Mechanisms of improved glycaemic control after Roux-en-Y gastric bypass

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This review has been accepted as a thesis together with three previously published papers by University of Copenhagen November 29th 2013 and defended on July 1st 2014.

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The thesis is based on 3 papers:

Study 1: Bojsen-Møller KN, Dirksen C, Jørgensen NB, Jacobsen SH, Serup AK, Albers PH, Hansen DL, Worm D, Naver L, Kristiansen VB, Wojtaszewski JFP, Kiens B, Holst JJ, Richter EA, Madsbad S 2014 Early enhancements of hepatic and later of peripheral insulin sensitivity combined with increased postprandial insulin secretion contribute to improved glycemic control after Roux-en-Y gastric bypass. Diabetes 63:1725–1737 (1).

Study 2: Bojsen-Møller KN, Dirksen C, Jørgensen NB, Jacobsen SH, Hansen DL, Worm D, Naver L, Kristiansen VB, Holst JJ, Madsbad S 2013 Increased Hepatic Insulin Clearance After Roux-en-Y Gastric Bypass. J Clin Endocrinol Metab 98(6):E1066–E1071(2).

Study 3: Dirksen C, Bojsen-Møller KN, Jørgensen NB, Jacobsen SH, Kristiansen VB, Naver LS, Hansen DL, Worm D, Holst JJ, Madsbad S 2013 Exaggerated release and preserved insulinotropic action of glucagon-like peptide-1 underlie insulin hypersecretion in glucose tolerant individuals after Roux-en-Y gastric bypass. Diabetologia 56 (12): 2679-87 (3).

1. INTRODUCTION

1.1 OBESITY AND TREATMENT OPTIONS

Obesity is a global health problem reaching epidemic proportions with a doubling in prevalence within the last 30 years and followed by increases in obesity related co-morbidities, e.g. type 2 diabetes, hypertension, dyslipidaemia, and increased mortality due to cardiovascular diseases and specific types of cancer (endometrial, breast and colon) (4). Weight loss through lifestyle interventions of dieting and increased physical activity is the recommended and obvious treatment (4), but a clinical relevant weight loss can be difficult to achieve and, especially, to maintain (5). Pharmacological treatment of obesity has not yet proven very successful, and several agents have been withdrawn from the market due to adverse side-effects (6). Bariatric surgery can induce weight loss, that is sustained for at least 15 years and amounts to 15-30% of total body weight, depending on the type of operation (7).

1.1.1 Criteria for bariatric surgery

At present, bariatric surgery is offered as a treatment option to severely obese patients, who have not been able to achieve or maintain weight loss through other interventions. International guidelines recommend bariatric surgery to these patients if BMI≥40 kg/m^2 or BMI≥35 kg/m^2 in combination with ≥1 obesity related co-morbidity (8). In Denmark, bariatric surgery has been a part of public health care since 2005, and the referral criteria were comparable to international recommendations until a legislative change in January 2011 reduced the number of operations by stricter referral criteria (BMI≥50 kg/m^2 or BMI≥35 kg/m^2 in combination with ≥1 obesity related co-morbidity diagnosed by medical specialist). Furthermore, all candidates for bariatric surgery are required by health authorities in Denmark to achieve a diet-induced 8% total body weight loss before surgery.

1.1.2 Roux-en-Y gastric bypass (RYGB)

Roux-en-Y gastric bypass (RYGB) is the most common bariatric procedure worldwide (9) and accounts for almost all bariatric operations in Denmark (10). The procedure combines stapling of the stomach and the creation of a pouch of approximately 25 mL with bypass of the remainder of the stomach, duodenum and upper part of the jejunum (biliopancreatic/secretory limb of approx. 75 cm) through the alimentary Roux limb of approx. 100 cm (figure 1). In Denmark, RYGB is only performed using the laparoscopic technique.
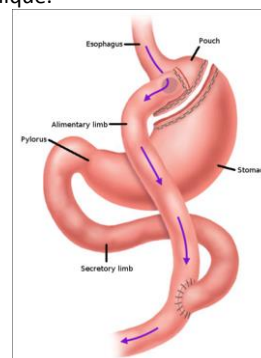

Figure 1: Schematic presentation of the gastrointestinal anatomy after Roux-en-Y gastric bypass (RYGB)

1.2 RYGB AND EFFECTS ON MORTALITY AND MORBIDITY

1.2.1 Mortality, cardiovascular events and cancer after RYGB

The Swedish Obese Subjects study (SOS-study) is an ongoing prospective intervention study of 2010 patients undergoing bariatric surgery matched on 18 variables to 2037 obese controls treated with usual care with a follow-up of 12-25 years to date (7). Only a subgroup in the SOS-study was operated with gastric bypass (13%), while the majority had procedures restricting gastric volume only; gastric banding (19%) or vertical-banded gastroplasty (68%) (7). Although not randomized, this study provides the best evidence so far for the long-term effects of bariatric surgery on mortality and morbidity.

Key results from the SOS-study include reductions in the surgery group of overall mortality (Hazard Ratio [HR] 0.76, 95% CI 0.54-0.92)(11), cardiovascular events (HR 0.67, 95% CI 0.54-0.83) (12) and cancer (in women only: HR 0.58, 95% CI 0.44-0.77) (13). Retrospective cohort studies including primarily data on RYGB have shown similar results as the SOS study on mortality (14), cardiovascular events (15,16) and cancer (17). There is, however, still a need for randomized controlled trials to establish long-term effects of RYGB on obesity-related comorbidities and especially to investigate the effects on cancer incidence.

1.2.2 Complications after RYGB

Peri-operative (≤30 days) mortality of bariatric surgery is low and for laparoscopic RYGB typically 0.2-0.5% (18). In Denmark, peri-operative mortality of RYGB was 0.05% in 2006-2011 (6 deaths after 11499 gastric bypass procedures), and in the same period 2.2% of RYGB patients were re-operated within the first month (10). Early complications consist of anastomotic leakage, bleeding, wound infections and pulmonary complications (18). Within the first year after RYGB, re-operations (including endoscopy) were performed in 7% in Denmark, most frequently because of cholecystolithiasis, bowel obstruction/internal hernia, incisional hernia, anal conditions and GI-ulcerations (10,19). On a longer term, "re-operations" were frequent (49% after 5 years), but this number also includes surgical removal of excessive skin and operations for conditions not related to RYGB (10).

Medical complications after RYGB consist primarily of micronutrient deficiencies, that often represent deterioration of pre-existing deficiencies, why monitoring and treatment pre- as well as post-surgery is important (20). Also protein malnutrition can occur, and patients are instructed to ingest a protein-rich diet (20). A very rare medical complication after RYGB is postprandial neuroglycopenic hypoglycemia, which in the most extreme cases can require surgical resection of parts of the pancreas (21,22).

1.2.3 RYGB and type 2 diabetes

In the SOS-trial, bariatric surgery resulted in remission of diabetes both after 2 years (Odds Ratio [OR] 8.4, CI 5.7-12.5) and 10 years (OR 3.5, CI 1.6-7.3), although a 50% relapse was observed at 10 years among the patients with initial remission (remission rate 72% at 2 years and 36% at 10 years) (23). In addition bariatric surgery prevented new cases of type 2 diabetes (HR 0.22, CI 0.18-0.27) in the follow-up period of up to 15 years (mean 10 years) and most efficiently in patients at high risk due to impaired fasting glucose (IFG) at baseline (Number needed to treat in order to prevent one case of type 2 diabetes was 1.3 in patients with IFG and 7.0 in patients with normal fasting glucose) (7,24).

A meta-analysis of bariatric surgery including 55106 RYGB patients (4973 with type 2 diabetes) estimated diabetes remission after RYGB to 80.3%, however the definition of remission was not very clear (25). In a consensus statement in 2009, complete diabetes remission was defined as HbA1c<6.0% and fasting P-glucose <5.6 mmol/L without antidiabetic medication and with at least 1 years' duration (26). Applying this definition, diabetes remission was reported to 40.6% after RYGB in a recent study of 160 patients with type 2 diabetes, and preoperative treatment with insulin (30.6% of patients) was associated with a lower remission rate (27). Nevertheless, glycaemic control improved after RYGB, and HbA1c declined from 8.1% to 6.2%, which is why RYGB could be seen as an intervention to achieve glycaemic control rather than complete remission of diabetes (27). Recently, three randomized controlled trials proved RYGB superior to conventional antidiabetic treatment in achieving glycaemic control within 1-2 years after surgery and demonstrated that lower HbA1c levels can be achieved with less medication after RYGB (28–30). The International Diabetes Federation (IDF) has recognized bariatric surgery as a cost-effective treatment of patients with type 2 diabetes with BMI>35 kg/m2, who are not able to achieve glycaemic control with medical therapy, and furthermore states that surgery can be considered under special circumstances in patients with BMI of 30-35 kg/m2 (18).

The time-course of the improvement in glycaemic control after RYGB is of particular interest, as glucose levels have been shown to decrease already within days after surgery (31) suggesting the presence of weight loss independent factors influencing glucose metabolism after RYGB. Understanding the physiological mechanisms behind the improvement in glycaemic control after RYGB could thus be a potential source of new knowledge of the pathophysiology in type 2 diabetes, as well as providing clues to future therapies.

1.3 PATOPHYSIOLOGY OF TYPE 2 DIABETES

Type 2 diabetes is characterized by development of insulin resistance, i.e. decreased insulin sensitivity, caused by obesity and/or sedentary lifestyle in a genetically predisposed individual (32). A core element in the pathogenesis of type 2 diabetes is the early development of insulin resistance in skeletal muscle and liver resulting in diminished glucose uptake and impaired suppression of hepatic glucose production (HGP), respectively (32). Insulin resistance of the fat tissue results in accelerated lipolysis increasing levels of fatty acids (FAs), which in turn can decrease insulin sensitivity in muscle, increase HGP and inhibit beta-cell function (lipotoxicity) (32).

In the pre-diabetic state normoglycaemia is maintained by a compensatory increase in insulin secretion (33), although changes in insulin metabolism in terms of decreased clearance also has been shown to contribute to the compensatory hyperinsulinaemia (34,35). However, impaired beta-cell function can be demonstrated years before the onset of hyperglycaemia and diagnosis of diabetes (33). Progressive beta-cell failure will lead to impaired glucose tolerance (IGT) and type 2 diabetes, and beta-cell dysfunction is thus a prerequisite to type 2 diabetes (32,33). The beta-cell dysfunction of type 2 diabetes is characterized by decreased insulin secretion in response to glucose with absent or severely impaired first phase secretion (0-10 min after glucose infusion) and diminished second phase insulin secretion (36). Furthermore, patients with type 2 diabetes exhibit impaired

insulinotropic action of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (37), while decreased postprandial secretion of particularly GLP-1 also have been demonstrated (38,39). Alpha-cell dysfunction in type 2 diabetes leads to hyperglucagonaemia both in the fasting and the postprandial state contributing to hyperglycaemia by raising HGP (32).

1.4 AIM OF THESIS

The aim of this thesis was to investigate the physiological changes in insulin sensitivity and beta-cell function after RYGB with a special focus on the early postoperative period in order to uncover the mechanisms responsible for the improved glycaemic control in type 2 diabetes.

We have prospectively studied different groups of patients with type 2 diabetes and normal glucose tolerance (NGT) before, 1 week, 3 months and 1 year after RYGB with assessment of hepatic and peripheral insulin sensitivity (1), insulin clearance (1,2) and beta-cell function in response to oral glucose and intravenous challenges (1,3). Furthermore, changes in the potentiating effect of incretin hormones have been studied in patients with NGT before, 1 week and 3 months after RYGB(3). Secretion of glucagon has been studied in response to oral glucose as well as during iv infusions of glucose and/or insulin (1,3). The thesis will be based on these three studies but will also review findings from other studies performed by our group at Hvidovre Hospital (40–45) as well as the many other relevant studies performed internationally, as recently reviewed by our group (46).

