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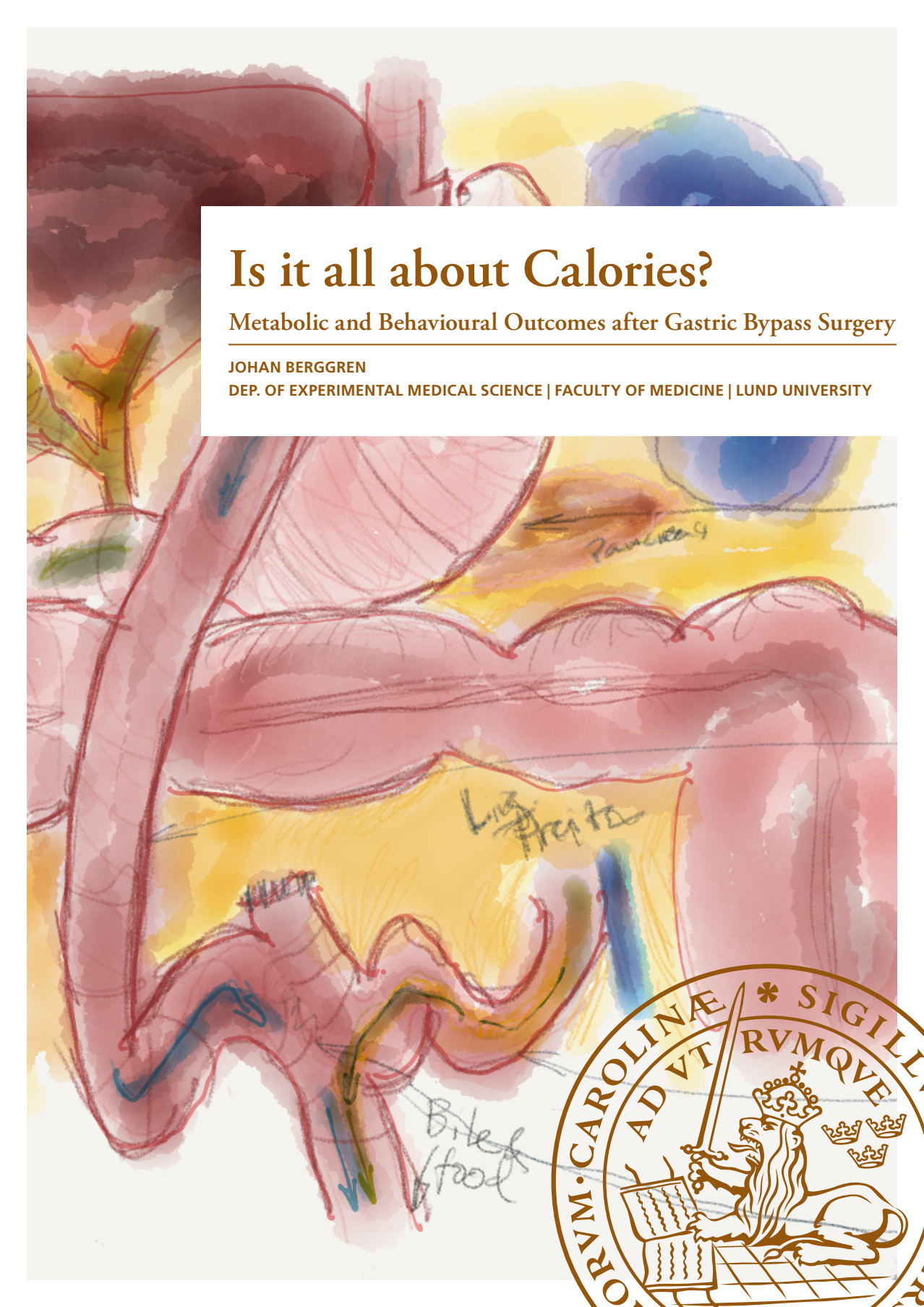
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Is it all about Calories?

Metabolic and Behavioural Outcomes after Gastric Bypass Surgery

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DEP. OF EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY



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Is it all about Calories?

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Johan Berggren



LUND
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DOCTORAL DISSERTATION

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Abstract:

Background: Roux-en-Y gastric bypass (RYGB) is the most effective treatment for severe obesity and often induces remission of type 2 diabetes (T2D) before significant weight loss, but whether this reflects the surgery itself or the accompanying caloric restriction is unclear. Increased alcohol consumption after surgery has been reported, mainly from self-report data.

Aims: To separate surgery-specific effects from those of caloric restriction after RYGB by examining (I) insulin and incretin responses, (II) metabolomic and lipidomic changes and (III) proneurotensin (pNT), and (IV) to quantify long-term alcohol consumption using phosphatidylethanol (PEth).

Methods: Papers I and II used standardised mixed-meal tests in 19 women with severe obesity (9 normoglycaemic, 10 with T2D) before and after a very-low-calorie diet (VLCD), on postoperative day 1, and 6 weeks after RYGB. In Paper III we measured pNT in three cohorts spanning VLCD, RYGB or sleeve gastrectomy (SG), and one year postoperatively. In Paper IV we followed 1,238 patients with PEth measurements up to 10 years after RYGB or SG.

Results: Caloric restriction had limited effects on insulin and incretins, whereas RYGB immediately increased insulin and GIP, with a marked rise in GLP-1 at 6 weeks (Paper I). The metabolomic pattern at 6 weeks largely resembled that after VLCD (Paper II). Fasting pNT fell acutely after both VLCD and RYGB but was elevated from 6 weeks to one year after surgery, and a postprandial pNT response emerged that GLP-1 and GIP infusion did not reproduce (Paper III). At 5 years, 37.5% of men exceeded the heavy drinking threshold versus 4.8% of women, a pattern that persisted at 10 years (Paper IV).

Conclusions: RYGB exerts immediate effects on insulin and incretin responses independent of caloric restriction, whereas the broader metabolomic changes are largely diet-driven. Proneurotensin shows a distinct postoperative pattern not secondary to incretin signalling. A substantial proportion of patients, particularly men, develop sustained heavy drinking, supporting PEth screening in follow-up.

Key words: Gastric bypass, Bariatric surgery, Caloric restriction, Incretins, Metabolomics, Neurotensin, Phosphatidylethanol, Alcohol drinking, Type 2 diabetes

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Is it all about Calories?

Metabolic and Behavioural Outcomes after Gastric Bypass Surgery

Johan Berggren



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*” The constancy of the internal environment is
the condition for a free and independent life.”*

Claude Bernard, *Leçons sur les phénomènes de la vie*, 1878

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Abstract

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List of Papers

Paper I

Berggren J, Lindqvist A, Hedenbro J, Groop L, Wierup N. Roux-en-Y gastric bypass versus calorie restriction: support for surgery per se as the direct contributor to altered responses of insulin and incretins to a mixed meal. *Surg Obes Relat Dis*. 2017 Feb;13(2):234-242. doi: 10.1016/j.soard.2016.09.017. Epub 2016 Sep 28. PMID: 27894746.

Paper II

Herzog K*, Berggren J*, Al Majdoub M, Balderas Arroyo C, Lindqvist A, Hedenbro J, Groop L, Wierup N, Spéjel P. Metabolic Effects of Gastric Bypass Surgery: Is It All About Calories? *Diabetes*. 2020 Sep;69(9):2027-2035. doi: 10.2337/db20-0131. Epub 2020 Jun 11. PMID: 32527768.*Equal contribution

Paper III

Miskelly MG, Berggren J, Svensson M, Koffert J, Honka H, Kauhanen S, Nuutila P, Hedenbro J, Lindqvist A, Melander O, Wierup N. The Effects of Calorie Restriction and Bariatric Surgery on Circulating Proneurotensin Levels. *J Clin Endocrinol Metab*. 2025 Jan 21;110(2):e497-e505. doi: 10.1210/clinem/dgae147. PMID: 38477483.

Paper IV

Berggren J, Lindqvist A, Isaksson A, Hedenbro J, Wierup N. Alcohol consumption patterns up to 10 years after bariatric surgery: using the highly specific biomarker phosphatidylethanol. *Surg Obes Relat Dis*. 2026 Jun;22(6):619-627. doi: 10.1016/j.soard.2026.02.011. Epub 2026 Feb 19. PMID: 41862348.

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
AUD	Alcohol use disorder
AUDIT	Alcohol Use Disorders Identification Test
BMI	Body mass index
BPD	Biliopancreatic diversion
BPD/DS	Biliopancreatic diversion with duodenal switch
CCK	Cholecystokinin
CI	Confidence interval
DALY	Disability-adjusted life year
DPP-4	Dipeptidyl peptidase-4
ELISA	Enzyme-linked immunosorbent assay
FDR	False discovery rate
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FXR	Farnesoid X receptor
GBD	Global Burden of Disease
GHSR	Growth hormone secretagogue receptor 1a
GIGD	Gastrointestinal-mediated glucose disposal
GIP	Glucose-dependent insulintropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1RA	GLP-1 receptor agonist
HbA1c	Glycated haemoglobin
HEC	Euglycaemic–hyperinsulinaemic clamp
HOMA-IR	Homeostasis model assessment of insulin resistance
HOMA- β	Homeostasis model assessment of β -cell function
IFSO	International Federation for the Surgery of Obesity and Metabolic Disorders
IGI	Insulinogenic index
IR	Insulin resistance
MetS	Metabolic syndrome
MHO	Metabolically healthy obesity
MMT	Mixed-meal test
NG	Normoglycaemic
NIH	National Institutes of Health

NT	Neurotensin
NTSR1	Neurotensin receptor 1
OGTT	Oral glucose tolerance test
OR	Odds ratio
OXM	Oxyntomodulin
PCA	Principal component analysis
PEth	Phosphatidylethanol
pNT	Proneurotensin
PYY	Peptide YY
RYGB	Roux-en-Y gastric bypass
SG	Sleeve gastrectomy
SOReg	Scandinavian Obesity Surgery Registry
T2D	Type 2 Diabetes
TBWL	Total body weight loss
UPLC–QTOF-MS	Ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry
VLCD	Very-low-calorie diet

Introduction

Obesity

Surgery for obesity has been a mainstay of treatments of obesity in the recent decades. The surgical techniques have been evolving both in terms of the effects on obesity itself but also, and perhaps equally importantly, their metabolic effects.

