDL-C (mmol/l)	1.19 (0.21)	1.14 (0.25)	0.591
f-S-LDL / HDL	2.97 (1.00)	3.03 (1.20)	0.868
f-S-TG (mmol/l)	1.61 (0.80)	1.65 (0.65)	0.896
S-Uric acid (umol/l)	352 (84)	348 (66)	0.841
S-Magnesium (mmol/l)	0.80 (0.06)	0.80 (0.07)	0.961

Data given are arithmetic means ±(SD).
ASD, abdominal sagittal diameter; ALT, alanine aminotransferase; LDC-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG triglycerides; *f,* fasting; *S,* serum; *P* plasma

Ethical considerations

The study protocols were approved by the regional ethics review board at Uppsala University.

Investigations

Clinical measurements

Weight (kg) was measured on standardised, calibrated scales.

Height (cm) was measured without shoes by a fixed vertical metric rule and a horizontal headboard. The mean of the two observations is recorded.

Body mass index (BMI)
BMI (kg/m^2) was calculated as weight (kg) divided by height (m) squared.

Abdominal Sagittal diameter (ASD)
ASD (cm) was recorded twice at the umbilical level as the height of the abdomen measured from the examination couch. The mean of each of these sets of values was used in the analyses.

Blood pressure
Systolic (SBP) and diastolic (DBP) blood pressure were measured using a sphygmomanometer, with a cuff of appropriate size, twice in the supine position after a rest of five minutes. The mean of each of these sets of values was used in the analyses.

Laboratory tests

Proinsulin and insulin
At the laboratory of the Department of Public Health and Caring Sciences/Geriatrics, University Hospital, Uppsala, plasma proinsulin and insulin concentrations were determined using the Proinsulin ELISA and the Insulin ELISA immunoassays (Mercodia AB, Uppsala, Sweden) on a Bio-Rad Coda automated EIA analyzer (Bio-Rad Laboratories, California, USA). Insulin sensitivity was assessed by calculating the HOMA-IR index ((fasting serum glucose x fasting serum insulin)/ 22.5) in paper IV [102].

Glucose
Plasma concentrations were determined using a routine glucose oxidase technique (Beckman Glucose Analyzers; Beckman, Fullerton, California).

Free fatty acids (FFA) and triglycerides (Tgs)

In test meal studies concentrations of plasma FFA were determined using the Wako NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany) and plasma Tgs by a lipase and quinoneimine dye method (Konelab) on a Konelab analyzer (Thermo Clinical Lab systems Oy, Vantaa, Finland).

Magnesium

Serum magnesium concentrations were measured by spectrophotometer with xylidyl blue (Architect, Abbott). The coefficient of variation is < 2% for this method.

Basal routine tests

Concentrations of HbA1c, alanin aminotranferase, creatinine, sodium, potassium, haemoglobin, albumine, creatinine, uric acid, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were analyzed using routine methods at the Department of Clinical Chemistry at the University Hospital, Uppsala.

Test meal composition and procedures (study I and II)

The meal was composed to reflect with the amount and content of food possible to eat due to the reduced gastric volume after bariatric surgery, as followed: a hamburger, containing minced bovine meat 75 g, oatmeal 8 g, potato flour 2.5 g, milk 28 g, boiled egg 10 g and onion 5 g. A brown sauce based on starch and wheat flour, (Blå Band®) 70 g. Boiled potatoes 130 g and carrot 75 g. As dessert, oat-bread 40 g with margarine 13 g and raspberry jam 17 g. For drink, 200 ml of water was ingested directly after meal. Total energy content was 2400 kJ (570 kcal), consisting of: carbohydrates 68.2 g, fat 22.3 g, proteins 24.6 g and fibre 6.4 g.

After an overnight fast the standardised test meal was ingested at 1 p.m. at the outpatient clinic for obesity care, allowing supervision of the food-intake which was finished within 15 minutes. Blood samples were collected, centrifuged and freshly frozen immediately before the test meal and thereafter at 30, 60, 90, 120 and 180 minutes after ingestion. Data was collected in pre-printed Case Report Forms.

Statistics

Statistical software JMP 3.0-3.2 for PC (SAS Corporation, Cary, Texas, USA) was used.

Results are given as arithmetic mean with standard deviation (SD) and standard error of the mean (SEM). ANOVA and Students t-test was used for group comparisons. Tests were two-tailed and a p-value <0.05 was considered.

In paper I and II test meal data are given as absolute concentrations (cf. table 4, 5) and changes in concentrations (figure 5 and 6). Statistical analyses were performed using changes from basal concentrations as absolute differences in basal concentrations of variables glucose, proinsulin, insulin, triglycerides and free fatty acids were expected between groups. Areas under the curves (AUC) were calculated, using the trapezoidal rule, for glucose, proinsulin, insulin, FFA and TG for group comparisons [103]. ANOVA was used in paper II for trend tests over three years of follow-up of BPD-DS patients. Paired t-test was used for comparisons between the 1st, 2nd, and 3rd follow-up. In paper IV adjusted analyses were made using ANCOVA. Baseline associations between continuous variables were analysed using Pearson product moment correlation coefficients.

Results

Paper I

The mean fasting plasma glucose concentration was 0.4 mmol/l higher (non-significant) in the RYGBP-group and 0.5 mmol/l higher in the MO-group as compared with the NW- controls.

Fasting proinsulin and insulin did not differ between the RYGBP-group and NW-controls, whereas proinsulin and insulin concentrations were significantly higher in the MO-group compared with the RYGBP-group and NW-controls, respectively.

Test meal results

Absolute concentrations in plasma for glucose, proinsulin, insulin, FFAs and TGs are shown in Table 4. Changes in glucose, proinsulin, insulin, FFA and TG from the basal state are shown in Fig. 5 a-e.

Glucose

The AUC (mean±SD) for glucose (mmol*minutes) did not differ between the MO-group (270±92), the RYGBP-group (310±110) and NW-controls (250±130), p for trend=0.27.

Data for changes in glucose is presented in Figure 5a. The glucose concentrations were markedly raised during the early phase (0-60 minutes) in the RYGBP-group (rapid stomach transit) as compared with the MO-group and NW-controls. During the intermediate (60-120 minutes) and the late (120-180 minutes) phases after ingestion the reduction in glucose concentrations was larger in the RYGBP-group as compared to the MO-group and NW-controls. The MO-group and NW-controls showed similar in postprandial glucose responses.

Table 4. Postprandial testmeal data in morbidly obese subjects, obese subjects after RYGBP surgery and for normal weight controls

	Time					
	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes	180 minutes
MO group						
P-glucose (mmol/l)	5.3 (0.6)	7.2 (0.7)	7.6 (0.8)	7.2 (1.0)	6.7 (0.7)	6.0 (0.9)
P-proinsulin (pmol/l)	19.4 (18.1)	36.3 (23.8)	59.7 (36.8)	73.1 (59.6)	79.4 (64.7)	70.1 (56.6)
P-insulin (pmol/l)	56.4 (28.2)	325 (120)	424 (92.4)	355 (145)	281 (132)	151 (70.2)
P-FFA (mmol/l)	0.96 (0.32)	0.86 (0.31)	0.50 (0.25)	0.35 (0.14)	0.30 (0.08)	0.33 (0.09)
P-TG (mmol/l)	1.97 (1.00)	1.97 (0.96)	1.91 (0.89)	2.14 (0.97)	2.26 (0.99)	2.51 (1.15)
RYGBP group						
P-glucose (mmol/l)	5.2 (0.7)	10.6 (1.7)	9.6 (1.9)	7.0 (1.7)	5.7 (1.2)	5.0 (0.9)
P-proinsulin (pmol/l)	7.0 (3.5)	41.0 (20.6)	57.9 (31.0)	46.2 (22.9)	33.2 (16.6)	19.8 (9.7)
P-insulin (pmol/l)	24.6 (13.8)	558 (316)	379 (245)	143 (69.6)	63.0 (30.6)	34.8 (17.4)
P-FFA (mmol/l)	0.74 (0.13)	0.54 (0.15)	0.20 (0.06)	0.18 (0.04)	0.23 (0.06)	0.50 (0.14)
P-TG (mmol/l)	1.37 (0.70)	1.42 (0.76)	1.41 (0.55)	1.44 (0.62)	1.49 (0.63)	1.59 (0.65)
NW controls						
P-glucose (mmol/l)	4.8 (0.6)	7.3 (1.0)	7.4 (1.3)	6.5 (1.0)	5.8 (0.7)	5.3 (0.7)
P-proinsulin (pmol/l)	5.9 (7.4)	17.1 (10.8)	27.8 (14.6)	29.7 (11.8)	26.3 (8.9)	19.9 (10.8)
P-insulin (pmol/l)	19.8 (13.2)	190 (91.2)	196 (97.2)	147 (67.8)	88.8 (37.8)	52.2 (38.4)
P-FFA (mmol/l)	0.78 (0.32)	0.69 (0.27)	0.38 (0.23)	0.26 (0.11)	0.29 (0.19)	0.29 (0.14)
P-TG (mmol/l)	0.91 (0.65)	0.95 (0.89)	1.03 (0.97)	1.07 (1.02)	1.07 (0.96)	1.08 (0.81)

Data given are arithmetic means (SD).
P, plasma; FFA, free fatty acids; TG, triglycerides; MO, morbidly obese; RYGBP, roux-en-y gastric by-pass; NW, normal weight

Proinsulin
The AUC (mean±SD) for proinsulin (pmol*minutes) was largest in the MO-group (7600±5300), intermediary in the RYGBP-group (5300±2700) and lowest in NW-controls (3000±1600), p for trend=0.02.

Data for changes in proinsulin is shown in Figure 5b. In the early phase, proinsulin concentrations were higher in the RYGBP-group and in the MO-group compared to NW-controls. In the intermediate phase plasma proinsulin started to decrease in the RYGBP-group but was still increasing in the MO-group. In the late phase concentrations of plasma proinsulin were significantly lower in the RYGBP-group, similar to the NW-controls, but were still high in the MO-group.

Insulin

The AUC (mean±SD) for insulin (pmol*minutes) was largest in the MO-group (41000±9900), intermediary in the RYGBP-group (32000±16500) and lowest in NW-controls (18000±8300), p for trend<0.001.