We propose that improved glycaemic control after RYGB can be explained by improvements in both insulin sensitivity and beta-cell function (46) according to the following hypotheses:

Hypothesis 1:

RYGB has a differential effect on hepatic and peripheral insulin sensitivity in the early postoperative period with early improvements in hepatic and later improvements in peripheral insulin sensitivity.

Hypothesis 2:

Improved beta-cell function after RYGB depends on the changes in gastrointestinal anatomy and is thus linked to the oral rather than the intravenous route of administration.

2. INSULIN SENSITIVITY AND CLEARANCE AFTER RYGB

2.1 METHODS

2.1.1 Estimation of insulin sensitivity

The hyperinsulinaemic euglycaemic clamp is considered gold standard for the estimation of insulin sensitivity and is based on the steady state principle, that glucose uptake in peripheral tissues during a constant infusion of insulin can be estimated as the glucose infusion rate required to maintain plasma glucose constant, assuming complete suppression of endogenous glucose production (EGP) (47). Simultaneously intravenous glucose tracer infusion makes it possible to assess EGP by the tracer-dilution method and thereby to estimate peripheral glucose uptake in conditions with incomplete suppression of EGP (47,48). The liver is responsible for the majority of glucose production, and EGP will thus to a large extent reflect hepatic glucose production, although renal glucose production may contribute (49). An index of hepatic insulin sensitivity can be calculated at basal conditions after an overnight fast as the inverse of the product of EGP and insulin concentration (HISI) (50), although the use of C-peptide probably reflects prehepatic insulin levels to a larger extent (51). Suppres-

sion of EGP by insulin during the hyperinsulinaemic clamp reflects the insulin responsiveness of the liver (although renal and hepatic tissues may respond differently to insulin (49)). EGP suppression occurs with lower insulin doses than typically used in studies aiming at quantifying peripheral glucose disposal (52). Tracer methods can also be applied to estimate rates of lipolysis e.g. by glycerol tracers, and can be combined with the hyperinsulinaemic clamp to estimate insulin sensitivity of fat tissue, although measurements of plasma fatty acids (FAs) or glycerol also provide information on this parameter.

The homeostasis model assessment of insulin resistance (HOMA-IR) is an often used parameter (53) and can be easily calculated from the product of glucose and insulin in the fasting state, thus probably to a larger extent reflecting hepatic insulin resistance than peripheral insulin resistance (50). Insulin sensitivity indices can also be obtained from oral tests (e.g. oral glucose tolerance test, OGTT) and have been validated against the hyperinsulinaemic clamp (50,54), however after RYGB, glucose absorption rates after oral ingestion are substantially increased (44,55–57), which could potentially compromise the validity of these indices. Only the OGIS index has been tested against the hyperinsulinaemic clamp and only after biliopancreatic diversion (BPD), an extensive surgical procedure inducing nutrient malabsorption due to bypass of large parts of the small intestine, showing a significant correlation between changes in OGIS and changes in clamp-derived measures >6 months from surgery (r=0.68, p<0.005) (58). After a frequently sampled intravenous glucose test (FSIGT), it is possible to obtain a measure of peripheral insulin sensitivity (Si) using mathematical modeling (minimal model) (59), but the model has never been validated after bariatric procedures.

2.1.2 Estimation of insulin clearance

Insulin clearance and insulin action are coupled mechanisms, as insulin degradation is mediated by insulin receptor binding by which insulin is internalized by endocytosis and degraded in endosomes or lysosomes (60,61). The major organ responsible for clearing insulin from the circulation is the liver removing 50-60% already by first pass metabolism and in total removing 70-80% from the circulation; while kidneys and peripheral tissues only remove smaller fractions (60,62). Direct estimation of insulin clearance requires invasive procedures with blood sampling in the portal and the hepatic veins, why indirect methods often are preferred. An indirect measure of insulin clearance relies on measurements of C-peptide, as it is secreted equimolarly with insulin from the beta-cell, but is not subjected to hepatic extraction (60,63–65). In steady state conditions the ratio of C-peptide concentration to insulin concentration provides an estimate of insulin clearance, while the different elimination kinetics of C-peptide and insulin makes it inaccurate during non-steady state conditions (64). However, during a meal or an intravenous stimulation, an estimate of insulin clearance can be calculated as the ratio of the averaged means e.g. area under the curves (AUCs) of C-peptide to insulin provided that both have returned to basal levels (64). Pre-hepatic insulin secretion rates (ISR) derived from mathematical modeling of C-peptide concentrations, by use of population-based C-peptide kinetics (66,67), can also be used to estimate insulin clearance by comparing ISR to peripheral insulin concentrations (60). Finally, clearance of exogenous insulin after bolus injection or during constant infusion can be calculated (60). Notably, when obtaining similar levels of peripheral insulin concentration, infusion of exogenous insulin will result in lower por-

tal concentrations of insulin than during stimulation of endogenous insulin secretion. High portal insulin levels are believed to cause decreased insulin clearance due to receptor saturation and down regulation (61), which has primarily been described during supra-physiological infusions (62), but seems to occur during physiological stimulation of endogenous insulin secretion as well (60,68).

2.2 BACKGROUND: INSULIN SENSITIVITY AND CLEARANCE AFTER RYGB

Weight loss induced by hypocaloric diet has been shown in several studies to improve insulin sensitivity in obese subjects regardless of glucose tolerance (69–72). RYGB induces sustainable weight loss (7), and improvements in insulin sensitivity after RYGB-induced weight loss are thus not surprising and have been demonstrated in several studies using the hyperinsulinaemic clamp (56,73–75). However, the role of improved insulin sensitivity in the early improvement of glycaemic control after RYGB is not clarified. Improvements in insulin sensitivity have been demonstrated in a study using hyperinsulinaemic clamp only 4 days after biliopancreatic diversion (BPD) (76). After RYGB there are also several reports with consistent findings of marked reductions in HOMA-IR within the first month after surgery (46) including studies performed within the first week (40,77). In contrast, studies using the hyperinsulinaemic clamp early after RYGB have not shown changes in insulin sensitivity within the first postoperative month (73,74,78,79) except after 4 weeks in a recent study (80). Only two of these studies used infusion of glucose tracer to measure EGP in order to differentiate between hepatic and peripheral insulin sensitivity (74,79). As HOMA-IR may primarily reflect hepatic insulin resistance, while the hyperinsulinaemic clamp primarily estimates peripheral insulin sensitivity (50), the discrepancy between the two measures in the early postoperative period after RYGB could indicate a differential effect on hepatic and peripheral insulin sensitivity with immediate improvements in hepatic and not in peripheral insulin action. When studying immediate changes in insulin sensitivity after RYGB, it is thus important to use glucose tracers to evaluate the separate effects on hepatic and peripheral tissues. The two previous clamp studies using glucose tracers have not been conclusive regarding the early effects of RYGB on EGP with one study showing reductions in basal EGP after 1 month (79), while the other did not detect changes at 2 weeks postoperatively (74). At 6 months and 1 year post-RYGB, reductions in basal EGP have been demonstrated (74,81) although not consistently in subjects with NGT (44,51). Changes in EGP suppression during a clamp have not previously been reported early after RYGB, since EGP was already fully suppressed preoperatively in the two clamp studies (74,79). Changes in insulin clearance after RYGB have been reported in a recent study demonstrating increased fasting insulin clearance at 3 weeks postoperatively (82), while none had assessed early postoperative changes in postprandial clearance or clearance of exogenous insulin, although some reported lower postoperative insulin concentration during hyperinsulinaemic clamps (74,78,83).

2.3 HEPATIC AND PERIPHERAL INSULIN SENSITIVITY AFTER RYGB (STUDY 1)

To assess the differential effect of RYGB surgery on hepatic and peripheral insulin sensitivity, we studied 10 patients with type 2 diabetes and 10 subjects with NGT using a 4 hour hyperinsulinaemic (40 mU/m^2/min), euglycaemic (5.5 mmol/L) clamp combined with infusion of [6,6-^{2}H$_2$]-glucose tracer to measure

glucose production at fasting and during the clamp (1). Glucose production and disposal were calculated as rate of appearance of glucose (Ra) and disappearance (Rd), respectively, using non-steady state equations (48). Hepatic insulin sensitivity was estimated based on basal glucose production and basal C-peptide concentration as the hepatic insulin sensitivity index (HISI$_{basal}$= 10^6/[Ra$_{basal}$ × C-peptide$_{basal}$]). Suppression of glucose production was calculated as the difference between basal and clamp levels expressed as a percentage of the basal level. Glucose disposal (Rd) was adjusted for changes in fat free mass measured by whole body DEXA as well as for clamp insulin concentration (Rd/I). Suppression of fatty acids and glycerol were calculated as differences between basal and clamp levels expressed as a percentage of the basal level and used as a marker of insulin sensitivity of the adipose tissue.

Participants were included after a preoperative diet-induced total body weight loss of −9.2±1.2%, but the majority of preoperative weight loss was achieved >3 months prior to surgery as depicted in figure 2. Patients with type 2 diabetes had a mean BMI of 38.7 at 2 months before surgery and 38.9 at the time of the preoperative clamp immediately before surgery and were thus weight stable in the immediate preoperative period. Glucose tolerant subjects had a mean BMI of 41.4 at 2 months before surgery and 40.2 immediately before surgery. However, the decline in BMI was faster after surgery as seen by the change of the slope in figure 2 underscoring a marked shift towards calorie restriction post-RYGB in both groups.

Participants were studied within the last weeks before RYGB and at 1 week, 3 months and 1 year after surgery. Initial weight loss was comparable between the groups, but at 1 year after surgery subjects in the NGT group had lost more weight than patients in the T2D group (figure 2; total body weight: −36% versus −28%, p=0.02; excess BMI loss (EBL): 64% versus 48%, p<0.01) [%EBL= (ΔBMI$_{pre-1years}$/ΔBMI$_{pre-25}$)×100%].
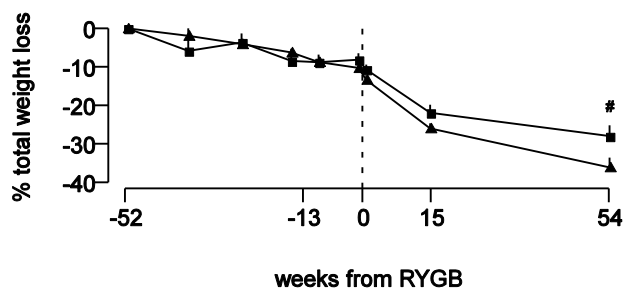
BMI	pre-diet	-1	+1	+15	+54
T2D	42.4±1.7	38.9±1.6**	37.3±1.8**	33.1±1.5**	30.8±1.7**
NGT	44.9±1.0	40.2±0.8**	37.9±0.9**	33.2±1.1**	28.5±1.5**

Figure 2 Preoperative and postoperative total body weight loss (%) in patients with type 2 diabetes (n=10, black squares) and NGT (n=10, black triangles) undergoing RYGB. Values are mean ± sem. Changes in BMI was analyzed with mixed-effects ANOVA (Time: p<0.001, Group: p=0.520, Time×Group: p=0.01). Figure from (1).
** p < 0.01 difference from baseline within the group.
 # p < 0.05 difference in changes between the groups

Glycaemic control improved after RYGB in patients with type 2 diabetes; fasting glucose declined after 1 week, decreased further at 3 months and remained low at 1 year, HbA1c and 2 hour glucose excursions after OGTT were also reduced after 3 months and 1 year. Correspondingly, antidiabetic medication was discontinued in all patients from the time of surgery throughout the study period except in one patient requiring metformin for a brief period at 4-11 months postoperatively. At 1 year after surgery fasting glucose was <5.6 mmol/L in 5 patients and HbA1c <6% in 8 patients. Thus, 50% (5/10) of the patients fulfilled the criteria of complete remission of type 2 diabetes (fasting glucose <5.6 mmol/L and HbA1c<6%) after RYGB while another 40% (4/10) experienced partial remission (fasting glucose <7 mmol/L and HbA1c<6.5%) (26). The NGT group experienced postoperative reductions in fasting glucose and 2 h glucose after OGTT, but not in HbA1c.