Obesity is defined, by the WHO, as the abnormal or excessive fat accumulation that presents a risk to health. Body mass index (BMI), calculated as weight in kilograms divided by height in metres squared is the most common measure to classify overweight and obesity. For western populations, several authorities define a BMI of 18.5-25 kg/m² as healthy weight, 25-30 kg/m² as overweight and BMI > 30 kg/m² as obesity, although more detailed classifications are also used (1). Although BMI is a generally used screening instrument it is not without its caveats. It does not differentiate between fat and lean mass or account for differences in body fat distribution. It can underestimate fat mass in elderly and in certain ethnic groups, while overestimating it in people with greater bone or skeletal muscle mass, such as athletes (2, 3).

Obesity is defined by the WHO and other authorities as a disease, or at least a precursor of disease. Anthropometric measures other than BMI, such as waist-to-hip ratio or waist circumference, may better capture the fat distribution relevant to risk stratification. Visceral adiposity is closely associated with metabolic risk and mortality, whereas lower-body and subcutaneous fat depots appear to confer less risk (4, 5).

Anthropometric measures can predict metabolic risk but do not in themselves indicate ongoing obesity-related organ dysfunction.

Although obesity is recognised as a disease, it can also be argued that it causes illness only when another condition, with its own pathophysiology, develops. From this perspective, obesity would not be considered an illness in its own right.

Despite this controversy, clear evidence shows that obesity increases the likelihood of numerous diseases, including type 2 diabetes, cancer, and cardiovascular disease (6-8). These conditions share pathophysiological features with obesity but also have their own clinical manifestations and evolution.

Epidemiology

The number of overweight and obese people in the world has risen during the late 20th century and the early 21st century. Between 1990 and 2021 the global prevalence of obesity has increased 150 % in males and 105 % in females, with the sharpest increase in North Africa and Middle East. No country to date has been able to halt the rising rates of adult overweight and obesity. In 2025, the “GBD adult BMI collaborators”, through data from the Global Burden of Disease (GBD), published a prevalence estimate and forecast to 2050 (9). They predict that nearly two thirds of adults over 25 will have overweight and obesity. As of 2021 the highest prevalence of overweight and obesity is observed in north Africa, Middle East, Oceania and in the United States, with some countries reaching almost 90 %. Among high-income countries, the United States had the highest prevalence of obesity (BMI ≥ 30 kg/m²), estimated at 41.5% in men and 45.6% in women. In Sweden, the prevalence of excess weight has risen steadily over the past two decades. In 2024, 58% of men and 48% of women had overweight or obesity. Obesity alone affected 18.0% of men and 18.4% of women, up from 10.8% and 11.2% in 2004. Overall, overweight is more common in men, whereas the prevalence of obesity is similar between the sexes (9-11).

Health implications of obesity

Overweight and obesity are major contributors to the global disease burden. In 2021, high BMI accounted for approximately 129 million disability-adjusted life years (DALYs), ranking it among the leading risk factors worldwide (12).

Obesity is associated with an increased risk of numerous diseases, many of which are linked to metabolic syndrome (MetS), while others are directly related to excess adiposity. Since the majority of individuals with obesity display components of MetS, it remains challenging to isolate the risks attributable solely to obesity. Nonetheless, a subgroup identified as having “metabolically healthy obesity” (MHO), characterised by the absence of typical metabolic risk factors, has been recognised. Despite this, meta-analyses demonstrate that individuals with MHO still possess a heightened risk for several conditions commonly associated with MetS, including cardiovascular disease and certain cancers (13, 14). These findings suggest that obesity should be addressed clinically irrespective of the presence of MetS-associated risk factors and may warrant a reassessment of criteria for bariatric intervention.

Metabolic syndrome

Metabolic syndrome (MetS) is a cluster of interrelated cardiometabolic abnormalities that increase the risk of cardiovascular disease, stroke, type 2 diabetes, chronic kidney disease, and premature mortality (15). Diagnosis requires any three of the following: 1) increased waist circumference (using population-specific cut-offs); 2) triglycerides ≥ 1.7 mmol/L (150 mg/dL) or drug treatment; 3) low HDL cholesterol, defined as < 1.0 mmol/L (40 mg/dL) in men or < 1.3 mmol/L (50 mg/dL) in women, or treatment; 4) blood pressure $\geq 130/85$ mmHg or antihypertensive treatment; and 5) fasting plasma glucose ≥ 5.6 mmol/L (100 mg/dL) or treatment for hyperglycaemia (16).

Although five criteria are used, visceral adiposity appears to be the principal driver of MetS. Its progression is modifiable, and often reversible, through targeted interventions (16, 17). Core pathophysiological components include insulin resistance, adipose-tissue dysfunction, and chronic low-grade inflammation (18). Normally, a rise in plasma glucose triggers insulin secretion from β -cells, promoting cellular glucose uptake through glucose transporters. In insulin resistance, peripheral tissues respond less effectively to insulin, leading to higher glucose concentrations and compensatory hyperinsulinaemia. Over time, β -cell dysfunction and impaired insulin signalling disrupt glucose metabolism, favour ectopic fat deposition, promote cardiometabolic toxicity, and sustain inflammation, the hallmark features of MetS (19-21).

Insulin resistance

Insulin resistance describes a state in which the biological effects of insulin are attenuated despite normal or elevated circulating concentrations. Although often discussed as a single entity, its manifestations are organ-specific: in the liver, impaired suppression of glucose production drives fasting hyperglycaemia. In skeletal muscle, reduced glucose uptake limits postprandial glucose disposal, and in adipose tissue, insufficient suppression of lipolysis increases circulating free fatty acids which propagate insulin resistance across tissues (22-24).

The causes of insulin resistance remain incompletely understood, and no single theory offers a full explanation. Several overlapping mechanisms have been proposed, including ectopic lipid accumulation and lipotoxicity in liver and muscle (25, 26), adipose tissue dysfunction with chronic low-grade inflammation (21), mitochondrial and endoplasmic reticulum stress under conditions of nutrient excess (27, 28), and alterations in gut microbiota composition and intestinal barrier function (29). Each of these is supported by experimental and clinical data, but none is sufficient in isolation. Current evidence favours a view of insulin resistance as an emergent property of converging perturbations in lipid handling, immune signalling,

and nutrient sensing in genetically and environmentally susceptible individuals, rather than the result of a single unifying defect (22-24).

From a clinical perspective, insulin resistance is central to the progression from obesity to type 2 diabetes and metabolic syndrome. It is also a key outcome measure in evaluating the metabolic effects of bariatric surgery, since improvements in insulin sensitivity are observed early after Roux-en-Y gastric bypass (RYGB), in some cases before significant weight loss has occurred. This raises the question of whether these improvements are driven by the surgery itself or by the accompanying caloric restriction.

Measurement of insulin resistance

The euglycaemic–hyperinsulinaemic clamp (HEC) involves infusing insulin to achieve a fixed high concentration and subsequently titrating glucose to maintain normoglycaemia. The glucose infusion rate (the “M value”) quantifies whole-body insulin sensitivity. This is the research gold standard for measuring insulin resistance (30).

The Matsuda (composite) index estimates whole-body or peripheral insulin sensitivity, with higher values indicating greater sensitivity.

The index has been validated against the clamp using data from an oral glucose tolerance test (OGTT), with samples commonly obtained at 0, 30, 60, and 120 minutes. It is calculated from fasting and mean glucose and insulin concentrations as follows: $10,000 / \sqrt{[(\text{fasting plasma glucose} \times \text{fasting plasma insulin}) \times (\text{mean OGTT glucose} \times \text{mean OGTT insulin})]}$, with glucose expressed in mg/dL and insulin in $\mu\text{U/mL}$ (31).

HOMA-IR, which primarily reflects hepatic insulin resistance, estimates the insulin required to maintain the fasting glucose concentration. It is calculated as $\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/L)} / 22.5$, with higher values indicating greater insulin resistance (32).

Neither the Matsuda index nor HOMA-IR is generally recommended for individual clinical decision-making because of limited validation and reproducibility for this purpose (33).

Type 2 Diabetes

Type 2 diabetes (T2D) is a chronic disorder of glucose homeostasis characterised by sustained hyperglycaemia arising from the combination of insulin resistance and inadequate insulin secretion. Diagnosis is based on an elevated fasting plasma

glucose concentration (≥ 7.0 mmol/L), an elevated two-hour value during an oral glucose tolerance test (≥ 11.1 mmol/L), or an elevated glycated haemoglobin (HbA1c ≥ 48 mmol/mol, 6.5%), with lesser elevations classified as prediabetes. T2D accounts for more than 90% of all diabetes and is among the fastest growing chronic diseases worldwide, driven largely by rising rates of obesity and physical inactivity (34).

The pathophysiology is commonly described as a dual defect. Insulin resistance in liver, muscle, and adipose tissue increases the demand for insulin, and for a time the pancreatic β -cells compensate by secreting more, maintaining near-normal glucose at the cost of hyperinsulinaemia. Overt diabetes develops only when β -cell function can no longer keep pace with this demand. Insulin resistance therefore sets the stage, but it is progressive β -cell failure that converts a compensated, insulin-resistant state into manifest hyperglycaemia (35, 36).