Data for changes in insulin is presented in Figure 5c. The RYGBP-group had a rapid increase in insulin concentrations in the early phase (peak at 30 minutes) as compared to the MO-group. Insulin rapidly decreased in the RYGBP-group in the intermediate phase. The late phase insulin response was similar in the RYGBP-group and NW-controls.

Free Fatty Acids

The AUC (mean±SD) for FFA (mmol*minutes) did not differ between the MO-group (84±31), the RYGBP-group (68±24) and NW-controls (63±54), p for trend=0.44. FFA absolute concentrations, shown in Table 4, were higher (statistically significantly at 30, 60 and 90 minutes; p=0.03-0.007) at each postprandial time point except at 180 minutes in the MO-group as compared with the RYGBP-group and NW-controls. Prior to 180 minutes, the FFA values were all lower in the RYGBP-group compared with the NW-controls and the MO-group.

Triglycerides

The AUC (mean±SD) for TG (mmol*minutes) was largest in the MO-group (32±29), intermediary in the RYGBP-group (16±22) and lowest in NW-controls (8±15), but not statistically significant, p for trend=0.07. TG absolute concentrations, presented in Table 4, were significantly higher (p=0.06-0.003) at each postprandial time point in the MO-group as compared with the RYGBP-group and NW-controls.
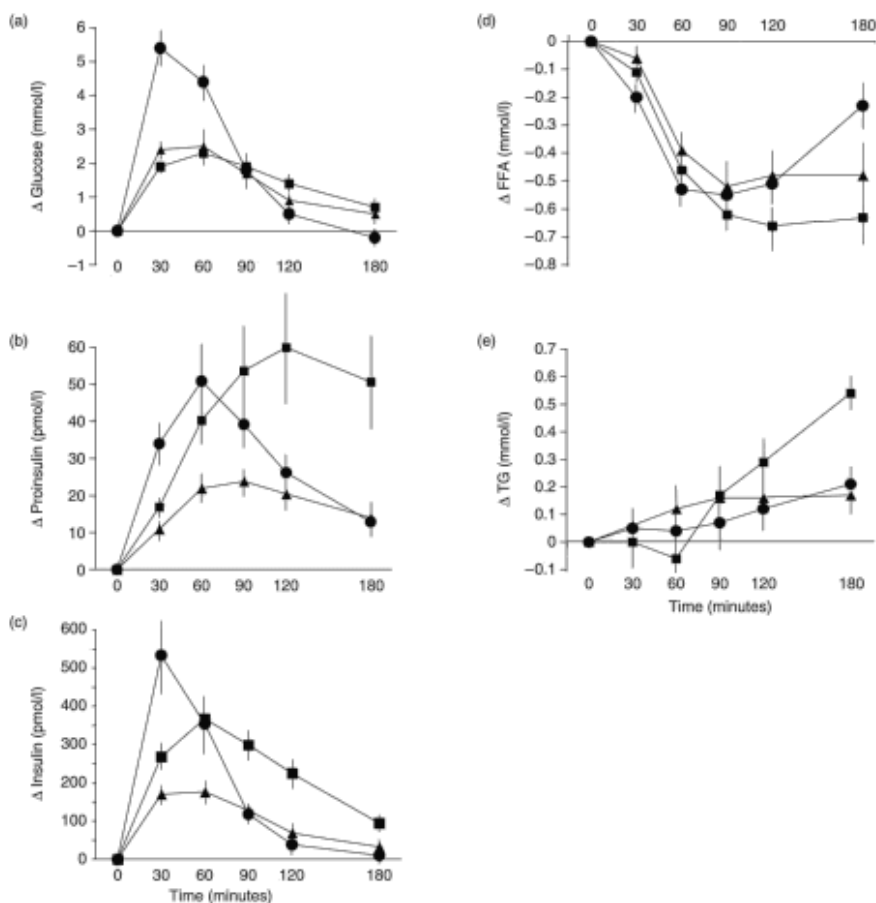
Figure 5a-e. The postprandial changes in in glucose (a), proinsulin (b), insulin (c), free fatty acid (FFA) (d) and triglyceride (TG) (e) concentrations are shown for 180 min after the ingestion of the standardized test meal (mean ±SEM). ■, Morbidly obese (MO) subjects; ●, MO subjects treated with roux-en-y gastric bypass (RYGBP) surgery; ▲, normal weight (NW) control subjects.

Paper II

Test meal results

Fasting data

The mean BMI was 5.8 kg/m^2 higher in the BPD-DS group compared with NW-controls. Fasting glucose concentrations were within the normal reference ranges in all participants. Fasting plasma glucose concentration was 0.4

mmol/l lower in the BPD-DS group compared with the NW-controls. Fasting proinsulin did not differ between the BPD-DS group and NW-controls, whereas insulin concentrations were 4.6 pmol/l lower in the BPD-DS group. TGs did not differ between groups.

Postprandial data

Absolute concentrations during the test meal are presented in Table 5. Changes in glucose, proinsulin, insulin, FFA and TG from the basal state are shown in Fig. 6 a-e.

Table 5. *Postprandial test meal data for obese patients, median 26 months (range 18-44), after BPD-DS surgery and for normal weights controls*

	Time					
	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes	180 minutes
BPD-DS group						
P-glucose (mmol/l)	4.2 (0.3)	6.2 (0.9)	6.7 (0.9)	6.0 (1.3)	5.0 (1.2)	4.2 (0.5)
P-proinsulin (pmol/l)	2.6 (1.0)	11.9 (6.2)	18.0 (6.0)	20.1 (10.2)	15.7 (7.9)	8.6 (4.7)
P-insulin (pmol/l)	15.2 (4.7)	177 (136)	136 (136)	85.6 (74.3)	39.8 (15.7)	26.2 (12.1)
P-FFA (mmol/l)	0.53 (0.22)	0.45 (0.20)	0.14 (0.06)	0.08 (0.02)	0.08 (0.04)	0.23 (0.10)
P-TG (mmol/l)	0.78 (0.3)	0.76 (0.29)	0.73 (0.28)	0.69 (0.28)	0.70 (0.30)	0.69 (0.28)
NW controls						
P-glucose (mmol/l)	4.8 (0.6)	7.3 (1.0)	7.4 (1.3)	6.5 (1.0)	5.8 (0.7)	5.3 (0.7)
P-proinsulin (pmol/l)	5.9 (7.4)	17.1 (10.8)	27.8 (14.6)	29.7 (11.8)	26.3 (8.9)	19.9 (10.8)
P-insulin (pmol/l)	19.8 (13.2)	190 (91.2)	196 (97.2)	147 (67.8)	88.8 (37.8)	52.2 (38.4)
P-FFA (mmol/l)	0.78 (0.32)	0.69 (0.27)	0.38 (0.23)	0.26 (0.11)	0.29 (0.19)	0.29 (0.14)
P-TG (mmol/l)	0.91 (0.65)	0.95 (0.89)	1.03 (0.97)	1.07 (1.02)	1.07 (0.96)	1.08 (0.81)

Data given are arithmetic means (SD).

 P, plasma; FFA, free fatty acids; TG, triglycerides; BPD-DS, bileopancreatic diversion with duodenal switch; NW, normal weight.

Glucose

The AUC (mean±SD) for glucose (mmol*minutes) did not differ between the BPD-DS group (230±150) and NW-controls (250± 130), (p=0.734).

Data for changes in glucose are presented in Fig. 6a. The glucose concentrations increased equally during the early phase (0-60 minutes) in both groups. During the intermediate (60-120 minutes) and the late (120-180 minutes) phases after ingestion the reduction in glucose concentrations was also similar.

Proinsulin

The AUC (mean±SD) for proinsulin (pmol*minutes) was numerically lower in the BPD-DS group (2000± 850) compared with NW-controls (3000±1600) but the difference was statistically not significant (p= 0.106).

Data for changes in proinsulin are shown in Fig. 6b. In the early phase, proinsulin concentrations increased equally in both groups but increased to a higher peak in the NW-controls. In the intermediate and late phases plasma proinsulin decreased similar in both groups in a parallel manner.

Insulin

The AUC (mean±SD) for insulin (pmol*minutes) did not statistically differ between the BPD-DS group (12000±9700) and NW-controls (18000±8300), (p=0.151).

Data for changes in insulin are presented in Fig. 6c. Both groups had a similar increase in insulin concentrations in the early phase but there was a higher peak in NW-weight controls. In the intermediate and the late phases insulin response was declining in a similar pattern in both groups and was significantly lower at 120 minutes (p=0.0079) in the BPD-DS group.

Free Fatty acids

The AUC (mean±SD) for FFA (mmol*minutes) did not differ between the BPD-DS group (57±29) and NW-controls (63±54) (p=0.764).

Data for changes are presented in Fig 6d. No significant differences were observed regarding postprandial changes in circulating FFAs concentrations between the two groups.

Triglycerides

The AUC (mean±SD) for TGs (mmol*minutes) was markedly lower in the BPD-DS group (-11±4), compared with NW-control subjects (8±15) (p=0.005).