Preoperatively, basal glucose production tended to be higher in patients with type 2 diabetes than in subjects with NGT (p=0.09) (figure 3), while basal hepatic insulin sensitivity (HISI) did not differ significantly between groups (1).

During the clamp, patients with type 2 diabetes had incomplete suppression of glucose production before RYGB, while clamp glucose production in subjects with NGT was not significantly different from 0 mg/min. Glucose disposal was higher in subjects with NGT than in patients with type 2 diabetes before surgery (figure 4).

After RYGB, the main finding was an immediate increase in hepatic insulin sensitivity at 1 week as evidenced by reduced basal glucose production (figure 3) and increased basal hepatic insulin sensitivity with comparable changes in patients with type 2 diabetes and NGT. During the clamp, glucose production was unchanged in patients with type 2 diabetes at 1 week, but reduced after 3 months and 1 year at which time glucose production was completely suppressed (figure 3). In subjects with NGT, we observed no postoperative changes in clamp glucose production or suppression of glucose production, and clamp glucose production was not significantly different from 0 mg/min at any time-point.
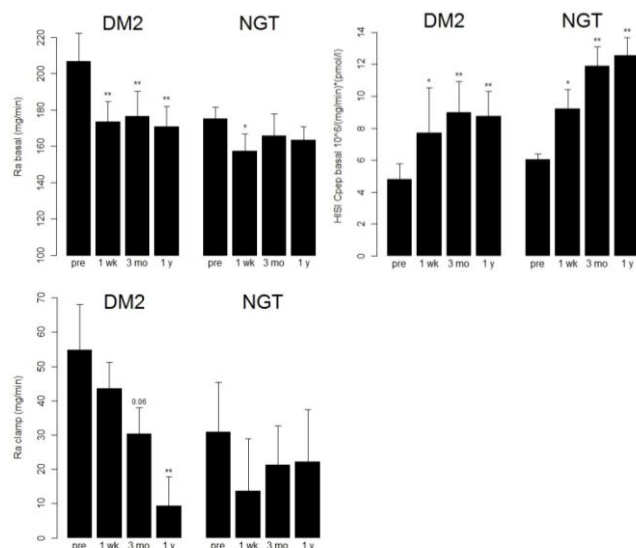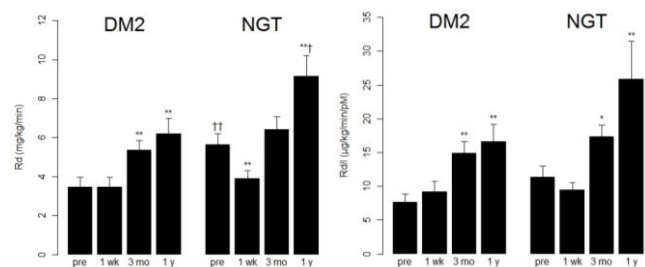

Figure 4 Glucose disposal during hyperinsulinaemic euglycaemic clamp (Rd) and adjusted for insulin concentration (Rd/I) in patients with type 2 diabetes (DM2) and normal glucose tolerance (NGT) before, 1 week, 3 months and 1 year after RYGB. Values are mean + sem.
*p<0.05, **p<0.01 from preoperatively within the group †p<0.05, †† p<0.01 for differences between the groups at a given study session
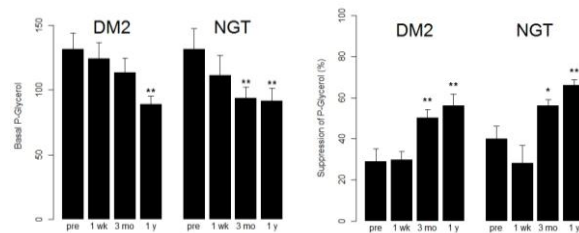

Figure 5 Basal concentration of plasma glycerol (left) and suppression of glycerol during the hyperinsulinaemic euglycaemic clamp (right) in patients with type 2 diabetes (DM2) and in obese subjects with NGT before, 1 week, 3 months and 1 year after RYGB.
*p<0.05, **p<0.01 for the change from preoperative level within the group

Glucose disposal corrected for clamp insulin levels (Rd/I) was unchanged 1 week after RYGB (the decline in Rd_{clamp} in the NGT group could be attributed to the lower clamp insulin concentration brought about by increased insulin clearance) (figure 4). Corrected glucose disposal (Rd/I) increased in both groups after 3 months and 1 year and the change in glucose disposal (Rd and Rd/I) correlated with the weight loss at 1 year but not at 3 months.

Fasting concentration of plasma FAs increased at 1 week after RYGB and suppression of FAs during the clamp decreased (in patients with type 2 diabetes only), however by 3 months fasting levels had returned to preoperative values and suppression had increased in both groups with further improvements by 1 year (1). FAs were also studied during a liquid meal test in 12 patients with type 2 diabetes and 11 patients with NGT at the same postoperative time points, but using another assay(2). In this study, fasting FAs decreased at 1 year after RYGB, while suppression of FAs during the meal did not change postoperatively at any time-point.

Plasma glycerol concentration showed a similar response as plasma FAs with lower fasting values at 1 year and increased suppression by insulin at 3 months and 1 year, however the concentration was unchanged at 1 week postoperatively both at fasting and during the clamp (figure 5).

2.3.1 Discussion: Hepatic and peripheral insulin sensitivity after RYGB
Improved glycaemic control in patients with type 2 diabetes is a clinical reality after RYGB (28–30), which is also confirmed in this selected group of patients, who still had overt type 2 diabetes after a preoperative weight loss (1). Complete remission of type 2

Figure 3 Endogenous glucose production in the basal state (Ra_{basal}, top left), basal hepatic insulin sensitivity (HISI, top right) and glucose production during the clamp (Ra_{clamp}, bottom panel) in patients with type 2 diabetes (DM2) and normal glucose tolerance (NGT) before, 1 week, 3 months and 1 year after RYGB. Values are mean + sem. *p<0.05, **p<0.01 from preoperatively within the group.

diabetes was achieved by 50% at 1 year corresponding well with previous reports using the same criteria for diabetes remission (27,84). Lower remission rates have been reported in patients using insulin preoperatively (27,85) most likely reflecting the severity of beta-cell dysfunction. It has been suggested that the postoperative treatment regime may influence the rate of diabetes remission (84). In general, there is a lack of consensus on the management of type 2 diabetes after RYGB, and randomized controlled intervention trials with different antidiabetic treatment strategies are warranted (84,86).

Before surgery, patients with type 2 diabetes tended to have slightly (18%) higher basal glucose production than obese subjects with normal glucose tolerance in accordance with studies using similar tracer methodology, where basal glucose production in patients with type 2 diabetes approximated +12% (range of 0-20%) (87). Glucose disposal during the clamp was reduced by ~30-40% in patient with type 2 diabetes compared to glucose tolerant subjects corresponding well to previous comparative studies of patients with and without type 2 diabetes and BMI ~30 kg/m^2 (52,88).

At 1 week after RYGB, we could confirm the hypothesis of early improvements in hepatic insulin sensitivity by improved basal glucose production and hepatic insulin sensitivity index. Glucose production during the clamp was already suppressed before surgery in most subjects with NGT, why further improvements were unlikely to occur in the NGT group. However, clamp glucose production and suppression of glucose production was also unchanged in patients with type 2 diabetes at 1 week, which could result from the negative influence by other factors such as increased FAs (89) or lower insulin concentration. In fact, unchanged suppression of glucose production despite ~20% lower insulin concentration during the clamp may be interpreted as a relative improvement in the suppressive effect of insulin on glucose production. Moreover, patients with type 2 diabetes had a suppression of glucose production during the clamp of 70% already preoperatively, which is higher than in similar clamp studies (90) and could be a result of the preoperative weight loss. Nevertheless, by 3 months and 1 year postoperatively, all measures of hepatic insulin sensitivity (Ra$_{basal}$, HISI$_{basal}$, Ra$_{Clamp}$, Supression of Ra) had improved in patients with type 2 diabetes.

Peripheral glucose disposal was not changed at 1 week after surgery, and suppression of FAs during the clamp even decreased in patients with type 2 diabetes. Glucose disposal at 1 week after surgery could be influenced by postoperative stress, which has been shown to reduce glucose disposal during hyperinsulinaemic clamp conditions in the first week postoperatively depending on the type of surgery (91,92). Thus postoperative stress could possibly counteract a beneficial effect of surgery. However, lack of improvement in glucose disposal has also been shown after 2-4 weeks postoperatively when surgical stress has abated (73,74,78,79). Elevated FAs also act to reduce peripheral glucose disposal and were seen in both groups 1 week after RYGB. Elevated FAs have previously been shown in the early period after RYGB both at fasting (74,78,82) and during insulin infusion (74) and to a similar extent after calorie restriction (82,83). Increased lipolysis could be an explanation for increased FAs and could be caused by calorie restriction and/or lower insulin levels.

An immediate improvement in hepatic insulin sensitivity is a common observation after calorie restriction in obese patients with type 2 diabetes (90,93–96) and NGT (72) and has been observed as early as after 48 hours (72,96) in absence of major weight loss and changes in peripheral insulin sensitivity (90,93,96). This rapid change in hepatic insulin sensitivity has been associated with decreased liver fat content measured by MR spectroscopy (72,90,93). Thus, not only cumulative weight loss but also changes in calorie restriction (e.g. rate of weight loss) exhibit impact on hepatic insulin sensitivity (94,97), which is important to consider when concluding on weight loss and/or diet independent effects of RYGB. Several studies have reported comparable changes in HOMA-IR after RYGB and calorie restriction (56,73,77,82,98,99), although some studies have found larger improvements in HOMA-IR after RYGB than after restrictive surgery (100,101) or diet alone (100,102). Incomplete matching of the diet between the groups in the previous studies could explain the observed differences due to better compliance in RYGB-operated patients. In a recent study, diet was rigorously controlled to obtain identical weight loss in RYGB operated and diet-treated patients with type 2 diabetes in 3 weeks, i.e. with matching of both weight loss and rate of weight loss, showing comparable changes in fasting glucose, HOMA-IR, insulin clearance and insulin sensitivity measured by FSIGT (82). In conclusion, calorie restriction is a likely explanation for our findings of early improvement in hepatic insulin sensitivity without changes in peripheral insulin sensitivity after RYGB. However, as we did not include a control group subjected to the same postoperative diet without surgery, we cannot rule out diet-independent effects of RYGB on hepatic insulin sensitivity per se. Comparing early changes in glucose production and liver fat content in patients undergoing RYGB to changes in non-operated patients subjected to the same postoperative diet would thus be very interesting, provided that diet-adherence can be controlled in a rigid manner.

Improved peripheral glucose disposal at 3 months and 1 year after RYGB was related to weight loss as demonstrated by the significant correlation between change in glucose disposal and weight loss at 1 year and demonstrated in studies of weight loss induced by diet (69–71,94) or RYGB (56,73,74). At the same time, suppression of plasma concentration of FAs and glycerol improved during insulin infusion indicating improved insulin sensitivity of adipose tissue, as previously described 6-7 and 12 months after RYGB (51,74,83). Suppression of FAs during a meal did not change, although this finding is somewhat unexplained, it has been shown in other studies after RYGB (44,75,101).

Weight loss after RYGB was initially comparable between groups, but at 1 year postoperatively weight loss was significantly larger in the NGT group, which also applied to a larger cohort adding patients, who at our research facility underwent meal testing (total T2D group n=23, NGT group n=22) (40). A preoperative diagnosis of diabetes has been associated with lower weight loss after RYGB in larger retrospective series (103–109), although a meta-analysis by Buchwald et al (2009) suggested otherwise (25). The use of anti-diabetic agents, the presence of other co-morbidities, reduced physical ability and higher preoperative BMI and age are only some of the proposed explanations (103–109), however given the relative poor quality of the studies, no firm conclusions can be derived. The different weight loss pattern of the T2D group and NGT group was reflected in the changes in glucose disposal between study sessions at 3 months and 1 year.