A recurring question is whether β -cell failure in T2D reflects a loss of β -cell mass or a loss of β -cell function. Both occur, but their relative contributions differ. Autopsy studies show a reduced β -cell mass in T2D (a deficit of roughly 40% in lean and 60% in obese individuals compared with weight-matched controls), attributable mainly to increased apoptosis rather than to impaired replication or neogenesis (37). Reported reductions vary widely across studies, however (from 0% to 63%), and this variable and often modest loss of mass is generally considered insufficient to account for the severe secretory deficit observed clinically, pointing to functional impairment of the surviving cells as the major contributor (20, 38). Contributing mechanisms include glucotoxicity, lipotoxicity, endoplasmic reticulum stress, and oxidative stress (39). A further concept is β -cell dedifferentiation, in which cells lose their mature identity and secretory phenotype without necessarily dying, providing a mechanism by which function can be lost, and potentially regained, without a corresponding change in cell number (38, 40).

This distinction matters because functional impairment is, at least in part, reversible. Sustained caloric restriction and weight loss can restore first-phase insulin secretion and normalise glucose in a substantial proportion of patients, in parallel with mobilisation of ectopic fat from the liver and pancreas (41). This reversibility is duration-dependent, however, diminishing in long-standing diabetes as recoverable β -cell dedifferentiation gives way to more fixed loss (38). Bariatric surgery, which imposes a pronounced and durable caloric deficit, frequently produces T2D remission, and the degree to which this reflects the caloric restriction itself rather than the surgery-specific hormonal changes that accompany it remains a central and unresolved question in the field.

Treatment of obesity

Pharmacological treatment

Historically, the pharmacological management of obesity relied on agents such as orlistat, sibutramine, and rimonabant. These medications typically produced only modest additional weight loss (approximately 2–4 kg at one year) and were frequently limited by gastrointestinal or psychiatric adverse effects, contributing both to withdrawal of some agents and to poor long-term adherence (42).

In contrast, the advent of the “incretin era” has introduced therapies based on glucagon-like peptide-1 (GLP-1), glucose-dependent insulintropic polypeptide (GIP), and glucagon. These treatments emerged from extensive research into the gut-hormone changes associated with bariatric surgery and are designed to reproduce aspects of their effects on weight loss and glucose homeostasis. Although many were initially developed for type 2 diabetes, gut-hormone-based treatments have become central to obesity care because of their robust effects on appetite regulation and body weight.

Among these, GLP-1 has been the primary focus because of its potent effects on satiety and glucose homeostasis. However, native GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), resulting in an active half-life of only 2–3 minutes (43). Therapeutic strategies therefore aim to circumvent this rapid inactivation, most effectively through long-acting GLP-1 receptor agonists (GLP-1RAs). These agents incorporate structural modifications (e.g., at the DPP-4 cleavage site) and pharmacokinetic extensions (e.g., albumin-binding side chains or fusion constructs), substantially prolonging half-life and enabling once-daily or once-weekly dosing (44, 45). Continued clinical progress has also driven development of multi-agonist approaches that combine GLP-1 activity with GIP and/or glucagon signalling.

In phase 3 trials, these newer therapies have demonstrated mean weight reductions of approximately 10–15% with semaglutide and up to approximately 20% with the dual GIP/GLP-1 agonist tirzepatide, clearly exceeding outcomes achieved with earlier pharmacotherapies (46, 47). Incretin-based treatments have also shown favourable effects on cardiovascular outcomes (48). However, randomised withdrawal data underscore that benefits depend on continued treatment. In STEP 4, participants who lost 10.6% of baseline body weight during a 20-week semaglutide run-in achieved 17.4% weight loss at week 68 with continued therapy, whereas those switched to placebo regained 6.9% from week 20, leaving a net loss

of only 5% from baseline despite ongoing lifestyle support (49). Similarly, in SURMOUNT-4, adults with obesity who achieved a mean weight loss of 20.9% after 36 weeks of tirzepatide either continued treatment or switched to placebo. Continued therapy produced an additional 5.5% reduction, while placebo recipients regained 14 percentage points (approximately two-thirds of the initial loss) over one year (50). A meta-analysis showed that weight regain was faster than after behavioural weight-management programmes (51). Collectively, these findings are consistent with evidence that discontinuing GLP-1RA-based therapy leads to substantial weight regain and reversal of cardiometabolic improvements, supporting the view that chronic pharmacotherapy is generally required to sustain its benefits in obesity management (52). The long-term effects remain to be fully elucidated. The most common adverse effects of GLP-1 receptor agonists are gastrointestinal such as nausea, vomiting, diarrhoea, and constipation. These symptoms are usually mild to moderate, dose-dependent, and most pronounced during dose escalation, and they tend to subside with continued treatment. Even so, gastrointestinal intolerance frequently leads patients to reduce the dose or discontinue treatment and, as noted above, discontinuation is followed by weight regain (53). Gallbladder and biliary events, such as gallstones and cholecystitis, are well-established complications and are most common with greater weight loss, higher doses, and longer treatment (54). The risk of acute pancreatitis is less settled. Meta-analyses of randomised trials have generally found no significant excess, and in the LEADER trial, the number of events were numerically fewer with liraglutide than with placebo (55). These trials are, however, poorly suited to detect a rare event. A more recent meta-analysis including newer agents reported a modest overall increase in risk that was not seen in subgroup analyses (56). Regulatory bodies have responded to this uncertainty. In January 2026 the UK Medicines and Healthcare products Regulatory Agency strengthened the product information for the whole class following rare reports of necrotising and fatal pancreatitis (57). An indirect pathway is plausible, since rapid weight loss promotes gallstone formation and gallstones are the commonest cause of acute pancreatitis, which would place any pancreatic risk downstream of the biliary effect rather than reflecting direct pancreatic toxicity.

Observational data, by comparison, suggest that metabolic bariatric surgery yields greater and more durable weight loss at lower long-term healthcare costs than continuous GLP-1RA treatment (58).

Bariatric surgery

History

Early bariatric procedures in the 1950s–60s typically used long-segment small-bowel bypasses (59). These operations achieved substantial weight loss but carried high rates of late complications. In 1967, Mason introduced the gastric bypass,

which evolved into today's minimally invasive Roux-en-Y gastric bypass (RYGB) (60). In 1979, Scopinaro described the biliopancreatic diversion (BPD), which combined a distal gastrectomy with a long intestinal bypass and a short common channel, producing substantial weight loss primarily through fat malabsorption (61). This was later modified by Hess and Marceau into the biliopancreatic diversion with duodenal switch (BPD/DS), which replaced the distal gastrectomy with a sleeve gastrectomy and preserved the pylorus, thereby reducing complications while maintaining efficacy (62). Although BPD/DS achieves the greatest weight loss of any bariatric procedure, its higher complication rates and nutritional demands have limited its use to selected cases. Sleeve gastrectomy (SG) originated as the gastric (restrictive) component of the biliopancreatic diversion with duodenal switch and was later adopted as a staged first step for high-risk patients (63, 64). Through the late 2000s and 2010s, evidence accumulated supporting SG as a standalone primary procedure (65). By around 2020, SG had become the most commonly performed bariatric operation worldwide (66).

Alongside sleeve gastrectomy (SG), laparoscopic RYGB is now a mainstay of 21st-century bariatric practice (67). Numerous high-quality studies show that RYGB produces durable weight loss, improves obesity-related comorbidities such as metabolic syndrome, and is associated with increased life expectancy (68-71).

Since the 1950s, several techniques have been proposed and used, some aiming to reduce risk, others tailored to special situations such as super-obesity. RYGB and SG account for the vast majority of the more than 500,000 bariatric operations performed worldwide each year.

Procedure type is central when discussing both outcomes and mechanisms. By altering gastrointestinal anatomy, RYGB and SG profoundly modify gastric emptying, intestinal transit, nutrient exposure and uptake, and enteroendocrine (humoral) signalling changes that underpin their distinct physiological and clinical effects.

Surgical anatomy and technique

Understanding the metabolic and hormonal effects of bariatric surgery requires a clear appreciation of how each procedure alters gastrointestinal anatomy. The operations discussed here differ fundamentally in the extent to which they modify gastric volume, intestinal transit, nutrient exposure to enteroendocrine cells, and biliopancreatic mixing (Fig.1). These anatomical differences underlie the procedure-specific endocrine profiles.

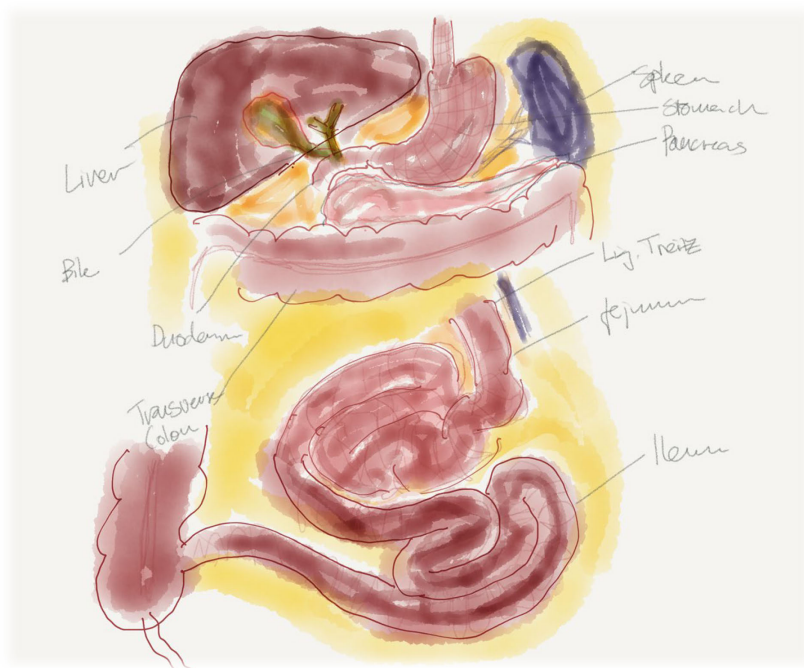


Figure 1: Normal GI-tract

Roux-en-Y gastric bypass

In RYGB (Fig.2), the stomach is divided to create a small proximal gastric pouch, typically 15–30 mL in volume, which is completely separated from the gastric remnant. The jejunum is then divided approximately 50 cm distal to the ligament of Treitz, and the distal end is anastomosed to the gastric pouch (the gastrojejunostomy), creating the alimentary (Roux) limb. The proximal end of the divided jejunum, which carries bile and pancreatic secretions from the duodenum, is reconnected to the alimentary limb further downstream (the jejunojejunostomy), creating the biliopancreatic limb. The distance from the gastrojejunostomy to the jejunojejunostomy defines the length of the alimentary limb, which in standard practice measures approximately 100–150 cm, with a biliopancreatic limb of 50–75 cm (59, 60).