Data for changes are presented in Fig 6e. Lower TG concentrations were observed in the BPD-DS group in the late phase (p=0.002) and a trend in the intermediate phase (p= 0.07-0.08).
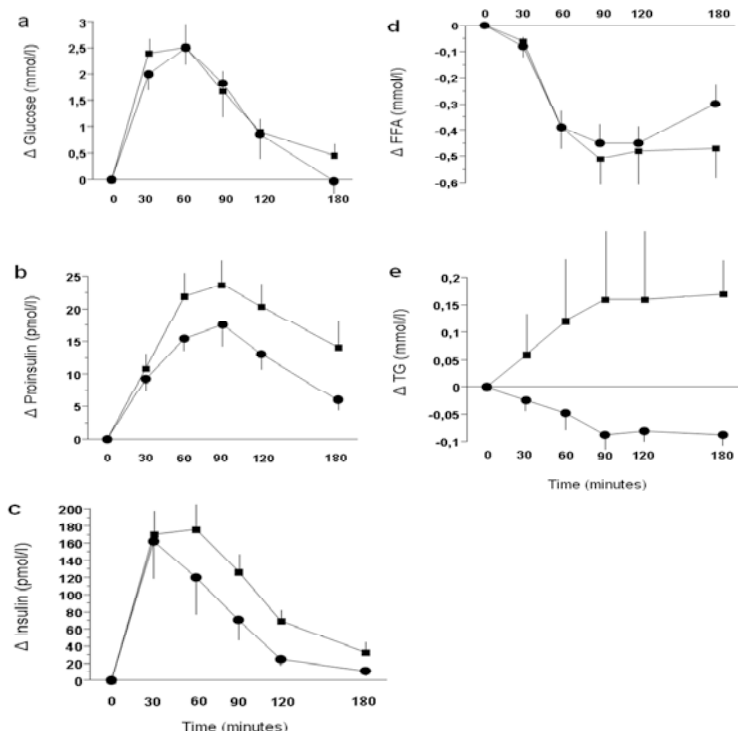
Figure 6a-e. The postprandial changes in in glucose (a), proinsulin (b), insulin (c), free fatty acid (FFA) (d) and triglyceride (TG) (e) concentrations are shown for 180 min after the ingestion of the standardized test meal (mean ±SEM). ■, normal weight control subjects; ●, morbidly obese subjects treated with bileopancreatic diversion with duodenal switch (BPD-DS) surgery.

Clinical characteristics during three years of follow-up study

Baseline data

Clinical characteristics for patients before they underwent BPD-DS surgery and at follow-ups over three years after surgery are shown in Table 6. No patient was identified with diabetes before surgery or at follow-ups. None of the patients in this study had any clinical complications during the surgical procedure or during the follow-up period.

Data from follow-ups 1, 2 and 3 years after BPD-DS surgery.

Over three years of follow-up there were significant trends in lowering of BMI, f-P glucose, HbA_{1c}, P-ALT, P-creatinine, P-uric acid, f-P-total cholesterol, f-P-LDL cholesterol, f-P-LDL/HDL cholesterol, f-P-triglycerides and increasing trends of P-magnesium, c.f. Table 6 and Figure 7 a-e.

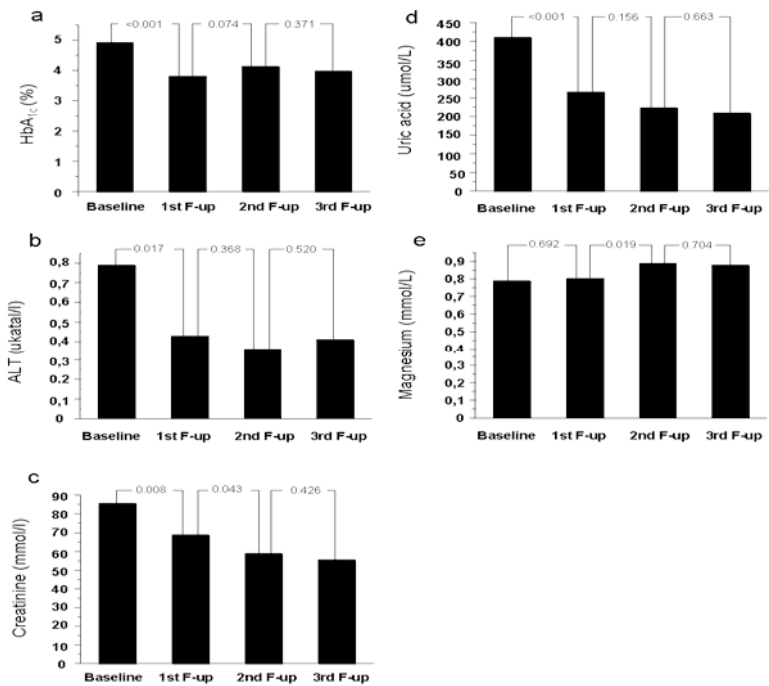*Figure 7a-e.* The changes in HbA1c (a) and concentrations of alanine aminotransferase, *ALT* (b), creatinine (c) uric acid (d) and magnesium (e) are shown at baseline, at first follow-up (1 year), at second follow-up (2 years) and at third follow-up (3 years) after bileopancreatic diversion with duodenal switch. Mean values are shown. Statistical significance is presented as p-values. F-up denotes follow-up.

Table 6. *Clinical characteristics for patients before BPD-DS surgery and at follow-ups 1, 2 and 3 years after surgery.*

	0	1 year	2 year	3 year	P for trend
Gender (women/men)	5/5	5/5	5/5	5/5	-
Age (years)	37.5				-
BMI (kg/m^2)	53.5 (3.8)	30.7 (4.6)	28.4 (3.8)	30.2 (5.0)	<0.001
Weight (kg)	161.3 (26.7)	92.9 (21.4)	86.0 (19.4)	91.9 (25.8)	<0.001
Height (cm)	173.0 (10.1)	173.4 (10.7)	173.3 (10.6)	173.4 (10.7)	0.999
f-P-glucose (mmol/l)	5.5 (0.9)	4.6 (0.58)	4.5 (0.35)	4.6 (0.22)	<0.001
HbA$_{1c}$ %	4.9 (0.39)	3.8 (0.28)	4.1 (0.45)	4.0 (0.29)	<0.001
P-ALT(μkatal/l)	0.79 (0.39)	0.43 (0.18)	0.36 (0.16)	0.41 (0.13)	0.001
P-creatinine (mmol/l)	85.6 (9.7)	68.8 (10.7)	58.9 (9.6)	55.4 (8.8)	<0.001
P-albumin (mmol/l)	40.4 (2.3)	38.9 (4.5)	39.8 (3.9)	39.3 (3.2)	0.322
P-uric acid (μmol/l)	411 (82)	266 (52)	233 (70)	209 (64)	<0.001
P-magnesium (mmol/l)	0.79 (0.10)	0.80 (0.07)	0.89 (0.07)	0.88 (0.06)	0.017
P-calcium (mmol/l)	2.30 (0.05)	2.26 (0.09)	2.18 (0.12)	2.20 (0.10)	0.058
P-sodium (mmol/l)	141 (3.5)	136 (4.9)	139 (0.6)	140 (2.1)	0.231
P-potassium (mmol/l)	3.8 (0.25)	3.4 (0.09)	3.6 (0.29)	3.7 (0.44)	0.106
f-P-total cholesterol(mmol/l)	5.0 (0.45)	3.38 (0.28)	3.38 (0.47)	3.20 (0.43)	<0.001
f-P-LDL-C (mmol/l)	3.18 (0.41)	1.96 (0.30)	1.99 (0.35)	1.93 (0.35)	<0.001
f-P-HDL-C (mmol/l)	1.07 (0.14)	1.10 (0.20)	1.13 (0.22)	0.98 (0.16)	0.316
f-P-LDL/HDL-C ratio	3.03 (0.61)	1.85 (0.58)	1.83 (0.58)	1.99 (0.56)	<0.001
f-P-TG (mmol/l)	1.74 (0.63)	0.92 (0.19)	0.93 (0.48)	0.90 (0.31)	<0.001

Data given are arithmetic means (SD). ALT, alanine aminotransferase; LDC-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; f, fasting; P, plasma.

Paper III

Baseline data

There were no statistically significant differences in mean fasting plasma concentrations of glucose, proinsulin, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, trigycerides, ALT or uric acid in the RYGBP group compared with the morbidly obese control (MOC) group.

Follow-up data

BMI

BMI is shown in Figure 8a. The mean BMI in the RYGBP group was reduced by 30%, from 42,3 kg/m^2 to 29.7 kg/m^2 at 1st follow-up and to 32.1 kg/m^2 at 2nd follow-up (p<0.001, respectively). The slight increase of 8 % between 1st and 2nd follow-up was significant (p<0.001). At 1st follow-up the intergroup difference (between the RYGBP group and MOC group) was significant (Fig.1a, p<0.001).

ASD

ASD was lowered by 22%, from 28.9 cm to 22.6 cm in the RYGBP group at 1st follow-up and increased to 23.8 cm at 2nd follow-up (p<0.001, respectively). The increase of 5%, between 1st and 2nd follow-up was significant (p=0.001). The intergroup difference at 1st follow-up was significant (p< 0.001).

Glucose

Glucose concentrations were lowered by 15 %, from 4.9 to 4.2 mmol/L (p< 0.001) in the RYGBP group at 1st follow-up but were increased to 4.9 mmol/L at 2nd follow-up (p=0.773). The increase of 15%, albeit within normal range, between 1st and 2nd follow-up was significant (p<0.001). The intergroup difference at 1st follow-up was significant (p< 0.001). In the MOC group glucose concentrations were increased between baseline and 1st follow-up by 6 % from 5.1 to 5.4 mmol/L, but not statistically significant (p=0.198).

Proinsulin

Proinsulin is shown in Figure 8b. The mean fasting proinsulin concentrations were markedly lowered by 75 %, in the RYGBP group from 13.5 to 3.5 pmol/L at 1st follow-up (p<0.001) and at 2nd follow-up, the mean fasting proinsulin concentrations were almost unchanged and only slightly higher than at 1st follow-up, 4.9 pmol/L, (p<0.001). There were no significant change between 1st and 2nd follow-up (p=0.091). A significant intergroup difference at 1st follow-up was noticed (Fig.1b, p<0.001).

Insulin

Insulin is presented in Figure 8c. The mean fasting insulin concentrations were markedly lowered by 70% in the RYGBP group from 83.4 to 24.6 pmol/L at 1st follow-up (p<0.001). At 2nd follow-up the mean fasting insulin concentrations were still low in the RYGBP group but now 48% higher than observed at 1st follow-up, 36.4 pmol/L (p<0.01). The increase between 1st and 2nd follow-up was significant (p=0.020). At 1st follow-up a clear intergroup difference was noticed (Fig.1c, p<0.001).

ALT

ALT is presented in Figure 8d. ALT in the RYGBP group was lowered by 45%, from 0.62 to 0.34 mmol/L at 1st follow-up and continued to lower to 0.24 mmol/L at 2nd follow-up (p<0.001, respectively). The further decrease of 29 %, between 1st and 2nd follow-up was also significant (p= 0.02). At 1st follow-up an intergroup difference was noticed (Fig.1d, p=0.001).