2.4 INSULIN CLEARANCE AFTER RYGB (STUDY 1 AND 2)

We investigated the changes in fasting hepatic insulin clearance (fasting C-peptide to insulin ratio) in a large group of patients with type 2 diabetes (n=32) and NGT (n=32) before, 1 week, 3 months and 1 year after RYGB, while postprandial insulin clearance was evaluated during a liquid meal test in a subgroup of the patients (n=12, n=11, respectively) at the same time points (2). Postprandial insulin clearance was estimated as the ratio between the incremental areas under the curves of insulin secretion rates (ISR) and insulin during the 4 hour meal test. The clearance rate of intravenously infused insulin was assessed during insulin infusion in the other subgroup of 10 patients with type 2 diabetes and 10 subjects with NGT throughout the first year after RYGB (1) with correction for endogenous insulin production estimated by C-peptide (Insulin infusion rate / (Insulin$_{clamp}$ − [C-peptide$_{clamp}$ × Insulin$_{basal}$/C-peptide$_{basal}$]) as described in (62).

We found no preoperative differences between patients with type 2 diabetes and NGT in fasting hepatic insulin clearance, postprandial insulin clearance or clearance of exogenous insulin (1,2).

After RYGB, fasting hepatic insulin clearance increased in both groups at 1 week postoperatively, increased further until 3 months and remained stable from 3 months to 1 year after surgery (1,2) and illustrated in figure 6 (upper panels). Postprandial insulin clearance was lower than fasting insulin clearance pre- and postoperatively (figure 6 lower panels). In patients with type 2 diabetes, postprandial insulin clearance increased slightly from 1 week after surgery and remained higher at 3 months and 1 year, whereas subjects with NGT experienced no changes in postprandial insulin clearance (figure 6, lower panels).

The clearance rate of intravenously infused insulin during the hyperinsulinaemic clamp was increased in both groups already from 1 week after surgery, which could be a likely reason for 20-30% lower insulin concentrations during the postoperative clamps (1).
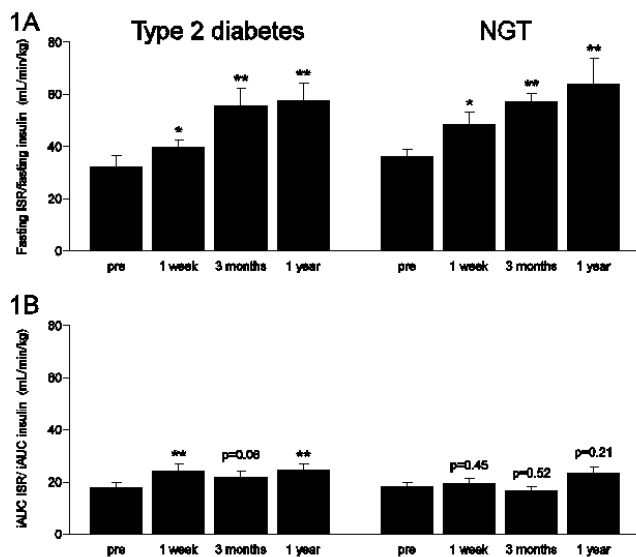
Figure 6 Fasting (fasting ISR/fasting insulin, upper panels) and postprandial (iAUC ISR/ iAUC insulin, lower panels) insulin clearance before (pre), 1 week, 3 months and 1 year after RYGB in patients with type 2 diabetes (left) and normal glucose tolerance (NGT, right). Values are mean + sem.* p<0.05, ** p<0.01 compared to preoperative value using paired t-test. Figure from (2).

2.4.1 Discussion: Insulin clearance after RYGB

Increased insulin clearance has previously been described after very low calorie diet in patients with type 2 diabetes and NGT (110,111), but to our knowledge we were the first to demonstrate increased insulin clearance already 1 week after RYGB (2). A few studies reported lower insulin concentration during hyperinsulinaemic clamps within the first month after RYGB although not reporting insulin clearance (74,78,83), and a small study moreover showed increased hepatic insulin extraction after 7 months in 6 subjects with NGT (51). Recently, a study confirmed the increase in fasting C-peptide to insulin ratio at 3 weeks post-RYGB and demonstrated a similar increase after 3 weeks of strict dieting (82). Calorie restriction is thus likely to explain the increased insulin clearance post-RYGB and could act by reducing liver fat content (112). Concomitant early increases in hepatic insulin sensitivity and insulin clearance after RYGB suggest a common mechanism, which seems reasonable as both insulin action and degradation require interaction with insulin receptors on hepatocytes (60,61).

Insulin secretion after a meal is enhanced and even more so post-RYGB (40) giving rise to high portal insulin concentrations, potentially changing insulin clearance due to saturation of insulin receptors (60). Indeed, postprandial insulin clearance rates were lower than at fasting, which has been shown previously (113). The unchanged postprandial insulin clearance after RYGB in patients with NGT could also be explained by saturation due to very high insulin secretion rates. In contrast, patients with type 2 diabetes experienced a small increase in postprandial insulin clearance despite increased insulin secretion, thus clearly not an effect of receptor saturation. However, we cannot rule out that the marked change in insulin secretion profile in patients with type 2 diabetes could influence hepatic insulin clearance per se, as the liver has been shown to respond rapidly to dynamic changes in insulin secretion (114).

At fasting and during infusion of exogenous insulin, portal insulin concentrations are much lower and thus unlikely to be influenced by saturation of the insulin receptor (60,62).

The increased postprandial clearance of insulin in patients with type 2 diabetes must be taken into consideration, when interpreting studies using peripheral insulin concentrations for estimation of insulin secretion after RYGB. Postoperative changes in postprandial insulin secretion will be underestimated in patients with type 2 diabetes when evaluated by insulin concentration (figure 7), which is why changes in ISR or C-peptide concentration will provide as a better estimation of the post-RYGB change in insulin secretion.
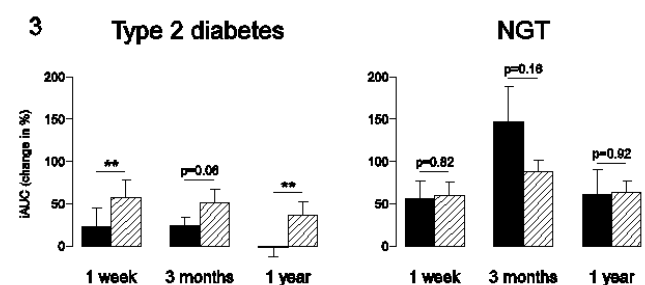

Figure 7 Relative changes in iAUC insulin (black) and iAUC insulin secretion rate (ISR) (diagonal striping) in response to meal test from before surgery to 1 week, 3 months and 1 year post-RYGB in patients with type 2 diabetes (left) and normal glucose tolerance (NGT, right). Values are means + SEM. *p<0.05, **p<0.01 by unpaired t-tests.

Figure 8 Relative changes in HOMA2-IR insulin (white) and HOMA2-IR C-peptide (diagonal striping) from before sur-gery to 1 week, 3 months and 1 year post-RYGB in patients with type 2 dia-betes (left) and normal glucose tolerance (NGT, right). Values are mean + SEM. * p<0.05, **p<0.01 by unpaired t-tests
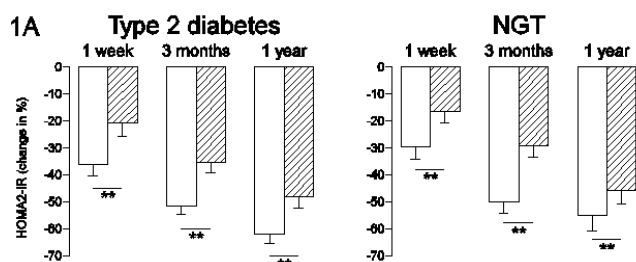
Changes in fasting insulin clearance may also affect interpretation of changes in HOMA-IR after RYGB as the magnitude of change depends greatly on whether insulin or C-peptide is used for the calculations (using the HOMA2-calculator available at www.dtu.ox.ac.uk/homa) (figure 8). Similarly, postoperative changes in hepatic insulin sensitivity index will be overestimated using insulin instead of C-peptide. Traditionally, it has been rec-ommended to use insulin when calculating these indices (50, 53), although the validity of HOMA-IR in conditions with decreased insulin clearance has been discussed (112). Also after RYGB, the validity of using peripheral insulin concentrations in the calcula-tion of insulin sensitivity indices may depend on whether hepatic or peripheral insulin action is estimated.

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glu-cose-dependent insulinotropic polypeptide (GIP) have been pro-posed as regulators of hepatic insulin clearance due to the larger suppression of clearance during oral than intravenous glucose (115). More recent studies did not observe changes in insulin clearance during infusions of GLP-1 or GIP and explained the lower insulin clearance after oral compared to intravenous glu-cose by the higher insulin secretion causing saturation of clear-ance to be more prominent (68,113). Changes in incretin hor-mones in response to the meal were largely unchanged in fasting in the immediate postoperative period, and changes in postpran-dial hormone secretion after RYGB were comparable in patients with type 2 diabetes and NGT (40). In contrast, the greatest change in insulin clearance was observed at fasting, and changes in postprandial clearance differed between the two groups, thus changes in the concentrations of incretin hormones do not seem to contribute to the findings. Changes in fatty acids (FAs) have also been associated to changes in hepatic insulin clearance as experimentally increased FA-levels lead to reduced hepatic insulin clearance in dogs (116), although human studies did not show a similar association (89). We found no early changes in fasting FAs in the subgroup of patients receiving meal tests and even increas-es in the patients undergoing clamps at 1 week after RYGB, thus it seems unlikely that changes in FAs explain the changes in hepatic insulin clearance.

Interestingly, we found no preoperative differences between patients with type 2 diabetes and NGT in insulin clearance. In contrast, a significant difference in C-peptide to insulin ratio between patients with type 2 diabetes and NGT has previously been reported in obese individuals (BMI ~30 kg/m^2) (88). Howev-er, with increasing levels of obesity the reduction in fasting hepat-ic insulin clearance becomes more prominent (117) and severe obesity, as present preoperatively in both groups in our study, could be an explanation for the lack of difference in insulin clear-ance between patients with type 2 diabetes and NGT.

2.5 SUMMARY: INSULIN SENSITIVITY AND CLEARANCE AFTER RYGB

Patients with type 2 diabetes and normal glucose tolerance had completed a similar diet-induced weight loss and had comparable BMI before RYGB. Diabetes status was confirmed by 2 hour post-prandial plasma-glucose measurements after standard oral glu-cose tolerance test at this time point, and normal glucose toler-ance was further defined by normal HbA1c levels. Preoperatively, patients with type 2 diabetes tended to have higher basal glucose production, whereas the hepatic insulin sensitivity index and insulin clearance did not differ between groups. Peripheral glu-cose disposal was lower in patients with type 2 diabetes, while suppression of fatty acids did not differ significantly between groups.

After RYGB, glycaemic control was improved, and 50% of patients with type 2 diabetes achieved complete diabetes remission de-fined by fasting glucose<5.6 mmol/l and HbA1c<6% at 1 year after surgery. Weight loss was initially comparable between groups, but larger at 1 year in subject with normal glucose tolerance. Basal glucose production and hepatic insulin sensitivity improved in both groups at 1 week after surgery, whereas suppression of glucose production only increased at 3 months and 1 year after surgery in patients with type 2 diabetes. Insulin clearance in-creased at fasting and during insulin infusion in both groups at 1 week, whereas postprandial insulin clearance only increased slightly in patients with type 2 diabetes. Peripheral insulin sensi-tivity in terms of insulin mediated glucose disposal and suppres-sion of fatty acids and glycerol was significantly improved at 3 months and 1 year after surgery in both groups. Changes in glu-cose disposal correlated significantly with weight loss at 1 year, but not at 3 months.

In conclusion, RYGB increases hepatic insulin sensitivity already 1 week after surgery in patients with type 2 diabetes and in obese glucose tolerant subjects. Concomitant increases in insulin clear-ance further highlight the liver as an important organ responsible for the early effects on glucose metabolism after surgery. Later improvements in peripheral insulin sensitivity 3 months and 1 year postoperatively are likely related to the substantial reduction in body weight.