This reconstruction has three main functional consequences. First, ingested nutrients bypass the duodenum and proximal jejunum entirely, reducing stimulation of K-cells (the principal source of GIP) and contact with the initial sites of nutrient absorption. Second, nutrients arrive earlier and in a less digested form in the mid and distal jejunum and ileum, where L-cells (producing GLP-1, PYY, and oxyntomodulin) and N-cells (producing neurotensin) are more densely distributed, leading to amplified and accelerated postprandial hormone responses. Third,

separating the biliopancreatic stream from the nutrient stream along the alimentary limb delays enzymatic digestion and bile-salt-mediated fat absorption until the two streams converge at the jejunojejunostomy. The small gastric pouch empties rapidly into the jejunum, further accelerating nutrient transit (72). Virtually all RYGB procedures are now performed laparoscopically.

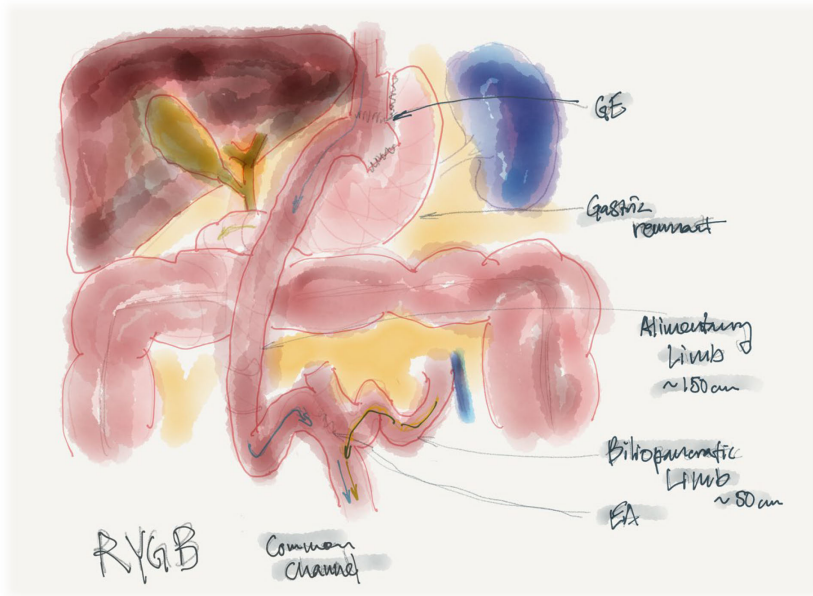


Figure 2: RYGB, 15-30 ml gastric pouch, a gastrojejunostomy and a 150 cm alimentary limb created through a jejunojejunostomy, with a 50-75 cm biliopancreatic limb.

Sleeve gastrectomy

Sleeve gastrectomy (SG) involves longitudinal resection of approximately 75–80% of the stomach along the greater curvature, leaving a narrow tubular remnant (the “sleeve”) calibrated over a bougie, typically 32–40 French in diameter (Fig. 3). The resected portion includes the majority of the gastric fundus and body, which contains the highest density of ghrelin-producing P/D1 endocrine cells. The antrum and pylorus are preserved, maintaining the normal gastric outlet (63).

Unlike RYGB, SG does not involve intestinal bypass or rerouting of the biliopancreatic stream. Nonetheless, the procedure alters gastrointestinal physiology in ways that extend beyond simple gastric volume reduction. The tubular gastric remnant generates higher intraluminal pressures and accelerates gastric emptying compared with the intact stomach (73). As a consequence, nutrients reach the duodenum and proximal jejunum more rapidly, and this accelerated transit

appears to increase stimulation of distally located enteroendocrine cells sufficiently to produce measurable increases in postprandial GLP-1 and PYY, although the magnitude of these changes is generally smaller than after RYGB (74). The most distinctive endocrine effect of SG is a marked and sustained reduction in circulating ghrelin, consistent with removal of the ghrelin-producing fundic mucosa (75).

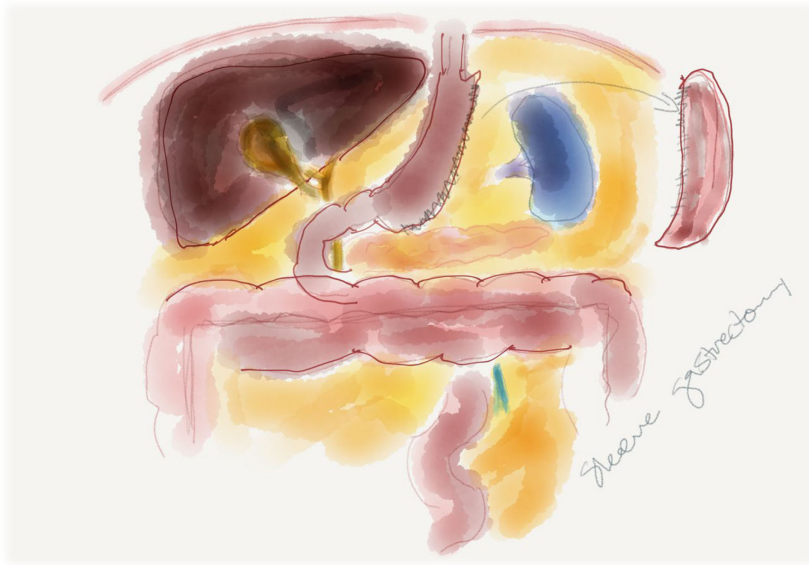


Figure 3: Sleeve gastrectomy is created by stapling off curvatura major using a bougie along curvature minor. Antrum is partly preserved.

Biliopancreatic diversion and duodenal switch

The biliopancreatic diversion (BPD), originally described by Scopinaro (61), combines a horizontal (distal) gastrectomy with an extensive intestinal bypass. The stomach is resected to leave a proximal gastric remnant of approximately 200–500 mL, and the small bowel is divided approximately 250 cm proximal to the ileocaecal valve. The distal segment is anastomosed to the gastric remnant (the alimentary limb), and the proximal segment carrying bile and pancreatic secretions (the biliopancreatic limb) is reconnected to the alimentary limb at a point 50–100 cm proximal to the ileocaecal valve, creating a short common channel where nutrients, bile, and pancreatic enzymes first meet. This short common channel is the defining feature of BPD and produces substantial macronutrient malabsorption, particularly of fat, which contributes directly to weight loss (76). However, classical BPD carries higher rates of protein-calorie malnutrition, fat-soluble vitamin deficiencies, metabolic bone disease, and dumping syndrome.

To address these complications while retaining the potent weight-loss efficacy of BPD, Hess and Marceau developed the biliopancreatic diversion with duodenal switch (BPD/DS) (62). The key modification is the replacement of Scopinaro's distal gastrectomy with a sleeve gastrectomy, preserving the pylorus and the first portion of the duodenum. The duodenum is divided just beyond the pylorus, and the distal ileum is anastomosed to the duodenal stump (duodenoileostomy), while the biliopancreatic limb is reconnected approximately 75–100 cm proximal to the ileocaecal valve, creating a common channel of similar length to classical BPD. By preserving the pylorus, BPD/DS maintains a more physiological gastric emptying pattern, reducing the incidence of dumping syndrome and marginal ulceration. The sleeve gastrectomy component produces ghrelin suppression analogous to standalone SG, and the extensive intestinal bypass generates a hormonal profile dominated by distal gut signals, with very large postprandial increases in GLP-1 and PYY, reduced GIP, and significant macronutrient malabsorption (77, 78). Today, BPD/DS has largely replaced classical BPD in clinical practice (67) (Fig. 4).

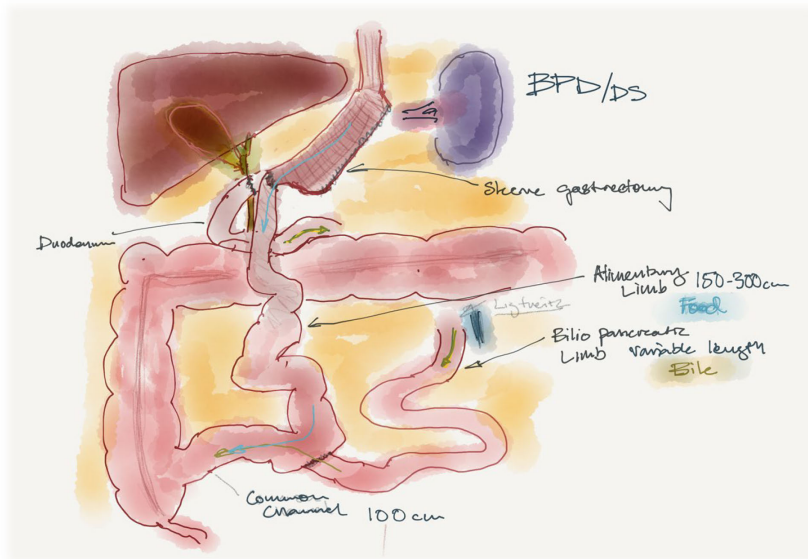


Figure 4: BPD/DS is created through combining a sleeve gastrectomy with long bypass with a much shorter common channel (Food + bile) of approximately 50-100 cm.