HDL cholesterol

HDL cholesterol is shown in Figure 8e. The mean HDL cholesterol in the RYGBP group increased by 25% from 1.16 to 1.45 mmol/L at the 1st follow-up and continued to increase at 2nd follow-up to 1.58 mmol/L (p <0.001, respectively). The further increase by 9%, between 1st and 2nd follow-up was also significant (p=0.006). The intergroup difference at 1st follow-up was significant (Fig.1e, p=0.04).

Total cholesterol

Total cholesterol was lowered by 12 %, in the RYGBP group from 5.2 to 4.6 mmol/L (p<0.001) at 1st follow-up but at 2nd follow-up cholesterol had increased to 4.9 mmol/L (p=0.03). The increase of 6% between 1st and 2nd follow-up was significant (p=0.049). The intergroup difference at 1st follow-up was borderline (p=0.05).
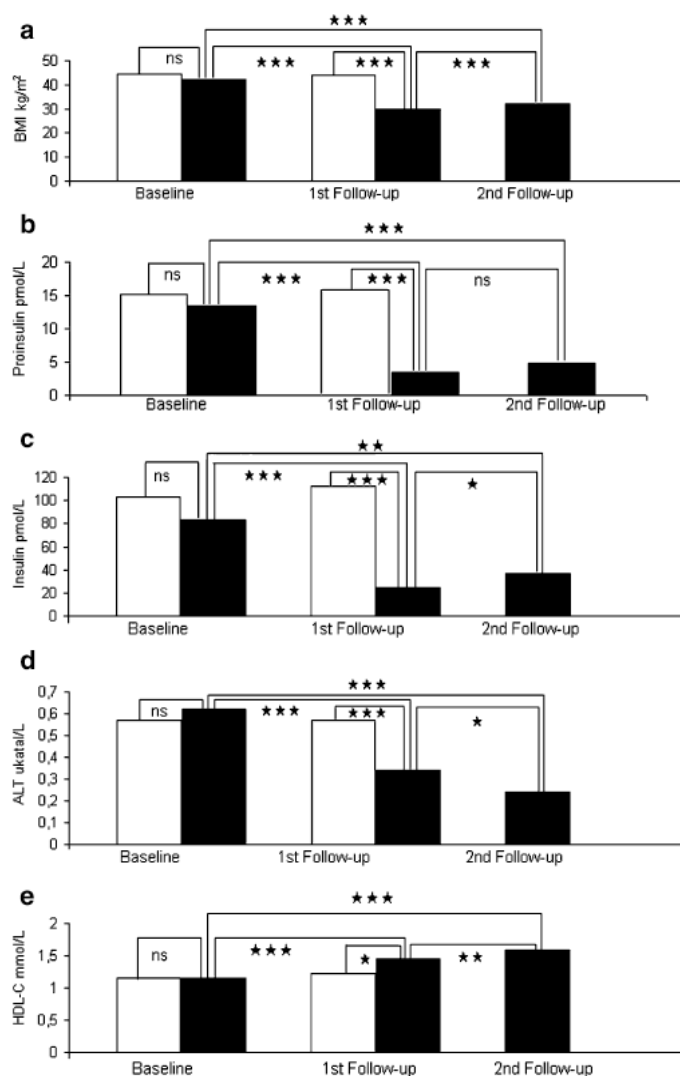
*Figure 8a-e.*The changes in BMI (a) and concentrations of proinsulin (b), insulin (c), alanine aminotransferase, ALT (d), and HDL cholesterol (e) are shown at baseline, at first follow-up (12 months), and at second follow-up (42 months) after RYGBP surgery. Group symbols are: morbidly obese control group, white bars; morbidly obese patients treated with Roux-en-Y gastric bypass surgery, black bars. In morbidly obese control group, data are available up to 12 months. Mean values are shown. Statistical significance is indicated by *p<0.05, **p<0.01, and ***p<0.001.

LDL cholesterol

LDL cholesterol decreased by 23%, in the RYGBP group from 3.5 to 2.7 mmol/L at 1st follow-up but increased slightly by 5% to 2.84 mmol/L at 2nd follow-up. Thus, at both time points the value was significantly improved (p<0.001, respectively). There were no significant change between 1st and 2nd follow-up (p=0.159). At 1st follow-up a significant intergroup difference was observed (p=0.02).

Triglycerides,

Fasting triglycerides were lowered by 31%, in the RYGBP group from 1.62 to 1.11 mmol/L at 1st follow-up and to 1.03 mmol/L at 2nd follow-up (p<0.001, respectively). There were no significant change between 1st and 2nd follow-up (p=0.304). The intergroup difference at 1st follow-up was borderline (p=0.051).

Uric acid

Uric acid was lowered by 17% in the RYGBP group from 356 to 294 mmol/ at 1st follow-up and was unchanged at 2nd follow-up (p<0.001 respectively). There were no significant change between 1st and 2nd follow-up (p=0.827). The intergroup difference at 1st follow-up was borderline significant (p=0.059).

Paper IV

Baseline data

At baseline, i.e., before patients underwent RYGBP surgery, there were no statistically significant differences between the group of patients directed for surgical treatment and the control group regarding weight, BMI, abdominal sagittal diameter, fasting plasma glucose and serum insulin concentrations, systolic and diastolic blood pressure, or serum magnesium concentrations.

Follow-up data

Magnesium

Serum magnesium concentration increased by 6% in the RYGBP-treated group, from 0.80 mmol/L to 0.85 mmol/L (p=0.009), while an opposite trend was observed during the corresponding period in the control group by 4%, from 0.80 mmol/L to 0.77 mmol/L (p=0.132). The intergroup difference in serum magnesium concentrations at the 1-year follow-up 0%, p=0.014) was significant cf. Figure 9.

BMI

In the RYGBP group, the mean BMI decreased from 42.3 kg/m^2 at baseline to 29.7 kg/m^2, i.e., by 30% (p < 0.001). In the control group no change in mean BMI was observed between baseline and 1-year follow-up, BMI 44.3 kg/m^2 and 44.2 kg/m^2, respectively. Abdominal sagittal diameter (ASD) decreased from 28.9 cm at baseline to 22.6 cm, i.e., by 22% in the RYGBP group (p<0,001), while no change was observed in the control group (30.0 cm at baseline as well as at the 1-year follow-up). BMI and abdominal sagittal diameter were significantly different in the two groups at the 1-year follow-up (both p< 0.001).

Glucose

Fasting plasma glucose concentrations decreased by 15%, from 4.9 mmol/L before RYGBP surgery to 4.2 mmol/L at the 1-year follow-up (p<0.001) and increased by 6% from 5.1 mmol/L to 5.4 mmol/L, (ns) in the control group. The intergroup difference regarding fasting plasma glucose at the 1-year follow-up was statistically significant (p<0.001).

Insulin

The fasting plasma insulin concentrations decreased by 75%, from 83.4 pmol/L before RYGBP surgery to 24.6 pmol/L at the 1-year follow-up after GBP surgery (p<0.001). In the control group the corresponding figures were 103.2 pmol/L at baseline and 112.8 pmol/L at the 1-year follow-up (ns). The intergroup difference in insulin concentration, at the 1-year follow-up, was statistically significant (p<0.001; Figure 9).

HOMA-IR

Insulin resistance assessed by HOMA-IR index decreased by 76%, from 3.1 to 0.75 in the GBP group (p<0.001) while an opposite non significant alteration from 4.1 to 4.9 was observed in the control group, implying a significant intergroup difference at the 1-year follow-up (p<0.001; Figure 9).

Blood Pressures

SBP decreased in the RYGBP group by 7%, from 123 mmHg to 114 mmHg (p=0.05). No significant change was observed in the control group, (121 mmHg at baseline and 123 mmHg at the 1-year follow-up, p=0.20). DBP showed a similar pattern, decreasing in the RYGBP group by 7%, from 75 mmHg to 70 mmHg (p=0.045). An opposite trend was observed in the control group (76 mmHg at baseline and 79 mmHg at the 1-year follow-up, p=0.06). The intergroup differences regarding SBP and DBP at the 1-year follow-up were statistically significant (p=0.019, p<0.001, respectively; Figure 9).

Associations within the RYGBP group

The increase in serum magnesium concentration at the 1-year follow-up in the RYGBP group was accompanied by a decreased ASD (r^2 = 0.32, p=0.009), a lowered BMI (r^2=0.28, p=0.021), a lowered glucose concentration (r^2=0.28, p=0.027) and lowered HOMA-IR index (r^2=0.24, p= 0.002).

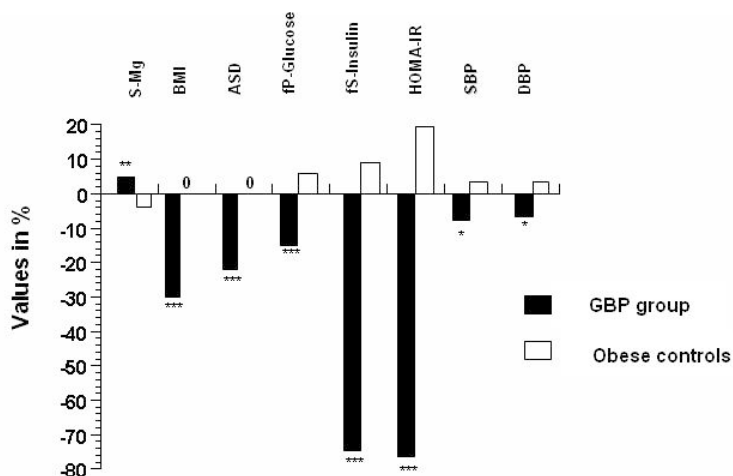*Figure 9.* The changes in percent from baseline to follow-up at 1 year after RYGBP in serum magnesium (s-Mg), body mass index (BMI), abdominal sagittal diameter (ASD), fasting plasma glucose (fP-glucose), fasting serum insulin (fS-insulin), Homeostatic model assessment (HOMA-IR), systolic (SBP) and diastolic (DBP) blood pressures. Statistical significance indicated * p<0.05, ** p<0.01, *** p<0.001

Discussion

Main findings and comparison with the litterature

Paper I

The high fasting proinsulin concentrations in non-diabetic morbidly obese individials, indicating insulin resistance, were significantly lowered in the RYGBP group and were similar to normal weight controls, indicating improved insulin sensitivity by the induced weight loss in the group treated with bariatric surgery. Furthermore, in the RYGBP group late postprandial proinsulin response was markedly lowered, and normalized at 180 minutes, indicating a lowered beta-cell demand throughout the test, meanwhile in the MO group, proinsulin concentrations were high during the entire 180 min test. This observation, taking into account the long half-life of proinsulin and that meals are often ingested 3 or more times a day, might indicate a high beta-cell demand and contribute to raised proinsulin concentrations over long periods of the day.