3. ISLET-CELL FUNCTION AFTER RYGB
3.1 METHODS

3.1.1 Estimation of beta-cell function
Assessment of insulin secretion is complex as beta-cells provide basal insulin secretion in the fasting state and can increase secre-tion many fold in response to dynamic challenges (118). Accord-ingly, a comprehensive assessment of beta-cell function cannot be performed using a single test or measurement, but requires a number of tests in combination (118). An important distinction should be made between intravenous and oral tests as the first only determines intrinsic regulation of the beta-cell while the latter also evaluates the extrinsic regulation, i.e. includes the potentiating effects of the hormonal responses released during a meal (incretin hormones) and the effects of the autonomous nervous system.

Some of the most frequently applied intravenous tests include the frequently sampled intravenous glucose test (FSIGT) and the hyperglycaemic glucose clamp, both assessing insulin secretion in

response to iv glucose (bolus and infusion, respectively), while non-glucose challenges include the arginine test and the glucagon test (119). Infusion of GLP-1 or GIP can be used in combination with hyperglycaemic clamps to assess the potentiating effect of the hormones on first phase (0-10 min) and second phase insulin secretion (i.e. insulinotropic action)(37,120). Oral tests can be performed using OGTTs or meal tests. As the beta-cell stimulus is not standardized during oral tests, it is necessary to normalize changes in insulin secretion to changes in glucose concentrations, i.e. calculating beta-cell glucose sensitivity (or insulinogenic index), which is especially relevant after RYGB due to marked changes in glucose absorption rates (44,55–57).

Another important consideration in the evaluation of beta-cell function is the relation to insulin sensitivity, as beta-cell function adapts to the prevailing insulin sensitivity in order to maintain glucose tolerance (33). The relation between insulin secretion and insulin sensitivity is best described by a hyperbolar function, and the product of insulin secretion and insulin sensitivity (disposition index, DI) is constant in NGT and declining in type 2 diabetes with the severity of the disease (33). Thus beta-cell dysfunction describes the inability of the beta-cells to produce sufficient insulin to maintain a normal plasma glucose concentration at a given level of insulin sensitivity. The hyperbolar relation of beta-cell function and insulin sensitivity has primarily been established in studies using the FSIGT (33,121), and it is not known whether all measures of beta-cell function display the same relation to insulin sensitivity (118), or whether adaptation relates to peripheral or hepatic insulin sensitivity or both (122).

3.1.2 Estimation of alpha-cell function
Plasma glucose is a main regulator of glucagon secretion, and low levels stimulate whereas high levels suppress glucagon secretion. Mixed meals and amino acids, e.g. arginine, stimulate glucagon secretion, whereas GLP-1 is a known inhibitor. The role of insulin in suppressing glucagon secretion is debated, especially whether intra-islet insulin inhibits glucagon secretion in a paracrine way (123). A supraphysiological dose of glucagon (bolus of 1 mg iv) is a potent insulin secretagogue used for decades to assess beta-cell capacity (119,124). The main physiological effect of glucagon is to increase hepatic glucose production, thereby opposing the suppressive effect of insulin. In type 2 diabetes, fasting and postprandial glucagon levels are high compared to subjects with normal glucose tolerance, particularly when considering concomitant hyperglycaemia (125).

3.2 BACKGROUND: ISLET-CELL FUNCTION AFTER RYGB
Insulin secretion after meal intake is changed after RYGB as demonstrated by higher and earlier peaks in C-peptide or ISR (40,43,80,98,101,126–129). The use of C-peptide or ISR is important in order to avoid the confounding effect of increased insulin clearance after RYGB (2). The changes occur early after RYGB (40,43) in both patients with type 2 diabetes and NGT and may be associated with the changed gastrointestinal anatomy after RYGB, as feeding through a tube placed in the gastric remnant does not elicit the same insulin response (127,130–132). In contrast, studies applying iv challenges have demonstrated a more gradual increase in insulin secretion occurring weeks to months after RYGB in patients with type 2 diabetes (80,82,133,134), while the insulin response after iv stimulation in subjects with NGT tends to decline with time (134,135). However, most studies have used the FSIGT, i.e. a single bolus of iv glucose,

and moreover only report changes in insulin; not C-peptide or ISR. None have previously studied post-RYGB changes in insulin secretion in response to iv non-glucose stimuli, e.g. arginine or glucagon, or changes in the ability of GLP-1 and GIP to potentiate insulin secretion, i.e the insulinotropic action of the hormones, and only one study has previously applied the hyperglycaemic clamp (101). In this study, first and second phase C-peptide and ISR during the hyperglycaemic clamp were unchanged in patients with type 2 diabetes at 1 and 4 weeks after RYGB, whereas beta-cell glucose sensitivity was 4 fold increased during a mixed meal test (101). Thus, when evaluating beta-cell function after RYGB, it is of particular importance to consider the route of administration of the stimulus. We hypothesized, that the marked increase in insulin secretion after RYGB is linked to the oral route of administration, while the responsiveness of the beta-cells to iv challenges is largely unchanged. Testing this hypothesis required simultaneous assessment of insulin secretion in response to both iv and oral challenges after RYGB, which have previously been performed in a few studies (80,101,133,135). A single recent study used the hyperinsulinaemic clamp to evaluate concomitant changes in insulin sensitivity (80).

Alpha-cell function has been examined during oral tests with glucose or mixed meals after RYGB generally shows increased postprandial glucagon secretion postoperatively (40,43–45,98, 136,137), although not reported by all (56). Paradoxically, the glucagon secretion is increased early after ingestion of nutrients, where concentrations of glucose and GLP-1 levels are high. Only a single study has examined the glucagon response to iv glucose and only post-RYGB and found preserved suppression after RYGB (136). During pharmacological blockade of the GLP-1 receptor by infusion of Exendin (9-39) after RYGB, glucagon concentration increases further, suggesting that GLP-1 mediated inhibition of glucagon secretion could also be preserved postoperatively (45,136,138). However, the glucagon response to iv infusion of GLP-1 after RYGB has not been assessed previously. Taken together, changes in alpha-cell function after RYGB may also depend on the route of administration with marked differences in the glucagon secretion response to oral versus iv challenges.

3.3 BETA-CELL FUNCTION AFTER RYGB (STUDY 1 AND 3)
Beta-cell function was investigated in 10 patients with type 2 diabetes and 10 subjects with NGT applying the iv glucose-glucagon test prior to and at 1 week, 3 months and 1 year after RYGB and the oral glucose tolerance test before, 3 months and 1 year after surgery (1). Another 11 glucose tolerant patients were studied with hyperglycaemic clamps with arginine bolus and co-infusion of GLP-1, GIP or saline before, 1 week and 3 months after RYGB as well as with oral glucose tolerance tests before and after 3 months (3).

Insulin secretion was evaluated using C-peptide concentration (1) or insulin secretion rates (ISR) (3). During the oral glucose tolerance tests, we used the insulinogenic index (IGI) to evaluate beta-cell function, calculated as $\Delta C\text{-peptide}_{0-30} / \Delta Glucose_{0-30}$ or $\Delta ISR_{0-30} / \Delta Glucose_{0-30}$ in (1) and (3), respectively. During the iv glucose-glucagon test, first phase insulin secretion was calculated as the mean increment in C-peptide above basal levels after 6-12 min (1), while three different indices of insulin secretion were calculated during the hyperglycaemic clamps (3): First phase insulin response to glucose (AIR_{glu}; mean increment in ISR above basal levels during the first 10 min), second phase response to

glucose (mean increment in ISR above basal levels during the first 20-40 min) and first phase insulin response to arginine (AIR_{arg}; maximal increment in ISR from pre-injection ISR within 5 min).

In patients with type 2 diabetes, insulin secretion after OGTT was markedly increased at 3 months and 1 year after RYGB with doubling of insulinogenic index (IGI) (figure 9) and iAUC of C-peptide (1). Relating insulinogenic index to insulin sensitivity (Rd/I) by calculating an oral disposition index demonstrated even larger changes due to concomitant improvements in insulinogenic index and insulin sensitivity (1). Despite the large postoperative changes in insulin secretion after oral glucose, insulinogenic index and the oral disposition index was markedly lower in the T2D group than in the NGT group at all pre-and postoperative time points.

First phase insulin secretion in response to iv glucose-glucagon was unchanged in patients with type 2 diabetes after RYGB (figure 10). However, due to improvements in insulin sensitivity at 3 months and 1 year post-RYGB, the iv disposition index increased at these time points (1). In subjects with NGT, the C-peptide response to iv glucose-glucagon declined significantly after surgery, but the iv disposition index was unchanged (figure 10).
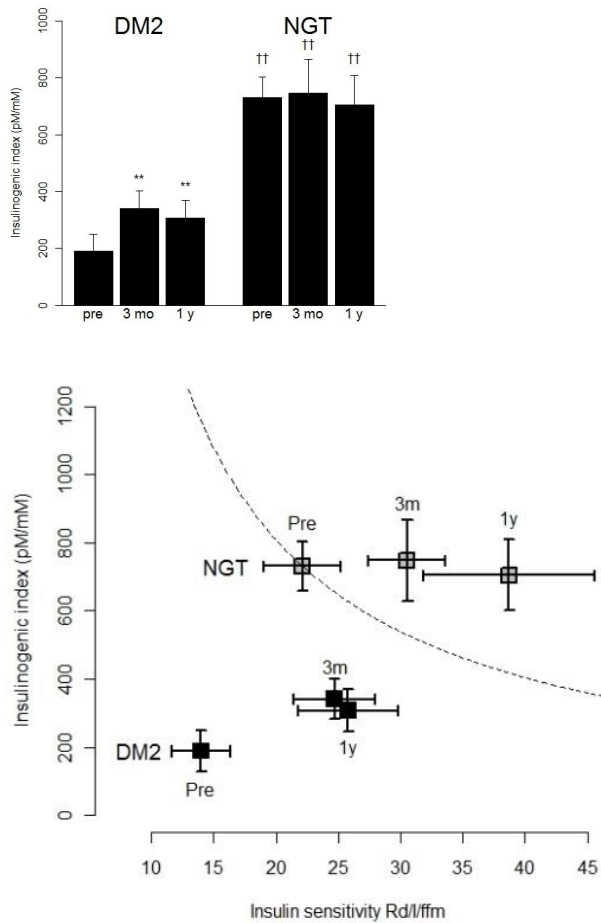
Figure 9: Insulinogenic index (top) and insulinogenic index plottet against insulin sensitivity (Rd/Iffm, bottom) in patients with type 2 diabetes (DM2) and normal glucose tolerance (NGT) before, 3 months and 1 year after RYGB. Values are mean + sem.
**p<0.01 from preoperative within the group
†† p<0.01 for differences between the groups at a given study session
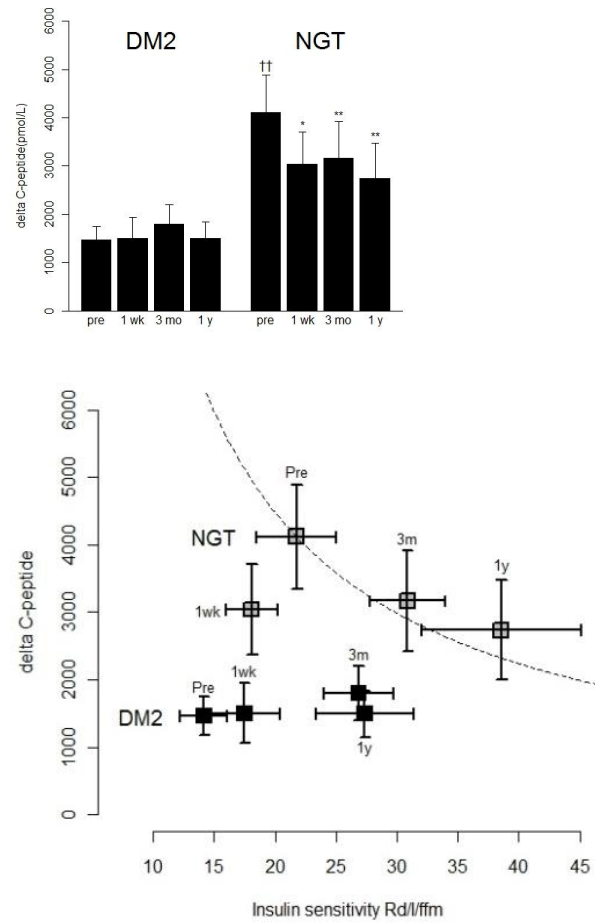
Figure 10 C-peptide response to iv glucose-glucagon test (top) and plottet against insulin sensitivity (Rd/Iffm, bottom) in patients with type 2 diabetes (DM2) and normal glucose tolerance (NGT) before, 1 week, 3 months and 1 year after RYGB. Values are mean+sem.
* p<0.05 **p<0.01 from preoperatively within the group
†† p<0.01 for differences between the groups at a given study session

During hyperglycaemic clamps in subjects with NGT, first and second phase insulin secretion rates were unchanged at 1 week and 3 months after RYGB (3). Insulin secretion rates in response to iv arginine were also unchanged after surgery, although a tendency towards a decline was observed (3). The iv disposition index was unchanged, when adjusting glucose mediated first phase insulin secretion to insulin sensitivity measured by OGIS, while it increased using 1/HOMA-IR at 1 week and 3 months (3).