In summary, these procedures can be understood along a continuum of increasing anatomical disruption and correspondingly greater enteroendocrine stimulation: SG alters gastric emptying and removes ghrelin-producing mucosa without intestinal rerouting. RYGB adds duodenal bypass and moderately amplifies distal gut hormone responses, and BPD/DS combines sleeve gastrectomy with an extensive

bypass and short common channel, achieving the most pronounced malabsorption and distal gut-hormone stimulation at the cost of the greatest nutritional risk.

Indications and procedure selection

The indications for bariatric surgery have evolved substantially since the 1991 NIH Consensus Conference, which established thresholds of BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with at least one obesity-related comorbidity for surgical eligibility (79). Over the past decade, however, growing recognition of the metabolic effects of surgery has led to broader indications. The 2nd Diabetes Surgery Summit (DSS-II) in 2015 recommended considering metabolic surgery for patients with T2D and a BMI of 30–35 kg/m² who have inadequate glycaemic control despite optimal medical therapy (80). The 2022 joint statement from the ASMBS and IFSO further lowered the threshold, recommending surgery for a BMI ≥ 35 kg/m² regardless of comorbidities or a BMI ≥ 30 kg/m² in the presence of metabolic disease (81). These criteria were also adopted in Swedish government national guidelines in 2023 (82).

In current Swedish practice, RYGB and SG are the dominant procedures. SG is sometimes favoured when a simpler operation is preferred, BMI in lower range, or when continued endoscopic access to the upper gastrointestinal tract is needed (e.g. IBD/Crohn's disease). RYGB may be preferred in patients with established T2D, given its generally higher remission rates (83, 84), and in those with severe gastro-oesophageal reflux disease, which SG can exacerbate. BPD/DS is reserved for patients with super-obesity (BMI > 50 kg/m²) and accounts for fewer than 1–2% of bariatric operations worldwide (67).

Because each procedure generates a distinct anatomical, endocrine, and metabolic milieu, findings from studies of one procedure cannot be directly extrapolated to another. This is an important consideration when interpreting research that compares outcomes across surgical techniques.

Bariatric surgery outcome

Pharmacological treatment of obesity-related risk factors is common, but many such treatments do not address the underlying risk conferred by obesity. Patients undergoing bariatric surgery, especially Roux-en-Y gastric bypass and sleeve gastrectomy, benefit not only from weight loss but, more importantly for long-term health and survival, from improved glycaemic control, reduced cardiovascular morbidity, and a lower risk of certain cancers (85-87). The results are often substantially better than those achieved with intensive pharmacological treatment (88-90). Although these operations were originally rationalised as producing weight loss through macronutrient malabsorption and/or mechanical restriction, contemporary evidence suggests those mechanisms play a limited role (91, 92). The

mechanisms that replace them are not established. Current hypotheses centre on a resetting of body-weight regulation, driven by changes in enteroendocrine signalling, alterations in bile-acid metabolism and FXR/TGR5 signalling (93), modulation of vagal and central neural circuits (94), and shifts in the gut microbiota (95, 96). Each has some experimental support, but their relative importance, and their relation to the sustained reduction in caloric intake that surgery imposes, remains unresolved.

Considering weight loss alone, Roux-en-Y gastric bypass yields greater long-term loss than sleeve gastrectomy: mean total body weight loss (TBWL) exceeds 30% at the 2-year nadir and is maintained at ~25% at 10 and 15 years, whereas SG reaches ~28% TBWL at 1 year and ~18.7% at 10 years (97).

From a metabolic perspective, particularly in relation to type 2 diabetes and associated metabolic dysfunction, bariatric surgery produces superior glycaemic outcomes compared with medical therapy, with larger reductions in HbA1c and substantially higher remission rates (88, 89). RYGB is generally more effective than SG, with diabetes remission reported in 30–85% depending on definitions and follow-up (83, 84). The likelihood of remission is strongly influenced by preoperative HbA1c, diabetes duration, and the type and intensity of antidiabetic treatment (98, 99).

Among individuals with obesity, the principal risk factors for cardiovascular disease, including atrial fibrillation, heart failure, myocardial infarction, and stroke, are hypertension and type 2 diabetes, with additional contributions from dyslipidaemia and chronic low-grade inflammation. Bariatric surgery (RYGB or SG) is associated with >40% reductions in cardiovascular mortality and an estimated 35–50% lower risk of CVD (68, 100).

Physiology of gut hormones

The gastrointestinal tract functions as the body's largest endocrine system, coordinating nutrient intake with metabolic responses through secretion of multiple peptide hormones (101). More than 30 such hormones have been identified, but they arise from a limited number of established enteroendocrine cell types, each traditionally denoted by a letter and classified by its principal secretory product (102). The principal types comprise enterochromaffin (EC) cells (serotonin), K-cells (GIP), L-cells (GLP-1, Oxyntomodulin and PYY), I-cells (CCK), S-cells (secretin), G-cells (gastrin), N-cells (neurotensin), D-cells (somatostatin), Mo-cells (motilin), enterochromaffin-like (ECL) cells (histamine), and P/D1-cells (ghrelin), the last of these historically termed X/A-like cells in rodent nomenclature. These populations are distributed regionally along the alimentary canal. Gastrin, ghrelin, histamine, and somatostatin producing cells predominate in the stomach, GIP, CCK, secretin,

and motilin producing cells in the proximal small intestine, and neurotensin and GLP-1/PYY-producing cells become increasingly common towards the distal small intestine and colon (102). The historical one cell, one hormone model is now recognised as an oversimplification because many enteroendocrine cells co-express and co-release several peptides (103, 104).

In the context of bariatric surgery and type 2 diabetes, several hormones are of particular interest: GLP-1 and GIP collectively termed the incretins, as well as peptide YY (PYY), ghrelin, and neurotensin (NT). Together, these hormones form an interconnected signalling network, often referred to as the gut–brain axis, that regulates appetite, digestion, glucose homeostasis, and long-term energy balance (105, 106).

GLP-1

GLP-1 is produced by enteroendocrine L-cells, which are most densely distributed in the distal small intestine and colon. GLP-1 is released postprandially in response to luminal nutrients, primarily carbohydrates, and acts as an incretin, potentiating glucose-dependent insulin secretion from pancreatic β -cells. Beyond its insulinotropic role, GLP-1 slows gastric emptying, suppresses glucagon secretion, and promotes satiety through vagal afferent pathways and direct actions on brainstem nuclei, including the area postrema and the nucleus of the solitary tract (107, 108). Fasting GLP-1 levels are generally low and appear largely unchanged after bariatric surgery (109), but it is the postprandial response that is markedly amplified after procedures such as RYGB (110, 111). In the physiological setting, gastrointestinal-mediated glucose disposal (GIGD) accounts for approximately two-thirds of the disposal of an oral glucose load, reflecting the combined actions of GLP-1 and GIP on β -cell function (112).

GIP

GIP is secreted from enteroendocrine K-cells concentrated in the duodenum and the proximal jejunum. GIP is released in response to ingested glucose and fat and is quantitatively the dominant incretin in healthy individuals, accounting for the larger share of the postprandial insulin response (113). In addition to its insulinotropic actions, GIP promotes lipid uptake and storage in adipose tissue and affects bone metabolism. Unlike GLP-1, GIP does not substantially slow gastric emptying and stimulates rather than suppresses glucagon secretion. Its proximal location in the gut means that GIP secretion is directly affected by surgical procedures that bypass the duodenum, such as RYGB.

Neurotensin

NT is a 13-amino acid peptide originally isolated from the bovine hypothalamus (114) but now recognised primarily as a gut hormone, produced by enteroendocrine N-cells concentrated in the ileum and distal jejunum (102). NT is released primarily by N-cells but is also coexpressed and co-released with GLP-1 and PYY in certain enteroendocrine cell populations (115). In the circulation, mature NT is rapidly degraded (with a half-life of approximately 30 seconds in rodents), which has led to the use of its stable precursor prohormone, proneurotensin (pNT), as a surrogate biomarker. pNT is produced in equimolar amounts with mature NT (116).

In the gut, NT inhibits gastric acid secretion and gastric emptying, increases intestinal blood flow and hepatic bile acid handling, and enhances pancreatic exocrine activity, collectively coordinating the postprandial response to lipid (117, 118). NT also facilitates intestinal fat absorption directly. Neurotensin-deficient mice are protected from high-fat-diet-induced obesity and hepatic steatosis (119). Intriguingly, peripheral and central NT appear to exert opposing but complementary effects on body weight: whereas intestinal NT promotes fat absorption and energy storage, central NT signalling in the hypothalamus and brainstem suppresses feeding and promotes energy expenditure, primarily through the NTSR1 receptor (120, 121). Peripherally administered NT activates brainstem and hypothalamic appetite centres, and the anorexigenic effect is partly vagus-dependent (122). Increased meal-induced NT secretion has also been associated with successful weight-loss maintenance in humans (123).

PYY, ghrelin, and other gut hormones

PYY is co-released with GLP-1 from distal L-cells following a meal. The full-length peptide (PYY₁₋₃₆) is rapidly cleaved by dipeptidyl peptidase-4 to PYY₃₋₃₆, which acts as a selective Y2 receptor agonist. PYY₃₋₃₆ inhibits appetite through actions on neuropeptide Y-expressing neurons in the hypothalamic arcuate nucleus, slows gastric emptying, and reduces intestinal motility, thereby contributing to the ileal brake mechanism. In human studies, peripheral infusion of PYY₃₋₃₆ at physiological postprandial concentrations reduced food intake (124). PYY concentrations rise in proportion to caloric intake and are particularly responsive to fat and protein.