   Previously, insulin concentrations but not proinsulin have been analysed in meal tolerance or glucose tolerance studies among obese or obese that had undergone RYGBP surgery [104, 105]. One pilot study that actually measured proinsulin concentrations after a test meal in obese compared with normal controls was according to the authors too small to be conclusive due to a large intra group variation [106]. The present study analysed glucose, proinsulin, insulin and the proinsulin to insulin ratio after a standardised test meal where effort was made to compose a test meal representing an "every day lunch" for RYGBP treated subjects. The caloric content of carbohydrates is almost similar to that of a 75 gram oral glucose tolerance test. The rapid rise in glucose observed in the RYGBP group induced a rapid insulin response that peaked at 30 minutes postload, followed by relative hypoinsulinemia during the latter half of the test. The first 30 to 60 minutes of a test meal or an oral glucose tolerance test reflects the first phase of insulin secretion, which has an important role in switching metabolic processes between fasting and postprandial states, mainly by inhibiting hepatic glucose production. A poor or inappropriate first phase of insulin secretion is associated with unsuppressed postprandial glucose production, leading to subsequent higher postprandial glycaemia [107].

Paper II

In the test meal study fasting glucose, proinsulin and insulin were lower than in NW-controls despite a higher BMI. Postprandial glucose, proinsulin, insulin, and FFA responses were similar in BPD-DS treated patients compared to NW-controls, whereas TGs were markedly lower in the PBD-DS patients. The reduction of TGs was unexpected as our earlier studies have shown increased postprandial concentrations during test meals in obese, NW-controls and patients who have undergone RYGBP surgery. The uptake of exogenous TGs from the intestine could be reduced due to fat malabsorption after BPS-DS surgery as nutrients are delivered more directly into the ileum and are rapidly transferred to the colon. In BPD-DS patients fat malabsorption may be explained by several factors: A smaller absorptive area in the common channel, a shorter and possibly faster food passage in the alimentary limb, reduced uptake in the common channel because of passage time difference between the long and short limbs and a longer passage through the bileopancreatic limb for pancreatic enzymes and bile salts.

Over three years of follow-up fasting glucose, HbA1$_c$, and lipids were lowered and uric acid and creatinine were markedly lowered following BPD-DS surgery; more prominently than previously reported following RYGBP. Uric acid is associated with obesity and insulin resistance, CVD and mortality [108, 109]. Weight loss changes uric acid metabolism by increasing clearance [110]. The observed improvement after BPD-DS was more pronounced than earlier data after BPD [111]. BPD-DS may induce a greater loss of muscle mass than other bariatric surgery procedures and thereby a possibility of a greater reduction in creatinine than other bariatric surgeries may have. We suggest further studies including more precise analyses of muscle mass on the one hand and renal function on the other hand to reveal the probable associations between a possible loss of muscle mass and associated creatinine changes after BPD-DS surgery. Findings on magnesium during follow-up will discussed further under paper IV below.

Previously, as far as we know, proinsulin has not been analysed in meal tolerance or glucose tolerance studies following BPD-DS surgery. Studies regarding insulin show restoration of the acute insulin response and insulin sensitivity evaluated by intravenous glucose tolerance tests after BPD without DS in morbidly obese with T2DM [112]. The effects of BPD on T2DM in patients with BMI<35 kg/m^2 compared to dieting T2DM patients have been evaluated by euglycemic insulin clamp. Results showed rapid post operative improvement in insulin sensitivity. One month after BPD, fasting and 2 h post-OGTT glycaemia decreased from 15.22 +/- 3.22 to 6.22 +/- 0.51 mmol/l (p = 0.043), while insulin sensitivity increased significantly. No significant changes were observed in the low-energy diet group. Diabetes amelioration and changes in HbA1c level were observed up to 18 months after BPD without pharmacological therapy [113].

Paper III

In the present long term study of up to 42 months, study improvements were observed in several metabolic variables. Proinsulin was markedly lowered and sustained normalized after RYGBP. The proinsulin/insulin ratio was lowered and improved over the long-term, indicating that RYGBP has long-term effects on improving the dynamics of proinsulin and insulin as markers of beta-cell function and insulin sensitivity. ALT, a marker for NFALD, and HDL-cholesterol were initially improved, and further improvements were followed over time, despite a slight increase over time in BMI. These improvements could be mediated by improved hepatic insulin sensitivity.

In three previous studies [19-21] with follow-up times of 16, 14 and 6 months, respectively, a pronounced weight loss following RYGBP was associated with a marked improvement in insulin sensitivity [19], lowered fasting hyperinsulinemia [20] and lowered postprandial hyperinsulinemia [21]. One study on patients who had undergone LAGB, reported lowered fasting insulin and proinsulin concentrations 14 months after surgery [114] but we observed an equal reduction in insulin but substantially larger reductions in proinsulin after RYGBP. We have previously reported, from a cross-sectional study, that RYGBP surgery induces markedly lowered fasting and postprandial proinsulin concentrations [115]. A recent pilot study including T2DM-patients, followed up-one month after RYGBP, reported that fasting proinsulin was lowered, but not normalized, from 32 to19 pmol/L already after one month [116]. Taken together these studies show that proinsulin after RYGBP is rapidly lowered and sustained low over long-term follow-up periods.

Paper IV

In the present study serum magnesium increased by 6% during the first year after RYGBP surgery. The increase in magnesium was associated with lowered BMI, lowered central obesity (ASD), reduced fasting glucose concentrations and a lowered HOMA-IR index.

Previous bariatric procedures such as JIB induced a weight loss similar to the weight loss observed after RYGBP. However, in comparison to the increased serum magnesium levels found after RYGBP a status of hypomagnesemia was often found after JIB [28]. Huerta et al have reported lower serum magnesium concentrations in obese non-diabetic children and also that lowered circulating magnesium concentrations are associated with increased insulin concentrations and impaired insulin sensitivity [99]. The serum magnesium concentrations have been shown to be inversely related to circulating glucose concentrations and to insulin resistance in diabetic patients as well as in non-diabetic patients [95-97]. The association between circulating magnesium concentrations and glycaemia has been investigated in studies by Djurhuus et al who managed to lower plasma glucose concen-

trations by 20% in type 2 diabetic patients by intensified insulin treatment which reduced the renal excretion of magnesium by 14% but did not change serum magnesium [117]. Similar findings have been reported by Schnack et al [118]. Their study showed that marked improvement of glycaemic control does not correct hypomagnesaemia in T2DM. Possibly the intervention time of three months in both studies was too short to result in changes in serum magnesium. Our observations of increased magnesium concentrations and improved glucometabolic status are seen at follow-ups one year after RYGBP and three years after BPD-DS c.f. paper II. In the BPD-DS group a significant increase in magnesium was first found at the second year follow-up. A reduced renal excretion of magnesium mediated via improved insulin sensitivity and lowered insulin concentrations could be the mechanism behind our results. However, after one year the change in magnesium was not associated with the huge change in insulin but instead with changes in ASD, BMI and glucose.

This difference in serum magnesium following different surgical procedures may be explained by altered magnesium absorption or induced side effects like diarrhoea, which was common after JIB surgery. The blood volume is increased in obesity and the question is if the magnesium increase after bariatric surgery is a direct effect of a reduced blood volume? The answer to such a question is at present not known. In the BPD-DS group magnesium is unchanged during the first year but weight is reduced by more than 40%. Further studies are needed to reveal the mechanisms. It may also be speculated whether increased serum magnesium concentration might contribute to the improvements observed in several of the metabolic variables, as supplementation with magnesium has been reported to improve insulin sensitivity [100]. Such findings however have not been reported consistently in other trials [119, 120]. One meta-analysis has recently shown that oral magnesium supplementation for 4-16 weeks may be effective in reducing plasma fasting glucose levels in patients with T2M diabetes, although the long-term benefits and safety of magnesium treatment on glycaemic control remain to be determined [101].

Strengths and limitations

Paper I-II

Paper I includes patients who underwent RYGBP who were matched by their pre-surgery BMI to the BMI of the control group. Further normal weight controls were included. Both sexes were represented, however longer follow-up time in the standardized meal test would have been desirable to observe changes late post-load in the morbidly obese group since, at 180 minutes proinsulin had not yet started to decrease in that group.

Previously, proinsulin has not been analysed in meal tolerance or glucose tolerance studies following BPD-DS surgery. The present studies analysed glucose, proinsulin and insulin after a standardised test meal where effort was made to compose a test meal representing an "every day lunch" for bariatric surgery treated patients. Age and gender distribution was similar in both BPD-DS patients and normal weight controls but the BPD-DS group weighted 20% more. The possible influence of weight on glucose metabolism, which is known to deteriorate with increasing weight, would have driven results towards the null-hypothesis, thus possibly underestimating differences rather than overestimating them. The diet regime after bariatric surgery is characterized by a limited caloric intake but with a high content of nutrients, however, no dietary registrations were carried out in the present studies.

Paper III-IV

The long term improvements in ALT and HDL-cholesterol, indicating reduced hepatic insulin resistance, need further evaluation as surrogate markers for NAFLD in RYGBP in comparison to computed tomography and biopsies. Physical activity on a regular basis, which was not recorded in this study, may increase HDL-cholesterol.

As insulin may increase the renal magnesium excretion, the pronounced decrease in circulating insulin concentrations observed after RYGBP surgery in the present study, might contribute to the increased serum magnesium concentrations observed, however, renal magnesium excretion was not measured in this study, nor was renal excretion of proinsulin.