The time course of insulin secretion after OGTTs changed in patients with NGT after surgery with an increase in peak and reduced time to peak and a more rapid decrease during the last hour of the test, however incremental area under the curve (iAUC) still increased after surgery (1,3). In study (3), insulinogenic index increased significantly by three months, while it was unchanged postoperatively in the patients in study (1). This difference was not related to the use of C-peptide concentrations or ISR, as calculation of insulinogenic index using ISR in study (1) gave a similar result (data not shown). The oral disposition index was calculated from insulinogenic index in both studies, while different measurements of insulin sensitivity (insulin corrected Rd, 1/HOMA-IR and OGIS) were used. Due to the differences in insulinogenic index in the two studies, the oral disposition index

was most markedly increased in study (3), while the increase in study (1) was solely driven by the increased insulin sensitivity. The two study populations of subjects with NGT undergoing RYGB differed with respect to the time-course of the glucose profile after OGTTs (study (1) and (3), respectively); peak glucose increased postoperatively in study (1) (NGT pre: 8.0±0.5 mmol/l, 3 months: 9.7±0.6 p<0.05, 1 year: 10.1±0.7 p<0.05) but not in study (3). Also in the last hour of the test, subjects in study (1) and (3) reacted somewhat differently with lower mean 2 hour plasma glucose in study (3) (study (1) at 3 months: 4.4±0.3 mmol/l vs study (3) at 3 months: 3.4±0.2) and more subjects with hypoglycaemia (% of patients with 2 h P-glucose <3.9 mmol/L: 33% in study (1) vs 80% in study (3)).

3.3.1 Incretin hormones after RYGB: secretion and insulinotropic actions

Secretion of incretin hormones was studied during oral glucose tolerance tests in 10 patients with type 2 diabetes and 10 subjects with NGT before and 3 months and 1 year after RYGB (1). Another 11 glucose tolerant patients were studied with oral glucose tolerance tests before and after 3 months, while the insulinotropic actions of GLP-1 and GIP were studied using 3 hyperglycaemic clamps with co-infusion of the incretin hormones or saline before, 1 week and 3 months after RYGB (3). The insulinotropic actions of GLP-1 and GIP were estimated by dividing the indices of insulin secretion (AIR$_{glu}$, Second phase insulin response, AIR$_{arg}$) from the hormone infusion day with the corresponding indices of the saline day.

Concentrations of GLP-1 or GIP did not differ before surgery between patients with type 2 diabetes and subjects with NGT neither at fasting nor in response to oral glucose (1). After RYGB, concentrations of GLP-1 and GIP were unchanged at fasting in both groups (1,3). Postprandial secretion of GLP-1 increased postoperatively in both groups with 5 fold increased peak concentrations and ~10 fold in-creased incremental AUC (1,3). Postprandial GIP secretion was unchanged after RYGB, although at 1 year peak GIP increased significantly in patients with type 2 diabetes and occurred earlier in subjects with NGT (p<0.01) (figure 11).
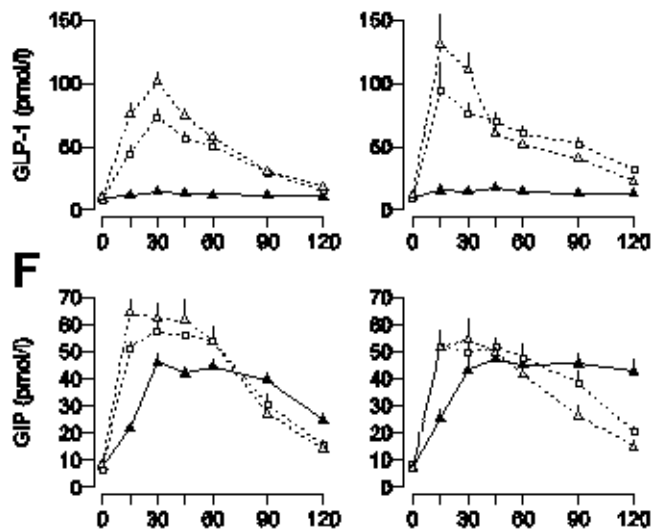

Figure 11 : Plasma total GLP-1 (upper panels) and plasma total GIP (lower panels) in response to oral glucose tolerance test in patients with type 2 diabetes (left) and NGT (right) before (solid line, black triangles), 3 months (dotted line, white squares) and 1 year (dotted line, white triangles) after RYGB. Figure from (1). Values are mean + sem.
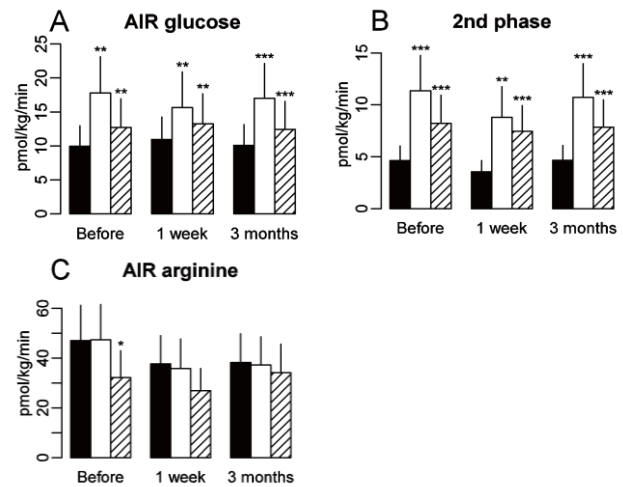

Figure 12 Acute insulin response to glucose (AIR$_{glu}$) (A), second phase insulin response to glucose (2nd phase) (B), acute insulin response to arginine (AIR$_{arg}$) (C) during the hyperglycaemic clamps with co-infusion saline (black bars), glucagon-like peptide-1 (GLP-1) (white bars) or glucose-dependent insulinotropic polypeptide (GIP) (hatched bars) in glucose tolerant subjects before and 1 week and 3 months after Roux-en-Y gastric bypass (RYGB). Overall effect of GLP-1 infusion: P <0.001 (A), P <0.001 (B), P = 0.957 (C). Overall effect of GIP infusion: P = 0.003 (A), P <0.001 (B), P = 0.031 (C). *P <0.05 **P <0.01 ***P <0.001 compared to saline co-infusion.

The insulinotropic action of GLP-1 and GIP was evaluated in glucose tolerant subjects by relating insulin secretion during the hyperglycaemic clamp with saline co-infusion to the insulin secretion during the hyperglycamic clamp with hormone co-infusion (3). Infusion of GLP-1 and GIP increased first and second phase insulin secretion in response to glucose before surgery, and the effect was preserved, but not altered, postoperatively (figure 12). Infusion of GLP-1 and GIP did not significantly alter the insulin secretion response to arginin pre- or postoperatively with the exception of a lowering of AIR$_{arg}$ by GIP before surgery.

3.3.2 Discussion: Beta-cell function and incretin hormones after RYGB

Beta-cell function is improved in response to oral glucose in patients with type 2 diabetes after RYGB, which confirms previous findings (40,45,80,101). At the same time, postprandial GLP-1 secretion is markedly enhanced indicating an association between exaggerated release of GLP-1 and improved beta-cell function. A causal relation has been further stressed in studies using pharmacological blockade of the GLP-1 receptor by infusion of Exendin (9-39), whereby the postoperative increase in insulin secretion could be reduced (45,136). The exaggerated GLP-1 secretion has been related to the changed gastrointestinal anatomy (127,130,131) and a similar response is not seen after diet or weight loss alone (73,77,98); it may therefore result from fast delivery of nutrients to the distal parts of the small intestine (41,137,139), where GLP-1 secreting L-cells are located (140). Secretion of other L-cell hormones are also enhanced after RYGB; peptide YY (PYY) and glucagon-like peptide 2 (GLP-2) (43,141) pointing towards a general stimulation of the L-cells after RYGB. Besides the insulinotropic effect, enhanced GLP-1 in combination with increased PYY may also be important in inducing and keeping weight loss post-RYGB through appetite reducing effects in the central nervous system (42,141,142).

After RYGB, overall postprandial GIP secretion is unchanged in many studies (37,40,42,66,70,90, 93,126), although some have shown increased secretion (80,143) or changes in the secretion pattern with leftward shifting of the GIP curve and/or increased peak (44,77,98,136,137), as was reported in (1). The importance of this finding is unknown, but may also be related to the faster glucose delivery to the intestine (44). K-cells secreting GIP are primarily located in the proximal part of the intestine (39) and therefore likely to be bypassed by nutrients postoperatively, possibly explaining why the overall secretory response is not increased after RYGB, and the divergent results could be a reflection of differences in the length of the bypassed limb (46).

Importantly, the insulinotropic effects of incretin hormones are preserved postoperatively in glucose tolerant subjects, and therefore changes in the secretion of incretin hormones should translate into effects on insulin secretion, which was clearly demonstrated in (3). The finding of unchanged insulinogenic index post-RYGB in glucose tolerant subjects in (1) with concomitant increased GLP-1 secretion is therefore somewhat unexplained, although it has been demonstrated in other studies (40,80). However, unchanged insulinogenic index in the light of increased insulin sensitivity could represent a lack of adaptation and therefore a relative hypersecretion of insulin (as demonstrated by increasing DI_{oral} in the NGT group in (1)). Indeed, subjects with NGT experienced postoperative declines in 2 h postprandial glucose concentration in study (1). Hypoglycaemia was more frequent in study (3), although not symptomatic in any of the studies. To date none have applied GLP-1 receptor antagonism to investigate the importance of GLP-1 mediated insulin release in glucose tolerant subjects before and after RYGB. Pronounced changes in glucose absorption rates (44,55–57) resulting in higher peak glucose in subjects with NGT after RYGB could also change the time course of insulin secretion independently of exaggerated GLP-1 secretion, thereby explaining increased peak and iAUC of C-peptide and unchanged insulinogenic index as seen in study (1).

In patients with type 2 diabetes, studies using GLP-1 receptor antagonism pre- and postoperatively (45) as well as post-RYGB in a cross sectional manner (136,138) have supported the link between exaggerated GLP-1 secretion and increased postprandial insulin secretion after RYGB. However, glucose tolerance is relatively weakly affected by the blockade of the GLP-1 receptor, especially early after meal intake (45,138). This could indicate, that non-insulin mediated glucose uptake (i.e. glucose-effectiveness (144)) could be of importance post-surgery for glucose tolerance. Glucose effectiveness has been shown to contribute considerable to glucose tolerance during meal intake (144) and given the fast and large postprandial glucose excursions after RYGB, it seems likely, that the relative importance of glucose effectiveness could increase postoperatively especially in the early phase of the meal, while insulin-dependent glucose disposal may be of larger importance during the later phase of the meal. Furthermore, it is not known whether the insulinotropic effects of incretin hormones are unchanged in patients with type 2 diabetes postoperatively, and the insulinotropic effects of GLP-1 and especially GIP are likely to improve as glucose levels normalize post-RYGB (37). Reduced glucotoxicity could possibly also increase glucose-mediated insulin secretion (36) and has been the explanation for improvements in first phase insulin secretion observed in response to iv glucose in patients with type 2 diabetes after RYGB, although typically reported after weeks to months (74,80,82,133,134) and not consistently (101).