Ghrelin, by contrast, is the only known orexigenic gut hormone. It is a 28-amino acid acylated peptide produced predominantly by P/D1 endocrine cells in the oxyntic mucosa of the gastric fundus (125) and to a smaller extent in the upper small intestine (126). Ghrelin levels rise during fasting, acting centrally through the growth hormone secretagogue receptor (GHSR) in the hypothalamus and brainstem to stimulate hunger and food-seeking behaviour. Postprandially, ghrelin levels fall, contributing to meal termination.

Ghrelin also stimulates growth hormone release, promotes gastric motility, and influences glucose homeostasis through a direct, glucose-dependent inhibitory action on insulin secretion, demonstrated in human islets (127).

Its production in the gastric fundus makes it particularly relevant to surgical procedures that involve resection of this area, such as sleeve gastrectomy.

Cholecystokinin (CCK) is produced by I-cells in the duodenum and proximal jejunum and is released in response to intraluminal fat and protein. CCK stimulates gallbladder contraction, pancreatic enzyme secretion, and slows gastric emptying. It was the first gut hormone shown to reduce food intake, acting as an acute satiety signal through CCK-A receptors on vagal afferent neurons (128). However, the satiating effect of CCK is short-lived and does not translate into sustained weight reduction with chronic administration, limiting its direct relevance to long-term energy balance.

Oxyntomodulin (OXM) is a proglucagon-derived peptide co-released with GLP-1 from L-cells. It acts as a dual agonist at both the GLP-1 and glucagon receptors. In human studies, OXM reduced food intake and body weight and increased energy expenditure (106). Like GLP-1, OXM is rapidly inactivated by DPP-4.

Gut hormones in obesity and T2D

Obesity and type 2 diabetes are associated with marked disturbances in gut hormone signalling, particularly of the incretins. The incretin effect, defined as the augmentation of insulin secretion by gut-derived signals following oral versus intravenous glucose administration, is substantially diminished in T2D. This impairment reflects a reduced β -cell response to these signals rather than impaired secretion, as GLP-1 secretion is typically only modestly reduced, if at all, in patients with obesity and early T2D (129-131). At the low concentrations reached after a meal, endogenous GLP-1 elicits a blunted insulinotropic response from the T2D β -cell, which contributes to the diminished incretin effect. The GLP-1 receptor pathway is not lost, however. Supraphysiological concentrations, such as those achieved with GLP-1 receptor agonists, still drive substantial insulin secretion and lower glucose effectively (132).

The physiology of GIP is altered more profoundly. Although GIP secretion is typically preserved or even elevated in insulin-resistant states, its insulinotropic effect is markedly impaired. This phenomenon, often termed “GIP resistance,” has been attributed to desensitisation of GIP receptors on β -cells (105, 133). In parallel, GIP increasingly acts on adipose tissue, promoting triglyceride storage and contributing to visceral fat accumulation, thereby reinforcing insulin resistance (134). This shift from a glucose-lowering to a lipogenic role illustrates the pathophysiological complexity of incretin dysfunction in T2D.

Other hormonal axes are also affected. Postprandial PYY secretion may be blunted, reducing satiety signalling and promoting increased energy intake.

Ghrelin signalling is also altered in T2D. Fasting plasma ghrelin concentrations are reduced, and work from our group has shown that this reflects a loss of pancreatic islet ghrelin cells, decreased by roughly three-quarters in T2D donors, whereas the number of gastric ghrelin cells, the main source of circulating ghrelin, is unchanged. Because ghrelin exerts a direct, glucose-dependent insulin-suppressing action in human islets, this reduction in islet ghrelin has been interpreted as an adaptation to the increased insulin demand of T2D (127).

Elevated fasting NT concentrations are associated with insulin resistance, metabolic dysfunction-associated steatotic liver (MASLD), and an increased risk of T2D, possibly through enhanced intestinal lipid absorption and subsequent ectopic fat deposition (135, 136). The epidemiological evidence linking NT to metabolic disease is discussed in greater detail in the section on gut hormones as predictors of metabolic syndrome. Together, these hormonal disturbances create a permissive environment for overeating, impaired insulin secretion, and worsening metabolic disease, and they provide an important rationale for why surgical modification of the gut hormone milieu can have such profound metabolic effects (137).

Gut hormones as predictors of MetS

A substantial amount of research has examined whether circulating gut hormones may serve as early biomarkers for the development of metabolic syndrome (MetS) and T2D. Although circulating levels of several enteroendocrine peptides correlate with obesity, insulin resistance, and cardiometabolic dysfunction, their predictive capacity varies considerably. Overall, only a subset appears to add independent information beyond established clinical risk markers.

Among the classical incretins GIP has shown modest but consistent associations with incident T2D (138). In prospective population studies, elevated fasting GIP predicted future diabetes independently of age, BMI, fasting glucose, and insulin resistance indices (138). These findings support the hypothesis that enhanced basal GIP secretion reflects early β -cell stress or altered nutrient handling. However, the incremental predictive value beyond conventional risk factors remains limited, and GIP has not achieved clinical utility for risk stratification.

Ghrelin, in contrast, demonstrates a more uniform relationship with metabolic risk (139, 140). Numerous cross-sectional and longitudinal studies show that low fasting ghrelin concentrations are associated with obesity, insulin resistance, MetS, and incident T2D (141, 142). Because ghrelin secretion is suppressed in states of chronic positive energy balance, low circulating concentrations likely reflect the cumulative metabolic load rather than acting as an independent upstream predictor. Nonetheless, ghrelin consistently marks a high-risk phenotype.

For GLP-1 and PYY, evidence for predictive value is considerably weaker. Cross-sectional data show modest reductions in postprandial GLP-1 responses in obesity, prediabetes, and type 2 diabetes (143), although meta-analytic evidence indicates that overall GLP-1 secretion differs little between individuals with and without T2D, with both reductions and elevations reported (144). Prospective evidence linking circulating GLP-1 to incident diabetes independently of standard metabolic risk variables remains limited. PYY concentrations tend to be reduced in obesity and T2D, yet prospective data linking PYY to future MetS or T2D are sparse, and no consistent independent predictive value has been demonstrated after multivariable adjustment (145, 146). Thus, while both peptides reflect aspects of metabolic health, neither currently supports a predictive role.

A notable exception is neurotensin, assessed using its stable precursor pro-neurotensin. A series of large, well-characterised Scandinavian cohort studies have consistently identified pNT as a strong, independent predictor of metabolic disease. In the Malmö Preventive Project, individuals above the median pNT concentration had a modestly but significantly increased risk of incident cardiovascular disease over 5.4 years of follow-up, with similar effect sizes in both sexes. Proneurotensin did not predict incident diabetes in the population as a whole, but did so in women (136, 147). Importantly, these associations persisted after adjustment for BMI, lipids, blood pressure, fasting glucose, lifestyle factors, and indices of insulin resistance, suggesting that neurotensin captures early pathophysiological changes not reflected using conventional biomarkers. Proneurotensin has also been associated with cardiovascular events, heart failure, and all-cause mortality, with particularly strong associations reported in women (136).

Mechanistically, neurotensin is released from enteroendocrine N-cells in response to dietary fat and facilitates intestinal lipid absorption, chylomicron assembly, and hepatic lipid accumulation. Experimental studies demonstrate that neurotensin promotes visceral adiposity, inflammation, and insulin resistance, whereas neurotensin-deficient mice are protected from diet-induced obesity, fatty liver, and glucose intolerance (119). These findings provide biological plausibility for neurotensin's predictive associations and suggest that it may participate directly in metabolic disease pathogenesis.

Finally, it should be mentioned that most knowledge of gut hormones is based on their circulating concentrations. However, how obesity and T2D affect the cells producing each hormone in different intestinal segments remains poorly understood.

Why does metabolic surgery work?

Historically, Roux-en-Y gastric bypass was thought to induce weight loss mainly through mechanical restriction, created by a small gastric pouch, and malabsorption resulting from bypass of the duodenum and proximal jejunum. Neither explanation has held up well. There is limited support for clinically meaningful macronutrient malabsorption as the primary driver of weight loss after RYGB (91). Studies comparing RYGB with non-surgical controls typically demonstrate only a small reduction in macronutrient absorption, which is unlikely to explain the magnitude of postoperative weight loss (148), and sleeve gastrectomy (SG) does not meaningfully alter macronutrient absorption at all (149). The restrictive account is equally difficult to sustain. In RYGB, formation of the gastric pouch and gastrojejunostomy accelerate rather than slow nutrient entry into more distal segments of the small intestine, although overall small-intestinal transit may be prolonged (72). Although SG appears restrictive from a surgical perspective, creating the sleeve likewise accelerates gastric emptying (73). As these explanations have proven insufficient, the question arises: what drives postoperative weight reduction?

Contemporary evidence supports a more integrated physiological model in which bariatric surgery induces substantial alterations in gut–brain communication. Surgery-related changes in enteroendocrine hormone secretion, modulating appetite regulation, energy intake, and food reward, appear to be key determinants of postoperative metabolic outcomes. Notably, each bariatric procedure generates a distinct endocrine and metabolic profile, which likely contributes to the observed variability in weight loss and glycaemic improvement across surgical techniques (Fig. 5). Because different operations modify nutrient flow and gastrointestinal physiology in unique ways, they elicit procedure-specific postprandial responses that shape the overall metabolic effect. Understanding the expected outcomes of different bariatric procedures requires focusing on their distinct anatomical alterations rather than the historical rationale behind their development.

In RYGB (Fig. 2), the gastrojejunostomy delivers nutrients to segments with a greater density of L-cells, while the duodenum and the other proximal regions in which K-cells predominate are bypassed. The result is a marked postprandial increase in GLP-1 and PYY, with earlier and higher peaks than before surgery.

Postprandial GIP responses after RYGB have been reported to fall, to remain stable, or to rise, and no consistent picture has emerged. The divergence has been linked to how the operation was performed, what was given as a test meal, how many participants were studied, and which comparison group was chosen. When the two procedures are compared directly in participants studied at least a year after surgery, the postprandial GIP response is lower after RYGB than after SG, which fits the exclusion of the K-cell-rich proximal intestine from the nutrient stream (150). Fasting GIP is generally unchanged or transiently lower (150, 151). Fasting GLP-1 levels, however, appear largely unchanged from the preoperative state (109).