After RYGBP surgery no extra supplementation was given with regard to magnesium. Since no dietary registration was carried out in the present studies, the possible influence of diet on magnesium concentrations remains open. The control group was significantly younger than the RYGBP group and the possible influence of age on glucose metabolism, which is known to deteriorate with increasing age, would have driven results towards the null-hypothesis, thus possibly underestimating differences observed rather than overestimate them.

Scientific and clinical relevance

Paper I-II

As proinsulin is a marker for the development of T2DM, CVD, stroke and mortality the present studies could have relevance when evaluating the effects of bariatric surgery. Our results suggest that proinsulin might be evaluated as a marker for diabetes risk before and after RYGBP surgery in future studies. It might be speculated that proinsulin status and postprandial re-

sponses could be used when evaluating the indication for bariatric surgery. To answer such questions, further research on proinsulin and insulin as markers for T2DM and CHD in long-term follow up studies following bariatric surgery is warranted and suggested.

Paper III-IV

As proinsulin is a better risk marker for T2DM and CHD, than insulin in epidemiological studies [6] and a markedly reduced conversion to T2DM was observed after bariatric surgery after long-term follow-up over 10 years [16]. Long-term follow-up studies following bariatric surgery with hard endpoints to evaluate if baseline concentrations of proinsulin are better than insulin concentrations for predicting future T2DM and CHD after bariatric surgery are warranted. Insulin determinations are comparable between different laboratories as methods are standardised and continuously quality assured. Such efforts are needed for proinsulin as well if proinsulin is to be used more frequently for these indications as presented in the thesis.

Obesity, insulin resistance and dyslipidemia are principal factors associated with NAFLD [34, 35]. Serum ALT and triglyceride concentrations increase and HDL-cholesterol decreases gradually before onset the of T2DM [36]. Further, individuals with elevated serum ALT activity have an increased risk of CHD [37]. In the present study ALT and triglycerides were lowered and HDL-cholesterol increased indicating a lower degree of NAFLD and possibly lower hepatic insulin resistance.

Regarding magnesium, our results show that RYGBP and BPD-DS do not induce hypomagnesemia which was common after JIB and that they induce similar or more prominent weight loss.

It may be speculated whether the increased serum magnesium concentrations observed after RYGBP might contribute to the improvements observed in several of the metabolic variables, similar to results obtained by supplementation with magnesium which has been reported to improve insulin sensitivity [100]. Such findings however have not been reported consistently in other trials [119, 120]. When adding electrolytes and especially serum magnesium to known risk factors to all-cause mortality, Håglin et al showed that low magnesium gave an unexpectedly high degree of prediction [27].
Proinsulin, as well as serum magnesium, could be a risk marker for later complications and may be involved in mechanisms related to decreased mortality among morbidly obese patients treated with RYGBP, as was observed in the SOS study in 2007 [17].

Conclusions

Paper I

Morbidly obese patients, free from diabetes, have elevated proinsulin concentrations in the fasting as well as in the postprandial phase. After RYGBP surgery markedly lowered fasting and postprandial proinsulin concentrations were observed although BMI was higher compared to NW-controls. RYGBP surgery improves insulin sensitivity and results in rapid meal stimulated secretion of proinsulin and insulin with sustained effects on glucose and lipid metabolism.

Paper II

Bileo-pancreatic diversion with duodenal switch, which is a combined restrictive and malabsorbtive surgical procedure, causes a large weight reduction in the obese and close to normalise both early and late phases of glucose, insulin and proinsulin responses after a meal, but with lowering of triglycerides.

Paper III

Morbidly obese patients who have undergone RYGBP surgery are characterized in long-term follow-ups (42 months) by marked and sustained improvements of proinsulin, ALT and HDL-cholesterol possibly indicating improved hepatic insulin sensitivity.

Paper IV

Morbidly obese patients threated with RYGBP surgery are characterized by reduced visceral adiposity, lowered plasma glucose and increased circulating magnesium concentrations. These findings might reflect an inverse association between central obesity and glucometabolic status on the one hand, and magnesium status on the other hand.

General discussion on bariatric surgery

In 1991, the National Institute of Health (NIH) arranged a consensus conference on surgery for obesity. More uniform indications for obesity surgery were established e.g. BMI 40 kg/m^2 or 35 kg/m^2 with co-morbidity [121, 122]. Prior to the consensus conference there was great ambiguity and different criteria were used for selecting patients for obesity surgery. The NIH conference consensus document achieved great impact worldwide and obesity treatment by bariatric surgery became more common. A new consensus conference 2004 held by the American Society of Bariatric Surgery confirmed much of the NIH consensus but also focused on methods of operation and follow-ups [123]. The European guidelines, as stated by the European Association for Endoscopic Surgery (EAES) and the European branch of the International Federation of Surgery for Obesity (IFSO), are similar to the NIH document of 1991 [124, 125]. In the Swedish national guidelines for obesity surgery from 2007 similar indications and content are presented as in the NIH document and the European guidelines [126].

Effects of surgery

Bariatric surgery results in decreased mortality and significantly lower incidence rates of several chronic conditions [17, 127-129]. The impact of bariatric surgery on T2DM is well documented and many patients show uniform normalization of glucose concentrations [22, 66, 67]. The improvement in glucose status seen after RYGBP or BBD-DS surgery is not only induced by weight reduction but also by improved entero-insular axis and glucagons-like–peptide secretion [66, 68, 130, 131]. Bariatric surgery reduces the onset of T2DM [16, 132] as well as inducing remission [16, 23, 24].

The relation between cardiovascular disease and obesity is complex and multifactorial. Obese patients often have the metabolic syndrome or part of it and it is difficult to separate risks mediated by the factors in the metabolic syndrome from risks caused by obesity per se. Today it is perhaps too early to definitely state the impact of obesity surgery on hypertension, cardiovascular disease and dyslipidemia. However, there are positive trends; bariatric surgery has been shown to reduce hypertension [16, 24, 133] and dyslipidemia [24, 133, 134]. The SOS study has reported decreased mortality in MI [17]. Confirmatory studies are needed.

Obstructive sleep apnoea (OSA), a common condition among obese patients, is often improved or cured by bariatric surgery [133]. The prominent weight loss after bariatric surgery often improves OSA and possibly also decreases the CVD risk associated with OSA.

PCOS can be successfully treated by RYGBP surgery which decreases intraabdomial fat and reduces insulin resistance which is often found in these patients [135]. Along with an improvement in the glucose status, the reduced intraabdominal fat may lead to the diminishing of the up regulated conversion of oestrogen to testosterone and possibly result in normalization of menstrual periods and ovulation.

Gastroesofagual reflux is common among the obese and RYGBP surgery often represents an effective treatment of this condition [136-138].

The effects on muscle pain, skeletal pain and life quality are vague and too early to state, even if improvements have been reported.

Complications

In general, complications are rare. Perioperative complications such as bleeding and leakage in anastomosis are seen in approximately 1- 2% of the patients. Mortality was reported to be around 0.25% in the SOS study [17]. Later complications like stenosis and ulcers may occur in 5-10%. Most patients have experienced dumping syndrome (fatigue, gastrointestinal symptoms, rapid heartbeat) especially when eating fast carbohydrates and fat. Usually dietary advice helps the patients and it is also recommended that they take vitamin and mineral supplementations. Regular controls are necessary to avoid vitamin B-12, folic acid, calcium, iron, zinc and vitamin-D deficiencies [139].

In general, bariatric surgery is considered for patients of ages between 18-60 years. New studies have shown that RYGBP can be successfully performed in the elderly, over 65 years [140, 141]. Thus a risk benefit analysis in each individual patient should be considered. RYGBP is more frequently performed in women than in men but no significant differences in results have been noticed between men and women [142]. Since the disease burden [143] and mortality [144] are higher already at BMI >30 kg/m^2 and many patients with BMI 35 kg/ m^2 have comorbities, the weight criteria for surgery is under discussion and suggestions have been made to lower it from 40 kg/m^2 to 35 kg/m^2.

Preoperative weight reduction decreases liver size as well as intraabdominal fat content [145] and promotes the surgical procedure [146, 147]. These observations have resulted in that preoperative treatment with low calorie diets will possibly be more frequently used.

Quality considerations

The outcome and complications after surgery depend to some extent on the surgeon's experience, operation volumes and also on hospital capacity [148-151]. Bariatric surgery is now performed in more than 30 clinics in Sweden. A suggestion from the workgroup of Swedish guidelines for obesity surgery (NBHW) to clinics performing bariatric surgery is to promote work with structural quality by ensuring admittance to intensive care units enabling reoperation when needed and by regularly performing safety analysis [126]. Further, suggestions are to encourage learning and to optimize the skill process as well as to achieve increased operating volumes and to have minimum acceptable operations per physician. Furthermore, participation in quality registers and long-term follow-up are very much encouraged. Sweden has from an international perspective a long tradition of bariatric surgery. Approximately 20 operations are performed per 100,000 individuals and year. Annually 4000 adults develop a BMI >35 kg /m^2 which might imply that the calculated annual need for bariatric surgery could be as high as 10,000-15,000 operations in Sweden [126].

Related areas of obesity surgery research

Peptides

Along with new knowledge and understanding on how neurohormonal gut-brain signalling regulates energy homeostasis, appetite and satiety new possibilities emerges for the treatment of obesity either by surgery or pharmacological therapies.

Digestion and nutrient absorption take place in the gastrointestinal tract, whereas food intake is controlled by the nervous system. The neuro-humural gut-brain axis is the communication pathway between the gastrointestinal tract and the central nervous system, e.g. the hypothalamus and the brainstem. The vagus nerve is the key neuronal connection. Further several peptides act as signal substances or hormones. Ghrelin is an orexigenic peptide produced by the stomach that stimulates appetite and food intake, while gut peptides such as cholecystokinin (CCK), pancreatic polypeptide (PP), peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM) reduce food intake and induce satiety [148]. In the central nervous system a new pair of neuropeptides has been found in the hypothalamus, Orexiens A and B (hypocretins) [152] which appear to be involved in the regulation of gastric and intestinal motility, food intake, and energy balance [152, 153]. Orexine A in rats decreases the rate of gastric emptying and alters glucagon and insulin levels and in humans decreases leptin concentrations [153, 154].