However, we did not find changes in first phase insulin secretion in patients with type 2 diabetes after iv glucose-glucagon throughout the first postoperative year after RYGB (1). The glucagon test has been extensively used to assess residual beta-cell function in patients with diabetes in clinical and experimental settings, and the C-peptide response has been shown to correlate well with maximal C-peptide levels during more physiological tests as meal tests (145). The combined glucose-glucagon challenge was chosen to minimize the importance of changes in fasting glucose levels post-RYGB, as the C-peptide response to iv glucagon depends on the pre-stimulatory plasma glucose, especially if glucose values are low (146). This influence of fasting plasma glucose on the C-peptide response is minimized using the combined glucose-glucagon challenge (147). However, it is likely, that insulin secretion in response to non-glucose stimuli (e.g. glucagon) is not influenced by glucotoxicity to the same degree as glucose-mediated insulin secretion (148), which could explain the discrepancy between our findings and studies using iv glucose. Changes in insulin secretion in response to iv non-glucose stimuli have not been reported previously in patients with type 2 diabetes after RYGB. However, diet induced weight loss with improved glycaemic control has been shown to increase the C-peptide response to iv glucagon in patients with type 2 diabetes (110), but in that study the combined glucose-glucagon challenge was only applied in the post weight loss protocol in order to compensate for the decrease in fasting hyperglycaemia. Furthermore, results may depend on whether applying the same increment in plasma glucose or reaching the same hyperglycaemic level (118), which may also explain the different results between studies using FSIGT (74,80,82,133,134) and the hyperglycamic clamp (101). Likewise, the same increment in glucose was applied in combination with a fixed bolus of glucagon in study (1), whereas we studied subjects at the same glycaemic level before and after surgery in study(3).

Peripheral insulin sensitivity improved at 3 months and 1 year after RYGB, thus allowing an unchanged insulin secretion to be more effective as underscored by the 2-fold increase in the DI_{iv} in patients with type 2 diabetes at these time-points (1). In contrast, patients with NGT experienced lower C-peptide response to iv glucose-glucagon after surgery, which could represent adaptation to peripheral insulin sensitivity resulting in unchanged DI_{iv} (1). In response to oral glucose in subjects with NGT, insulinogenic index did not adapt to the improved insulin sensitivity, and the oral disposition index increased (1,3), which may explain the reduced postprandial glucose concentration and increased risk of hypoglycaemia, as previously discussed. It should, however, be noted, that low plasma glucose levels in the postprandial period are frequently seen after RYGB, but severe symptomatic hypoglycaemia is rare and usually develops years after RYGB (149). Inappropiately enhanced GLP-1 action is likely to be involved in the development of hypoglycaemia after RYGB (21,132,136,143), but whether other factors contribute to the development of symptomatic hypoglycaemia remain unresolved.

Taken together, we did not find evidence of post-RYGB improvements in beta-cell function per se, as insulin secretion after iv stimulation was unchanged postoperatively in patients with type 2 diabetes and even declined in glucose tolerant subjects after iv glucose glucagon. Furthermore, the insulinotropic effects of iv infused GLP-1 and GIP were unchanged after RYGB in glucose tolerant subjects. In contrast, relative increases in insulin secretion after oral glucose exceeded changes after iv stimulation in both groups, highlighting the importance of changes in gut anat-

omy and increased GLP-1 secretion for increased postprandial insulin secretion after RYGB.

3.4 ALPHA-CELL FUNCTION AFTER RYGB (STUDY 1 AND 3)
Glucagon secretion was investigated in 10 patients with type 2 diabetes and 10 subjects with NGT with oral glucose tolerance tests before, 3 months and 1 year after surgery (1) as well as in another 11 glucose tolerant patients before and after 3 months (3). Suppression of glucagon during hyperglycaemic clamps was studied in 11 glucose tolerant subjects with co-infusion of GLP-1, GIP or saline before, 1 week and 3 months after RYGB (3), while glucagon suppression in response to hyperinsulinaemic euglycaemic clamps was studied in 10 patient with type 2 diabetes and 10 subjects with NGT before, at 1 week, 3months and 1 year after RYGB (1). Glucagon suppression was calculated as the difference between basal and end-clamp levels expressed as a percentage of the basal level in both studies. The glucagon response to arginin (AGR$_{arg}$; maximal increment in glucagon from pre-injection glucagon within 5 min) was studied in 11 glucose tolerant subjects during co-infusion of GLP-1, GIP or saline before, 1 week and 3 months after RYGB (3).

Glucagon in the basal period was similar preoperatively between groups (1). Nevertheless, basal glucagon was inappropriately high in patients with type 2 diabetes before RYGB given the concomitant fasting hyperglycemia. Furthermore, during the hyperinsulinaemic clamp, glucagon was more suppressed in glucose tolerant subjects than in patients with type 2 diabetes (1). Thus, alpha-cell dysfunction was present preoperatively in patients with type 2 diabetes.
Basal concentrations of glucagon decreased in patients with type 2 diabetes at 3 months and 1 year after RYGB, but were largely unchanged in subjects with NGT, except for a marked increase at 1 week postoperatively (1,3). The basal C-peptide to glucagon ratio decreased postoperatively in subjects with NGT, while it was largely unchanged in patients with type 2 diabetes after RYGB (1), (1) thus not explaining the changes in basal glucose production. Before surgery, glucagon secretion was suppressed in response to oral glucose in patients with type 2 diabetes and NGT, while the response was reversed after surgery in both groups, i.e. incremental AUC changed from negative preoperative values to positive postoperative values (1,3). In response to iv arginin, glucagon secretion increased transiently at 1 week after surgery, but returned to preoperative values by 3 months (3).

In contrast, glucagon suppression was unchanged postoperatively in response to iv insulin- and glucose-infusion during the hyperinsulinaemic clamp (1) and was slightly higher post-operatively in response to iv glucose-infusion during the hyperglycaemic clamp (3). Before RYGB, glucagon suppression was greater on the days with GLP-1 and GIP than during saline co-infusion (3). Postoperatively, no significant changes in glucagonostatic effect of the incretin hormones were observed (i.e. main effect of time was insignificant), although suppression of glucagon was only significantly affected postoperatively during infusion of GIP, and not GLP-1 (3). Infusion of GLP-1 or GIP did not affect the glucagon response to iv arginin pre- or postoperatively (3).

3.4.1 Discussion: Alpha-cell function after RYGB
Fasting glucagon declined in patients with type 2 diabetes (1) after 3 months and 1 year, which has been reported previously after improving glycaemic control in response to diet-induced

weight loss (94) and after RYGB (40). At these time-points, the lower glucagon could contribute to the lower basal hepatic glucose production, although not reflected in the basal C-peptide to glucagon ratio. In contrast, fasting glucagon did not decline at 1 week after surgery and even increased in subjects with NGT (1,3), thus obviously not mediating the early effect of RYGB on basal hepatic glucose production or fasting glucose. Short term calorie restriction does not change glucagon levels (94,96), but increases are seen after days of fasting (150). The transient increase in glucagon secretion in response to iv arginine at 1 week could also reflect the raised glucagon level at this time point. Interestingly, the response to iv glucose and/or insulin was preserved postoperatively even at 1 week in NGT.

We could confirm previous findings of increased postprandial glucagon secretion after RYGB in both patients with type 2 diabetes and NGT (1,3) at 3 months and 1 year. This is not a typical response to weight loss (94) and has furthermore been shown as early as 1 week after surgery (40). Paradoxically the increased glucagon occurs within 1 hour of nutrient intake, where levels of glucose, insulin and GLP-1 are high. In some studies with longer postprandial blood sampling, the postoperative glucagon response has been biphasic with an additional peak occurring later after meal intake, where glucose levels are low and thus possibly serving as a counter-regulatory response to postprandial hypoglycaemia indicating intact alpha-cell responsiveness to low glucose concentrations (43,44). However, none have assessed the counter-regulatory response during controlled hypoglycaemia, e.g. clamp, which could be interesting given the occurrence of postprandial hypoglycaemia after RYGB. We have shown preserved glucagon suppression during hyperglycaemic and hyperinsulinaemic clamps after RYGB confirming intact alpha-cell responsiveness to iv glucose and/or insulin (1,3); a finding not previously reported in a prospective design (136). Furthermore, the inhibitory effect of GLP-1 on glucagon secretion seems to be present after surgery, also supported by the finding of increased glucagon release during GLP-1 receptor blockade (45).
A glucagonotropic action of GIP has been suggested as an explanation for the early postprandial increase in glucagon after RYGB (151), however this is not supported by our findings, where GIP displayed glucagonostatic actions both before and after surgery(3). Increased postprandial secretion of GLP-2 after surgery (43) has also been proposed as mediator of the exaggerated release of glucagon (152). Alternatively, the excess plasma glucagon levels could originate from the L-cells as a byproduct of the exaggerated postoperative hormone release, perhaps reflecting biologically inactive proglucagon forms that interfere with glucagon assays (137). Further studies investigating the source and potential impact of increased postprandial glucagon secretion after RYGB would be of major interest, but changes in circulating glucagon are not likely to contribute positively to the improved glycaemic control in patients with type 2 diabetes after RYGB.

3.5 SUMMARY: ISLET-CELL FUNCTION AFTER RYGB
Beta-cell function was significantly reduced in patients with type 2 diabetes compared to normal glucose tolerant subjects in response to iv glucose glucagon and oral glucose both before and after RYGB. Insulin secretion increased postoperatively in patients with type 2 diabetes in response to oral glucose, whereas insulin secretion was unchanged in response to the iv glucose-glucagon tests throughout the first postoperative year. In subjects with normal glucose tolerance, the insulin secretion response after iv

glucose glucagon declined after surgery likely as an adaptation to increased insulin sensitivity, whereas insulin secretion during the hyperglycaemic clamps and in response to iv arginine was unchanged. In response to oral glucose, insulinogenic index was either unchanged (1) or increased postoperatively in subjects with normal glucose tolerance (3). GLP-1 secretion increased substantially in both groups in response to oral glucose, whereas GIP secretion was largely unchanged postoperatively. The insulinotropic effects of the incretin hormones were unchanged after surgery during intravenous infusion in glucose tolerant subjects. Increased insulin secretion was thus linked to the oral and not the intravenous route of administration, highlighting the importance of the changed gastrointestinal anatomy and the exaggerated GLP-1 secretion and not supporting major changes in intrinsic beta-cell function after RYGB.

Preoperatively, patients with type 2 diabetes had inappropriately high fasting glucagonaemia given the level of hyperglycaemia and moreover had lower suppression of glucagon during the hyperinsulinaemic clamp. Fasting glucagon decreased in patients with type 2 diabetes after 3 months and 1 year, while levels were unchanged in subjects with NGT except for at transient increase at 1 week. Glucagon suppression during hyperinsulinaemic euglycaemic conditions and hyperglycaemic conditions were unchanged after surgery, while glucagon secretion during the oral glucose tolerance tests was paradoxically increased after surgery in both patients with type 2 diabetes and normal glucose tolerance. Preserved glucagonostatic effects of the incretin hormones were observed during iv infusion of the hormones in glucose tolerant subjects after surgery. In conclusion, changes in alpha-cell function are not likely to contribute positively to the improved glycaemic control in patients with type 2 diabetes after RYGB.

4. CONCLUSION AND PERSPECTIVES
4.1 CONCLUSION
Roux-en-Y gastric bypass improves glycaemic control in patients with type 2 diabetes, and the glucose-lowering effect of surgery is superior to conventional antidiabetic treatment (28–30). We studied patients with type 2 diabetes and glucose tolerant subjects prior to and throughout the first year after RYGB in order to elucidate the physiological mechanisms responsible for the improved glycaemic control.