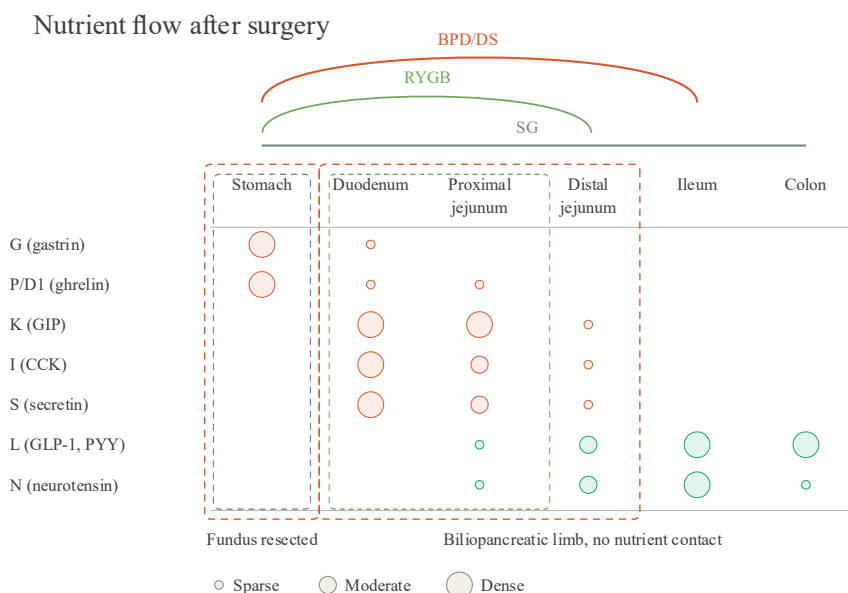


Figure 5: Nutrient flow after surgery. BPD/DS bypassing a larger portion of small intestine than RYGB. SG is not bypassing, but delivery of food to small intestine is accelerated.

In SG, the same acceleration increases nutrient delivery to the small intestine without any intestinal bypass, and postprandial GLP-1 rises in proportion to the acceleration of gastric emptying (152). The characteristic endocrine feature of SG is instead a marked, and often sustained, reduction in fasting ghrelin, consistent with resection of ghrelin-producing fundus mucosa (75), providing a plausible mechanism for reduced hunger and altered meal initiation. In contrast, RYGB may induce an early decrease in fasting ghrelin that tends to return toward preoperative levels, or higher, in the longer term despite continued weight loss (153).

Procedures with a much longer bypass, such as biliopancreatic diversion (BPD) or duodenal switch (DS), create a substantially shorter common channel and therefore produce a more pronounced macronutrient malabsorption that can contribute more directly to weight loss (76), an effect also demonstrated experimentally (77). The extensive bypass and the more distal delivery of nutrients also produce distinct changes in gut-hormone secretion. In a series of 19 patients restudied during the second postoperative year after BPD/DS, the postprandial GIP response was reduced by 42%, consistent with exclusion of the GIP-rich proximal K-cells from the nutrient stream, whereas the L-cell products GLP-1 and PYY were markedly amplified, with a 6.2-fold increase in the area under the curve for GLP-1 and a threefold increase for PYY (154). The two are not equivalent, however: the GLP-1 rise reflects a large early postprandial peak, whereas fasting PYY was tripled and the area under the curve, calculated from

zero, therefore reflects the higher basal concentration as much as the meal response. Fasting ghrelin was lower after surgery, in keeping with the sleeve gastrectomy component. The authors noted that the ratio of the 30-minute to the fasting concentration was essentially unchanged for both GIP and GLP-1, and interpreted this as a change in the number of secreting cells reached by nutrients rather than in their secretory properties. There is no head-to-head comparison of BPD/DS with RYGB or SG, so the apparently greater magnitude of the GLP-1 and PYY responses after BPD/DS rests on comparisons across studies that differ in meal composition, assay, and time since surgery.

GLP-1, PYY, ghrelin, and neurotensin all influence appetite regulation, but available evidence suggests that postoperative changes in eating behaviour cannot be attributed to any single hormone in isolation. In human studies using receptor antagonists, blocking individual gut-hormone pathways tends to produce modest or inconsistent effects on food intake after RYGB or SG, whereas combined manipulations may reveal clearer contributions (155, 156). At the same time, GLP-1 receptor agonists produce clinically meaningful weight loss (49), highlighting an important distinction between physiological, meal-tied hormone signals and sustained pharmacological receptor activation. Neurotensin affects food intake in animal models (120, 121) and predicts successful weight-loss maintenance in humans (123), but data in humans are still insufficient.

Finally, a subset of patients experiences suboptimal long-term outcomes after RYGB. While many patients regain some weight after an initially successful loss, a smaller subgroup exhibits primary weight-loss failure with limited initial response. It has been hypothesised that impaired gut-hormone signalling may contribute in some individuals. In one study, so far reported in abstract form only, postprandial GLP-1 and PYY levels did not differ between groups, and combined blockade increased food intake only among those with successful weight loss (157). These findings are compatible with heterogeneity in the downstream responsiveness to gut-hormone signalling, potentially involving central anorectic responses or vagal pathways, rather than differences in hormone secretion per se.

Other factors and pathways, such as bile acids and FXR/TGR5 activation, gut microbiota, gut blood flow and energy expenditure have been explored, but their contribution remains unclear (158).

Table 1. Changes in circulating gut hormone concentrations after caloric restriction and after bariatric surgical procedures.

Hormone and response	Caloric restriction without surgery	Roux-en-Y gastric bypass	Sleeve gastrectomy
GLP-1, <i>fasting</i>	↔ at 10 and 62 wk (159, 160); ↔ after 3 to 4 wk VLCD (Paper I)	↔ (74, 161)	↔ (74, 75)
GLP-1, <i>postprandial</i>	↔ no rise, total tended to fall in T2D (159, 160); ↔ after 3 to 4 wk VLCD, identical test meal (Paper I)	↑↑ several-fold peak within 1 mo, sustained at 12 mo (111, 160)	↑ smaller than RYGB, but head-to-head difference not significant (75, 109, 111)
GIP, <i>fasting</i>	↑ at 10 and 62 wk (159); ↔ after 3 to 4 wk VLCD (Paper I)	↓ in meta-analysis, mainly in T2D; earlier reviews report ↔ (161, 162)	Insufficient data, pooled estimate rests on two small studies (75)
GIP, <i>postprandial</i>	↑ in the first 60 min (159); ↔ after 3 to 4 wk VLCD (Paper I)	Contested, time-dependent: ↑ in the first month, not sustained at 6 to 12 mo; meta-analysis reports overall ↓ (161, 162)	Limited data, pooled analysis covered fasting only (75)
PYY, <i>fasting</i>	↓ at 10 wk, further ↓ at 62 wk (159)	↔ to modest ↑ from 3 mo, higher than SG (74, 109)	↔, lower than RYGB (74, 109)
PYY, <i>postprandial</i>	↓ at every time point, pattern preserved (159)	↑↑ approximately doubled through year 1; one meta-analysis found no rise at 12 mo (74, 109)	↑ at 6 mo, not sustained at 12 mo (74)
Ghrelin, <i>fasting</i>	↑ at 10 and 62 wk, counter-regulatory (159)	Variable: ↔ first month, ↑ 3 to 12 mo, ↔ at 24 mo (74, 109)	↓↓ consistently, fundus resected; lower than RYGB (74, 75, 109)
Ghrelin, <i>postprandial</i>	Meal suppression preserved, from a higher baseline (159)	↓ early, fluctuation restored by 12 mo (111)	Still attenuated at 12 mo, no postprandial decline (111)
CCK, <i>fasting and postprandial</i>	↓ at 10 and 62 wk (159)	Meal response rises less than after SG (111)	Higher peak than RYGB, integrated response did not differ (111)
Neurotensin, <i>fasting (pNT)</i>	↓ acutely by 52 to 63%, as much as immediately after surgery (Paper III)	↓ acutely, then ↑ above baseline from approximately 6 wk, elevated at 12 mo (163), Paper III	No difference from RYGB (Paper III)
Neurotensin, <i>postprandial</i>	No meal-stimulated rise, and none before the diet (Paper III)	↑↑ meal response emerges where none exists before surgery, not reproduced by GLP-1 or GIP infusion (Paper III); higher than after SG and in matched controls (164)	Meal response emerges, smaller than RYGB (Paper III); early postprandial rise only (164)

↑ increased, ↑↑ markedly increased, ↓ reduced, ↓↓ markedly reduced, ↔ unchanged. All changes are stated relative to preoperative or pre-diet values in the same individuals unless otherwise indicated. Follow-up intervals differ between studies, and the timing of assessment materially affects the direction reported for several hormones, particularly GIP, PYY and ghrelin.

The caloric restriction column combines published diet studies with the diet arm of this thesis. The published data are a prospective study of a 10-week very-low-energy diet in 50 patients with overweight or obesity and without diabetes, reassessed at 10 and 62 weeks (159), and a matched-weight-loss comparison of gastric bypass and a hypocaloric diet in women with type 2 diabetes (160). Mean weight loss in the former was 13.5 kg at 10 weeks but only 7.9 kg below baseline at 62 weeks, so the 62-week values reflect partial weight regain rather than maintained weight loss. In the latter, energy intake was 1000 kcal per day in the diet group versus 600 to 800 kcal per day after surgery. Entries marked Paper I and Paper III derive from the cohort of this thesis, 19 women studied before and after a 3 to 4 week very-low-calorie diet of approximately 858 kcal per day, each serving as her own control, with an identical mixed meal given on every occasion.