Also adipose tissue peptides such as leptin and adiponectin may also play roles in thermogensis and glucose metabolism. Adipose tissue, intestinal and gastric peptides are exemplified below.

Adipose tissue peptides

Leptin has been shown to regulate weight and thermogenesis in humans [155]. Increased levels are observed in the obese and leptin resistance in obesity has been under discussion [156]. RYGBP surgery in obese patients lowers leptin levels [157].

Adiponectin is involved in glucose metabolism and inhibits gluconeogenesis and improves insulin sensitivity. Adiponectin is suppressed in humans with obesity and RYGBP surgery has been shown to increase adiponection levels which may contribute to long term homeostasis [158].

Intestinal peptides

CCK is the prototypical satiety hormone, and it inhibits food intake [159, 160]. Due to a short half-life it has been difficult so far to develop as a pharmacological therapy.

Peptide YY increases the absorption of fluids and electrolytes from the intestine after a meal and inhibits pancreatic and gastric secretions and gastric emptying [161]. Lower basal concentrations in the obese are observed [162]. RYGBP surgery increases plasma PYY [131].

GLP-1 is the incretin in focus for the moment, since manipulation of the GLP-1 system, by dipeptidyl peptidase 4 inhibitors (DPP-IV) or administrating GLP-1 agonists, forms the basis for new T2DM treatment. The latter is now being tested for effects on weight loss in clinical trials. Patients with T2DM have improper postprandial GLP-1 responses which are improved by DPP4 inhibitors, by blocking the conversion of active GLP-1 to inactive forms. The actions of GLP-1 take place in several organs and improve insulin secretion, inhibit glucagons secretion, reduce hepatic glucose production induce satiety and possibly improve beta-cell neogenesis and inhibit apoptosis [163]. GLP-1 levels are lower in obesity and RYGBP surgery increases plasma GLP-1 concentrations [131].

Pro-NT is a precursor for neurotensin. Is released after food intake and is involved in the regulation of gastrointestinal motility and pancreatic and biliary secretion [164].

Gastric peptides

Ghrelin is the "hunger" hormone and the only circulating gastrointestinal peptide with orexigenic effects. It stimulates appetite, food intake and weight gain [159, 165]. Ghrelin peaks before meal and may be a meal initiator and play a role in the long-term regulation of energy balance [153]. Circulating ghrelin levels are decreased in human obesity [166], perhaps on the basis of overfeeding.

Discussion on obesity surgery related research

Already in 1955 Friedman et al reported amelolioration of diabetes mellitus following subtotal gastrectomy [167]. Later studies have shown that obesity surgery normalizes glucose tolerance in morbidly obese patients with T2DM. It improves insulin sensitivity and restores the acute insulin response to glucose. This contributes to rapidly restore glucose intolerance and T2DM.

Studies in rodents have shown that gastrojejunal and dudenonjejenal-bypass improves diabetes, both in obese Zucker rats [168] and lean Goto-Kakizaki (GK) T2DM rats [169]. By measuring oral glucose tolerance, food intake, body weight and intestinal nutrient absorption in GK T2DM rats who had undergone duodenal-jejunal bypass, Rubino et al suggested that intestinal bypass could be considered for T2DM treatment and that undiscovered factors from the proximal bowel might contribute to the pathophysiology of T2DM [170].

These findings are consistent with several clinical observations of T2DM remission when RYGB or BPD is performed in moderately obese patients [171, 172] or even in lean patients [69, 173].

The effects of incretins on glucose homeostasis have been studied and discussed during recent years [174-176]. GLP-1 promotes its glucose lowering effects through inhibiting gastric emptying, improving insulin sensitivity, inhibiting glucagon secretion and lowering hepatic glucose production [177]. Apoptosis of beta-cells has also been reported in rodents [178]. In patients with T2DM, the incretin effect is weakened. Fasting GLP-1 concentrations are normal or lowered in T2DM patients and postprandial responses are low. Treatment with GLP-1, GLP-1 analogues or dipeptidyl peptidase-4 (DPP-4) inhibitors improves postprandial glucose responses in T2DM.

After RYGBP surgery GLP-1 levels are unchanged or increased and postprandial GLP-1 responses and concentrations have been more consistently increased after mixed meals and oral glucose tolerance tests in obese and T2DM patients [179, 180]. Gastric banding and restrictive procedures do not result in an increase in fasting or postprandial GLP-1 [181, 182]. Results for glucose-dependent insulinotropic peptide (GIP) have not been as consistent as for GLP-1 or an initial increase in GIP has not persisted over time. The effects of RYGBP as compared with hypocaloric diet on glucose and incretin levels in patients with T2DM have been evaluated and revealed an 5-6 fold increase in GLP-1 following RYGBP but not after a hypocaloric diet [116]. Most important, Laferrère et al suggest that the release of incretin is not related to weight loss, but rather to the surgical procedure. The surgical intervention leads to both weight loss and direct metabolic effects e.g. the weight loss is one consequence of surgery

and improved metabolic status is another. In this aspect weight loss is not considered an intervention. Bariatric surgery has effects on T2DM but omentum resection or liposuction does not improve T2DM.

Central obesity with visceral fat depots leads to higher risks of developing insulin resistance and metabolic disturbances and cardiovascular diseases, while subcutaneous fat distributed around the gluteofemoral region is less metabolically active. Visceral fat predominates in the production of adiponectin, inflammatory cytokines and plasminogen activator inhibitor. Subcutaneous fat has a higher basal rate of lipolysis, lipase activity, anti-lipolytic activity and higher leptin expression than visceral fat which may be involved in regulating satiety [183]. The visceral adipose tissue is drained by the portal venous system with direct connection to the liver. Visceral adipose tissue has higher lipolytic activity than subcutaneous tissue due to inhibition by insulin and endocrine regulation, resulting in high mobilisation of FFA. In the obese the mobilisation of FFAs from visceral adipose is high, leading to higher portal concentrations which may possibly have negative effects on the liver, the synthesis of glucose and triglycerides and the inhibition of insulin degradation[184, 185].

The peroxisome proliferators-activated receptor gamma (PPAR-γ) is involved in adipocyte differentiation and the gene acts as a transcriptiptional regulator. By the relative down regulation of PPAR- γ receptors in visceral fat in the obese, redistribution of fat from visceral to subcutaneous depots has been observed when using drugs such as PPAR-γ agonists (gitazones) which also affects lipid and glucose-metabolism [186].

Omentectomy in animal models using dogs shows, after the removal of visceral fat, a reduction of liver glucose production by 40% and an increased glucose uptake in skeletal muscle [183]. In rat models improved hepatic insulin sensitivity has been reported [187]. In humans one pilot study has reported improvements in oral glucose tolerance, insulin sensitivity and fasting plasma glucose and insulin that are 2-3 times greater in omentectomized patients than in controls. Both groups had undergone adjustable banding and no effects on lipids were observed through omentectomy [188]. Data on resolving of T2DM is lacking.

Liposuction is one of the most common cosmetic surgery procedures. Adipose tissue transplantation by liposuction has even been proposed in severe cases of T2DM and NAFLD. One hypothesis proposed is to remove, by liposuction, inactive adipocytes and transplant or repopulate with new cultured adipocytes in severe cases of T2DM and NAFLD to resolve these conditions [189]. Liposuction has been reported to have a positive impact on insulin sensitivity [190, 191] but these observations have not been confirmed in other studies [192, 193]. Klein et al reported that liposuction of deep sub-

cutaneous visceral fat in obese women showed no improvement in insulin resistance or fasting glucose [193]. The question thus remains open if liposuction really can improve glucometabolic status. Even minor surgical procedures can improve the patient´s self confidence, their habits and lifestyle and thereby may have an influence on insulin sensitivity. Today evidence is lacking regarding effects on T2DM.

If weight loss per se was the only explanation for positive effects on glucometabolic status then all types of weight loss intervention would give the same result on T2DM per kilogram weight loss. Many specific effects are mediated by the bowel which may be considered as a potential endocrine organ. Billroths operation of type I and type II, first performed by Theodore Billroth 1881 in Wienna, has been used for more than 100 years. It has been performed on many normal weight ulcer patients with a minimum of side effects that include leakage from the duodenal stump, intraabdominal abscess, anastomotic leak, wound infection, anastomositis and bowel obstruction but has positive effects on T2DM [194, 195]. Thereby the rational for BMI> 35 kg/m^2 with T2DM as comorbidity for acceptance to bariatric surgery may be discussed.

In recent years, it has been debated that the standard BMI cutoff of 35 kg/m^2 [122, 196] for considering bariatric surgery should be lowered to 30 kg/m^2 in patients with T2DM or even lowered to 28 kg/m^2. Although this strategy may perhaps be a reasonable approach, it reflects the tendency to consider surgical treatment of T2DM just as a mere extension of bariatric surgery. Although initially it will be necessary to use BMI ranges when including patients in future clinical trials of diabetes surgery, the aim of such studies should be to find better criteria for patient selection if changing the focus from BMI to novel T2DM-specific parameters including considering evaluations of insulin resistance, acute insulin responses and including proinsulin. In fact, BMI alone is not ideal for accurately evaluating the risk-to-benefit ratio in obese or overweight T2DM patients. Surrogate markers such as saggittal diameter, which is a better marker for evaluating insulin resistance than BMI should also be used [45, 197]. Overweight study participants should also be evaluated in relation to bariatric surgery. There is at present no scientific evidence that any clear BMI cutoff can distinguish between patients for whom surgery can resolve diabetes and patients for whom surgery would be ineffective for this purpose. This highlight that the present guidelines of 35 kg/m^2 as cut of for T2DM patient is to be followed but if surgery is considered for T2DM patients with lower BMI, then it has to be strictly motivated. As already discussed RYGBP and BPD-DS surgery have effects on T2DM in moderately obese and close to normal weight patients [69, 171-173].