Already at 1 week after surgery, basal glucose production and hepatic insulin sensitivity had improved in patients with type 2 diabetes as well as in glucose tolerant subjects. Insulin clearance also increased at 1 week postoperatively in both groups at fasting and during exogenous infusion of insulin, whereas postprandial insulin clearance during a liquid meal only increased in patients with type 2 diabetes. As insulin predominantly is cleared by the liver, this could indicate a common mechanism responsible for the early improvements in hepatic insulin sensitivity and insulin clearance. The improvements in hepatic insulin sensitivity and clearance were sustained or further improved at 3 months and 1 year after RYGB; only glucose production in glucose tolerant subjects had returned to preoperative values.

Peripheral glucose disposal was unchanged at 1 week postoperatively in both groups, whereas the concentration of fatty acids increased at fasting as well as during insulin infusion in patients with type 2 diabetes. At 3 months and 1 year after RYGB, glucose

disposal as well as suppression of fatty acids and glycerol by insulin had improved, which was likely caused by the weight loss as demonstrated by a significant correlation between weight loss and changes in glucose disposal by 1 year.

Taken together, we have shown, that RYGB has a differential effect on hepatic and peripheral insulin sensitivity in the early postoperative period with early improvements in hepatic and later weight loss dependent improvements in peripheral insulin sensitivity. A similar response with rapid improvements in hepatic insulin sensitivity is a common observation after calorie restriction in obese patients with type 2 diabetes (90,93–96) and normal glucose tolerance (72) and has been observed as early as after 48 hours (72,96) in absence of major weight loss and changes in peripheral insulin sensitivity (90,93,96). Thus, calorie restriction is a likely explanation for our findings of early improvement in hepatic insulin sensitivity and clearance after RYGB.

Beta-cell function increased after RYGB in patients with type 2 diabetes in response to oral glucose, whereas insulin secretion was unchanged in response to an iv glucose-glucagon test throughout the first year after surgery. In glucose tolerant subjects, the insulin response to iv glucose-glucagon declined after surgery likely as an adaptation to increased insulin sensitivity, whereas insulin secretion during hyperglycaemic clamps and in response to iv arginine was unchanged. In glucose tolerant subjects, insulinogenic index was either unchanged or increased postoperatively in response to oral glucose. GLP-1 secretion increased substantially in both groups in response to oral glucose, whereas GIP secretion was largely unchanged postoperatively. The insulinotropic effects of the incretin hormones were preserved after surgery during intravenous infusion in glucose tolerant subjects. Increased insulin secretion was thus linked to the oral and not the intravenous rote of administration highlighting the importance of the changed gastrointestinal anatomy and the exaggerated GLP-1 secretion and not supporting major changes in intrinsic beta-cell function after RYGB.

Changes in alpha-cell function did not seem to contribute substantially to the improved glycaemic control after RYGB, as glucagon secretion increased paradoxically after oral glucose, while suppression of glucagon in response to iv infusions of glucose, GIP, GLP-1 and insulin was largely unchanged postoperatively. The origin and importance of excess postprandial glucagon remain unexplained, but could be linked to an excess stimulation of the L-cells after RYGB leading to exaggerated release of gut-derived glucagon.

In conclusion, improved glycaemic control after Roux-en-Y gastric bypass can be explained by early enhancements of hepatic insulin sensitivity and later improvements in peripheral insulin sensitivity in combination with increased postprandial insulin secretion linked to exaggerated postprandial GLP-1 secretion.

4.2 PERSPECTIVES
Roux-en-Y gastric bypass is at the moment one of the most efficient treatments of type 2 diabetes in obese patients and is superior to pharmacological treatment (28–30). Gaining knowledge of the physiological mechanisms responsible for this dramatic effect on glycaemic control offer a unique opportunity to provide a further understanding of the pathophysiology of type 2 diabetes in order to identify new targets for or optimize pharmocological and/or surgical treatment.

The rapid correction of fasting hyperglycaemia after RYGB is a phenomenon that has received much attention (31). We have shown that decreased hepatic glucose production could contribute to this rapid improvement in fasting glucose metabolism. Although calorie restriction has not yet been proven to be the only explanation for the improved hepatic insulin sensitivity, it seems reasonable to conclude that it is of major importance. It is perhaps not surprising, that calorie restriction is efficient at treating type 2 diabetes, but what remains intriguing is the fact that RYGB patients seem to sustain the metabolic improvement for long term. Although changes in fasting glucose and glucose production were impressive after very low calorie diet in the study by Lim et al. (2011), improvements were only sustained for 8 weeks and after another 12 weeks, patients had gained weight and fasting plasma glucose had increased (90). In contrast, RYGB induces weight loss for up to 12-18 months after surgery and leads to weight maintenance over 15-20 years thereafter (7).

Physiological factors involved in appetite and weight control after RYGB are therefore important to study as neither food restriction nor malabsorption seem to be of major importance (41,139). Of particular interest is the increased secretion of L-cell hormones (especially GLP-1 and PYY) as potential mediators of decreased appetite after RYGB (42,141). Further studies are required to investigate the role of gut hormones in weight reduction and weight maintenance after RYGB to uncover the potential for targeting these hormones individually or in combination as a future treatment of obesity. In that aspect, treatment with GLP-1R agonists has been shown to induce mean weight loss of 2-4 kg (153); but still far from the weight loss seen after RYGB. An important distinction between the exogenous administration of a GLP-1R agonist and the physiology after RYGB could be the stimulation of endogenous GLP-1 postoperatively. Exaggerated release of endogenous GLP-1 is likely to exhibit effects not related to the resulting level of circulating hormone, but local actions in the gut or in the portal vein by stimulating GLP-1 receptors on vagal sensory afferents neurons could affect appetite regulation (142) as well as insulin secretion as demonstrated in rodents (154,155). Notably, an important lesson learned from RYGB is the great capacity of increasing endogenous gut hormone secretion even in patients with type 2 diabetes. Thus enhanced endogenous secretion of gut hormones could be a promising drug target, if the precise mechanisms behind the exaggerated release are elucidated. Further studies are therefore required to identify the optimal stimulus as well as the enteroluminal receptors involved in the increased gut hormone secretion after RYGB. Furthermore, it may be of importance to investigate the interplay of different hormones in controlling appetite and glucose metabolism, as RYGB is characterized by simultaneous changes in insulin, glucagon, GLP-1, PYY, CCK and ghrelin (42,43) with potential synergistic effects (142).

A further understanding of the mechanisms involved in appetite reduction and improved glycaemic control after RYGB could also lead to improvements in surgical techniques or new less invasive procedures. In that aspect, it is interesting that the gastric sleeve operation, involving resection of large parts of the stomach creating a tube but without redirection of nutrients, seems to elicit a gut hormone response resembling the response after RYGB (151). Studies on surgical complications and long term effects on weight and glycaemic control after gastric sleeve compared to RYGB will be informative in order to identify the best procedure. Another possibility is to optimize the selection of patients to surgery in terms of choosing patients, who are more likely to lose weight and/or to improve/resolve type 2 diabetes. Identifying factors capable of predicting the response to surgery would be beneficial in order to avoid operation and the potential risk of complications in patients not likely to benefit from surgery. In that respect, patients with type 2 diabetes are a heterogeneous population with different degrees of insulin resistance and beta-cell dysfunction, and perhaps patients with severe hepatic and peripheral insulin resistance but only minor beta-cell dysfunction could be more likely to benefit from RYGB. The heterogeneous spectrum of type 2 diabetes is also important to consider when treating persistent type 2 diabetes after RYGB, but also postoperative changes must be considered: Is metformin the best antidiabetic drug post-RYGB given the large improvements in hepatic insulin sensitivity after surgery? Or could DPP-IV inhibitors be more efficient in the light of the exaggerated GLP-1 secretion postoperatively?

In conclusion, the metabolic changes after RYGB remain to be an inspiration for new intervention strategies in the treatment of type 2 diabetes and obesity.

LIST OF ABBREVIATIONS
AGR: Acute glucagon response, AIR: Acute insulin response, AUC: Area under the curve, BPD: Bilio-pancreatic diversion, CCK: cholecystokinin, DI: Disposition index, DM2: type 2 diabetes mellitus, DPP-IV: Dipeptidyl peptidase 4, EBL: Excess BMI loss, EGP: Endogenous glucose production, FA: Fatty acids, FSIGT: Frequently sampled intravenous glucose test, GIP: Glucose-dependent insulinotropic polypeptide, GLP-1: Glucagon-like peptide-1, GLP-2: Glucagon-like peptide-2, HGP: hepatic glucose production, HISI: Hepatic insulin sensitivity index, HOMA-IR: Homeostatis model assessment of insulin resistance, IGI: Insulinogenic index, ISR: Insulin secretion rate, MR: Magnetic resonance, NGT: Normal glucose tolerance, OGTT: Oral glucose tolerance test, PYY: Peptide YY, Ra: Rate of appearance, Rd: Rate of disappearance, RYGB: Roux-en-Y gastric bypass, T2D: Type 2 diabetes

SUMMARY
Roux-en-Y gastric bypass (RYGB) surgery induces weight loss of 20-30% that is maintained for 20 years. In patients with type 2 diabetes, the glucose-lowering effect of RYGB is superior to conventional antidiabetic therapy and often occurs within days after surgery. The aim of the thesis was to investigate the physiological mechanisms responsible for improved glycaemic control with special focus on the early postoperative period. We therefore investigated insulin sensitivity, insulin clearance and pancreatic islet-cell function in patients with type 2 diabetes and in glucose tolerant subjects prior to and at 1 week, 3 months and 1 year after RYGB.

Hepatic insulin sensitivity measured with a glucose tracer increased already 1 week after RYGB, whereas peripheral insulin sensitivity estimated with the hyperinsulinaemic euglycaemic clamp was unchanged. Concomitant increases in insulin clearance at 1 week further highlights the liver as an important organ responsible for the early effects on glucose metabolism after surgery since insulin predominantly is cleared by the liver. Rapid improvements in hepatic insulin sensitivity is a common observation after calorie restriction in obese patients and has been observed as early as after 48 hours in absence of major weight loss and changes in peripheral insulin sensitivity. Thus, calorie restriction is a likely explanation for our findings of early improve-

ments in hepatic insulin sensitivity and insulin clearance after RYGB. Peripheral insulin sensitivity increased along with weight loss at 3 months and 1 year after RYGB.

Beta-cell function increased after RYGB in patients with type 2 diabetes in response to oral glucose, whereas insulin secretion was unchanged in response to an intravenous (iv) glucose-glucagon test throughout the first year after surgery. In glucose tolerant subjects, the insulin response to iv glucose-glucagon declined after RYGB likely as an adaptation to increased insulin sensitivity. The secretion of glucagon-like peptide 1 (GLP-1) increased substantially in both groups in response to oral glucose, whereas the secretion of glucose-dependent insulinotropic polypeptide (GIP) was largely unchanged postoperatively. The insulinotropic effects of the incretin hormones were preserved after surgery during iv infusion in glucose tolerant subjects. Increased insulin secretion postoperatively was thus linked to the oral and not the iv rote of administration highlighting the importance of the changed gastrointestinal anatomy and the exaggerated GLP-1 secretion and not supporting major changes in intrinsic beta-cell function after RYGB. Changes in alpha-cell function did not seem to contribute substantially to the improved glycaemic control after RYGB, as glucagon secretion increased paradoxically after oral glucose, and suppression of glucagon in response to iv infusions of glucose, GIP, GLP-1 and insulin was largely unchanged postoperatively.

In conclusion, improved glycaemic control after Roux-en-Y gastric bypass can be explained by early enhancements of hepatic insulin sensitivity and later improvements in peripheral insulin sensitivity in combination with increased postprandial insulin secretion linked to exaggerated postprandial GLP-1 secretion. Surgical changes in gut anatomy are likely to explain the increased GLP-1 secretion and hence the increased postprandial insulin secretion, whereas calorie restriction and subsequent weight loss may be the major cause of improved insulin sensitivity.

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