Future Perspectives

Peptide dynamics

Buchwald et al reported in 2009 impressive data from a systematic review from nineteen studies including 11175 patients regarding weight loss and T2DM after bariatric surgery. Mean weight loss was 38.5 kg or 55.9% excess body weight. T2DM was resolved in 78.1% of patients and resolved or improved in 86% [198]. Still, the mechanisms underlying the dramatic effects of bariatric surgery on insulin sensitivity and β-cell function are poorly understood. Possible mechanisms are weight loss due to gastric restriction and some degree of malabsortion due to separation of ingested food from digestive enzymes (gastric acid, amylase and lipase) which provide a caloric restriction. Further, regarding changes in intestinal hormones, much of the focus has been on GLP-1 and gastric inhibitory polypeptide (GIP) as discussed earlier, and the rearrangement of gastrointestinal anatomy. The later has resulted in the foregut and hindgut hypothesis to explain the immediate impact on glucose homeostasis after RYGBP [199, 200].

The rearrangements of the gastrointestinal anatomy after bariatric surgery may affect intestinal and/or gastric peptides and thus influence glucose uptake and beta-cell secretion. Future studies would perhaps benefit from simultaneous measurements of a broad spectrum of peptides including determinations of beta-cell secretion of insulin and proinsulin. This should include fasting and glucose tolerance test stimulated concentrations as well as determinations of proinsulin and insulin before obesity surgery, early in the follow-up, then several times when weight is stable and also after long term follow-up to reveal the dynamics of peptides and beta-cell secretion over time. Proinsulin has been suggested as a valuable risk marker for T2DM and CHD in epidemiological settings. We have shown that bariatric surgery ad modem RYGBP reduces the fasting as well as the postprandial proinsulin concentrations cross sectionally and longitudinally for up to four years. However, long-term follow-up studies in obese patients with hard endpoints are needed to confirm the benefits of surgery associated with changes in proinsulin concentrations. We have demonstrated that morbidly obese patients after BPD-DS show close to normal postprandial responses of proinsulin and glucometabolic status compared to NW-controls, despite a higher BMI. As meals are ingested 3-4 times a day, 24 hour measurement of the renal excretion of proinsulin would be of interest to evaluate total daily levels of proinsulin.

Inflammation markers and fatty acid

Obesity also results in the deposition of fat in muscle, the liver and the pancreas which may result in chronic inflammation reflected by highly sensitive C-reactive protein [201]. The adipose tissue produces atherogenic biomarkers such as C-reactive protein, interleukin-6 and interleukin-18 which are associated with CHD. Obesity surgery reduces insulin resistance and reduces markers of chronic vascular inflammation [202]. Long-term studies on inflammation markers, as well as if possible, biopsies from muscle and liver are warranted. Further, adipose tissue biopsies are easier to perform repeatedly and should be performed on a long-term study basis.

Fasting FFA was lower and the postprandial AUC for TG markedly lower, possibly caused by higher degrees of malabsorption due to the surgery per se. FFAs may contribute to insulin resistance by inhibiting glucose uptake and by increasing hepatic output. We have described long term improvements in lipids and ALT, a marker for liver fat content. The link between increased FFA and insulin resistance might involve an accumulation of TGs and FFA metabolites in muscle and the liver [203]. Rosa et al have shown that reversibility of diabetes after malabsorptive bariatric surgery is dependent on the improvement of skeletal muscle insulin sensitivity, mediated by genes regulating glucose and FFA metabolism in response to nutrient availability, although other mechanisms might intervene [204]. Further, an altered FFA composition is related to insulin resistance and CVD and associations can be found with degree of inflammation [205].

We suggest future studies following BPD-DS on the uptake of FFAs in the intestine as well as bowel transition times and B48 regulation of chylomicrons and TGs to possibly confirm a lower uptake and effects as prevoiusly discussed. All the mechanisms described show the complexity of obesity and the difficulties in developing new strategies to treat obesity. Most likely bariatric surgery will develop into "metabolic surgery" and even more focus on specific metabolic effects or targets than weight loss per se.

Svensk sammanfattning

Förekomsten av fetma och typ 2 diabetes (T2DM) ökar och går ofta hand i hand. Internationella diabetes federationen beräknar att år 2025 det skall finns ca 333 miljoner diabetiker i världen. Ökningar sker främst i Indien, Asien och Sydamerika. Vid insjuknande i T2DM är ca 80 % överviktiga eller feta. Fetma är en av de starkaste riskfaktorerna för T2DM. Uppemot en 40-faldig ökning av risken för T2DM har beräknats vid BMI 35 kg/m^2. Många har metabolt syndrom, vilket innebär övervikt, T2DM eller nedsatt glukostolerans, insulinresistens, hypertoni och lipidrubbning. Var och en av komponenterna i det metabola syndromet ökar risken för hjärtkärlsjukdom.

Kirurgisk behandling av fetma i Sverige har ökat kraftigt över en tioårsperiod och uppvisar idag bra resultat. SOS studien har visat goda resultat över tid beträffande viktnedgång, remission och nyinsjuknande i T2DM samt även minskad dödlighet. Gastric bypass kan idag räknas som gold standard bland överviktsoperationer. Icke kirurgisk behandling som omfattar livsstils åtgärder, kostråd, beteende terapi och läkemedel såsom Xenical ® och Reductil® har utveclas, förbättrats och är viktiga men hittills har det varit svårt att nå lika bestående resultat som efter överviktskirurgi.

Proinsulin är en riskmarkör för T2DM och hjärt-kärlsjukdom. Vid fetma noteras såväl förhöjda faste som måltids koncentrationer av insulin och proinsulin. I denna avhandling har glukos, insulin och proinsulin halterna studerats i fasta samt under måltid med hjälp av en standardiserad måltid hos patienter med fetma, normalviktiga individer samt patienter som genom gått överviktskirurgi av olika typ. Överviktsopererade patienter har visat en markant viktnedgång, normalisering av faste koncentrationer av glukos, insulin och proinsulin som även kvarstått vid långtidsuppföljning trots en kvarstående övervikt jämfört normalviktiga kontroller. Måltidsbeslastningarna har visat sänkta och förbättrade postprandiella svar after gastric bypass samt närmast normaliserade svar efter bileopankreatisk divergering med duodenal switch.

Långtidsuppföjlning efter överviktskirurgi visar förbättrat glucometabolt status kvarstår över tid och parallellt med detta kunde kvarstående och fortgående förbättringar noteras i ALAT, ett leverenzym som en markör för leverförfettning samt i HDL-kolesterol trots att patienten passerat viktned-

gångsmaximum och sakta stigit i vikt. Den avfettade levern med minskad insulinresistens spelar sannolikt en central roll, möjligen på basen av minskad visceral belastning av fria fettsyror för uppvisade metabola förbättringar. Ytterligare studier krävs dock för att fastsälla närmare mekanismer.

Magnesium är en cofaktor som är inblandat i en rad enzymsteg och är också involverad i glukosmetabolismen. Diabetiker har lägre magnesium koncentrationer än icke-diabetiker. Vi har visat att magnesium stiger efter såväl gastric bypass som bileopancreatisk divergering med duodenal switch, parallellt med förbättringar i glukos, insulin och proinsulin halter. Vid uppföljning efter kirurgi var förändringarna i magnesium kopplade till minskat bukomfång, minskat BMI och förbättrat glukos.

Acknowledgements

I wish to express my sincere gratitude and appreciation to everyone who has contributed to this thesis. Especially I would like to thank:

Björn Zethelius, my principal supervisor, for outstanding scientific teaching, sharing his knowledge, for patience, support, faith and good discussions, all with enthusiasm and structure. Keep up the good work and the sky is the limit. It has also been a great pleasure to clinically work together at the outpatient clinic for obesity, Samariterhemmet.

Arvo Hänni, my co-supervisor, fellow-colleague and head of clinical trials, for teaching me all about good clinical practice, good discussions from- life in general to technical problems, showing what an extremely wide range of knowledge and experience he possesses, not to forget good laughs and expertise on magnesium.

Margareta Öhrvall my co-supervisor, former head of the Outpatient Department for metabolic diseases at Samariterhemmet, for recruiting me to the team and giving me opportunities to clinically learn about the metabolic syndrome and introducing me to scientific work.

Lars Lanfeldt my co-supervisor**,** head of the Department of Geriatrics, for support and enthusiasm.

Magnus Sundbom, co-author and surgeon, for support and good clinical discussions regarding preoperative evaluations.

Anders Karlsson, co-author, professor in endocrinology at Medical sciences for support and discussion and introducing me to your "Monday seminars".

Britt Eden-Engström, co-author and colleague, for support and advice.

Bengt Vessby former head of the Section for Clinical Nutrition Research and expert in lipids for sharing his knowledge

Siv Tengblad, Barbro Simu and late Eva Seiby, for great laboratory skills and work, without you, this would not have been possible.

The entire staff at the Outpatient Department for metabolic diseases for support and assistance. It all started here in 2003 with clinical work, learning from you and senior colleagues. Thank you!

Enheten för kliniska prövningar (clinical trials) with nurses **Ida Eklöf, Maritha Andersson and Maria Forslund** for conducting several metabolic trials together with me. Sometimes the confusion was big regarding follow-ups or medication and the e-CRF could be tuff to fulfil but with united efforts it succeeded. I wonder why all investigators meetings were held in Prague.

Friends and former colleagues at the Outpatient Clinic for Metabolic Diseases for support and enthusiasm, and especially: **Per-Erik Andersson**, **Per Hallberg**, **Klara Halvarsson-Edlund and Katarina Lunner** for great clinical teamwork and good company.

The dieticians **Susanne Freden**, **Agneta Nilsson**, **Britt Stålnacke**, **Helena Pettersson, Erika Olsson, Karin Andersson and Brita Karlström** (head of dieticians) and the cook **Mariana Spoverud** for good teamwork.

Friends and colleagues at the section for Geriatrics: **Ann-Christin Åberg, Bernice Wiberg, Helena Petersson, Achraf Daryani, Tamanna Ferdous, Anja Saletti, Ulf Holmbäck, Ulf Risérus and Per Sjögren** for support.

Svante Wester for IT support and a great 50 year party.

Last but not least my beloved family, **Ulla, Erica and Frida** for their patience, understanding and support for all these years.

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Editor: The Dean of the Faculty of Medicine

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