The GIP entries for caloric restriction diverge between sources. Sumithran et al. report higher fasting and meal-stimulated GIP after a 10-week diet (159), whereas Paper we found no change after 3–4 week diet. Three differences are relevant: the shorter diet, the weaker glucose stimulus of a mixed meal ingested over 20 minutes, and a sample of 19 women, in which a modest effect could escape detection. The incretin response to caloric restriction therefore appears to depend on the duration and severity of the diet and on the stimulus used, rather than being a fixed property of energy deficit.

Changes after biliopancreatic diversion with duodenal switch are not tabulated. They rest on two small single-centre cohorts (154, 165) and are described in the running text instead.

Alcohol and bariatric surgery

Recent studies have identified an increased risk of alcohol use disorder (AUD) and hazardous drinking as adverse outcomes following bariatric surgery, particularly after RYGB and, to a lesser degree, SG (166-170). Most investigations rely on self-reported instruments such as the AUDIT, or on indirect indicators such as healthcare encounters or treatment-seeking behaviour for AUD (169, 171). Both approaches risk underestimating changes in alcohol consumption. Objective biomarkers offer an alternative. Phosphatidylethanol (PEth) is a group of abnormal phospholipids formed in erythrocyte membranes by phospholipase D, and only in the presence of ethanol, which makes it a highly specific biomarker for alcohol consumption (172). Because concentrations rise in proportion to intake over the preceding weeks, PEth also distinguishes moderate from heavy drinking, which established markers such as carbohydrate-deficient transferrin and gamma-glutamyl transferase do not reliably achieve.

At a first glance, alcohol use and gut hormone physiology may appear unrelated. However, emerging evidence indicates that GLP-1RA reduce alcohol intake and craving (173-175). Given that endogenous GLP-1 secretion increases markedly after RYGB, it may seem paradoxical that bariatric procedures are associated with higher, not lower, rates of AUD.

Several mechanisms help explain this apparent contradiction. Both RYGB and SG significantly increase alcohol bioavailability. These procedures reduce gastric alcohol dehydrogenase activity, thereby decreasing first-pass metabolism. In RYGB specifically, alcohol bypasses the pylorus and reaches the jejunum almost immediately, resulting in rapid and efficient uptake. Multiple studies demonstrate that RYGB leads to a shorter time to peak blood alcohol concentration, a higher peak concentration (C_{max}), and prolonged elimination time (176-178). Consequently, alcohol may trigger a faster and stronger dopaminergic reward response, an effect that can surpass or negate any potentially protective influence of elevated postprandial GLP-1.

Aims

General aim

To delineate the relative contributions of caloric restriction and surgery-specific effects to postoperative improvements in metabolic health after bariatric surgery by integrating (i) mixed-meal hormonal profiling (GLP-1, GIP, and proneurotensin), (ii) fasting and postprandial metabolite profile, and (iii) long-term objective measurement of alcohol exposure using PEth in RYGB and sleeve-gastrectomy cohorts.

Specific aims

1. **Disentangle surgery-specific from diet-induced effects on enteroendocrine function**

Isolate the immediate impact of RYGB on insulin secretion, insulin sensitivity, GLP-1 and GIP responses to a standardised mixed-meal, and compare with the effect of preoperative very-low-calorie diet (VLCD), in individuals with and without T2D. (Paper I)

2. **Map metabolite signatures of VLCD vs. RYGB**

Profile fasting and postprandial plasma metabolites and lipids across VLCD, the first post-RYGB meal, and 6 weeks post-surgery to determine whether metabolic changes previously attributed to RYGB are primarily driven by caloric restriction and relate key metabolites to insulin sensitivity. (Paper II)

3. **Define how RYGB or SG and caloric restriction modulate proneurotensin**

Assess fasting and postprandial pNT before and after VLCD and after RYGB or SG. Test whether postoperative increases in meal-stimulated pNT are related to GLP-1 or GIP signalling and assess fasting pNT levels up to one year after surgery. (Paper III)

4. **Quantify long-term postoperative alcohol using PEth**

Track PEth-based alcohol consumption up to 10 years after RYGB/SG, estimate the prevalence of heavy drinking, evaluate sex and procedure as predictors, and generate evidence to inform routine postoperative alcohol screening. (Paper IV)

Table 2. Overview of the four papers: design, cohort, n, intervention, primary outcome and main findings.

Paper	Design	Cohort and setting	n	Intervention or exposure	Primary outcome	Main findings
I	Prospective, paired within-subject study with four standardised mixed-meal tests (MMT).	Women with severe obesity scheduled for RYGB, Kaimar County Hospital. Nine normoglycaemic (NG), 10 with type 2 diabetes (T2D).	19	VLCD 858 kcal/day for 3-4 weeks, then RYGB. MMT at 4 weeks and 1 day before surgery, on postoperative day 1, and at 6 weeks.	Postprandial glucose, insulin, GIP and active GLP-1. Insulin sensitivity by four indices (insulin sensitivity index, Matsuda, HOMA-IR, HOMA-beta).	Caloric restriction had limited effects on insulin and incretins. RYGB raised insulin and GIP immediately, with a marked rise in GLP-1 at 6 weeks. The insulin sensitivity index improved after surgery but not after VLCD, whereas Matsuda, HOMA-IR and HOMA-beta were unchanged.
II	Untargeted metabolomics and lipidomics on the design and cohort of Paper I. Co-first authorship.	Same as Paper I.	19	As in Paper I.	Fasting and postprandial metabolite and lipid profiles, related to insulin sensitivity.	Of 719 detected features, 107 were altered by VLCD and 26 by RYGB immediately. Of the 58 features altered by the whole intervention, 40 were governed by VLCD and only 7 by RYGB. Acylcarnitines rose markedly with VLCD and were partly reversed after surgery. The metabolomic fingerprint was largely diet-driven.
III	Multi-cohort study with paired MMTs, hormone infusions and longitudinal fasting sampling.	C1 (Kaimar): as Papers I and II. C2 (Turku): obesity and T2D. RYGB or SG; plus normal-weight NG controls. C3 (Lund): longitudinal fasting cohort.	C1: 19 C2: 10 operated (RYGB 5, SG 5), 10 controls C3: 161, 92 and 118 at the three time points	VLCD and RYGB (C1). RYGB or SG; with separate GIP and GLP-1 infusions (C2). RYGB with fasting samples at baseline, 2 and 12 months (C3).	Fasting and meal-stimulated proneurotensin (pNT).	pNT fell after VLCD and immediately after RYGB, then rose again when solid food was reintroduced. A meal response emerged after RYGB and SG that GIP and GLP-1 infusion did not reproduce. Fasting pNT was still elevated at one year, irrespective of glycaemic status.
IV	Retrospective longitudinal cohort study on registry and hospital records, in two cohorts.	All patients undergoing RYGB or SG at Kaimar County Hospital 2010 to 2020, registered in SOReg. Over 75% women.	1,238 (327 with and 911 without preoperative PEth)	RYGB or SG. PEth measured up to 10 years after surgery, preoperative values in Cohort 1 only.	Heavy drinking, defined as PEth at or above 0.30 $\mu\text{mol/L}$.	At 5 years, 37.5% of men and 4.8% of women exceeded the threshold, and mean PEth rose from 0.07 to 0.38 $\mu\text{mol/L}$ in men. Male sex was the only significant predictor (OR 7.89, 95% CI 3.76 to 16.57). The pattern persisted at 10 years.

Methodology and findings in present studies

Ethical approval

All studies were approved by the relevant ethical review boards. Papers I–III were approved by the ethics committee at Lund University; Cohort 2 in Paper III was approved by the ethics committee of the Hospital District of Southwest Finland (Turku); and Paper IV was approved by the ethical review board at Linköping University. For further discussion, see the section on ethical and societal implications.

Statistical analysis

The four papers differ in design, and the statistical approach followed from that design rather than from any single unifying method. Three broad approaches were used across the thesis, and the detailed procedures for each analysis are given under the respective paper below.

Papers I to III share a repeated-measures, within-subject design, in which each participant was studied on several occasions before and after the intervention and therefore served as its own control. This design removes interindividual variation from the comparison and increases sensitivity in the small cohorts studied. In Paper I, the postprandial response of each analyte was summarised using the fasting value, 90-minute value, area under the curve, and peak-to-basal ratio. These measures were compared across occasions using one-way ANOVA within each glycaemic group, and two-way ANOVA to test the effects of glycaemic status, occasion, and their interaction. In Paper II the same repeated-measures structure was modelled using a linear mixed-effects model fitted for each metabolite and lipid, with subject as a random intercept and study occasion, glycaemic status, and their interaction as fixed effects. In Paper III, changes between time points were assessed using *t* tests, and comparisons involving more than two groups were performed using one-way ANOVA with Dunnett's post hoc test against a single prespecified reference group.

Paper II additionally required control of multiplicity, as hundreds of metabolites and lipids were tested simultaneously. Significance was therefore defined by false

discovery rate correction ($q < 0.05$) rather than an unadjusted p-value threshold, and variance estimates for individual features were stabilised using empirical Bayes moderation, which borrows information across features and is particularly important when the sample is small.

Paper IV used a registry-based observational design. Baseline characteristics of the two cohorts were compared using the Mann-Whitney U test for continuous variables and the chi-squared test for categorical variables. Dichotomous outcomes including heavy drinking (defined as $PEth \geq 0.30 \mu\text{mol/L}$) were analysed using multivariable logistic regression adjusted for age, and the proportions exceeding each $PEth$ threshold at follow-up were evaluated using logistic regression. Longitudinal changes in continuous $PEth$ concentrations were evaluated using a repeated-measures ANOVA with time as the within-subject factor, and Tukey's adjustment for post hoc pairwise comparisons.

Across all papers, descriptive data are presented as mean $\pm$ SEM or as median with range. Linear associations between continuous variables were assessed using Pearson correlation, and a two-sided significance threshold of $p < 0.05$ was applied except when false discovery rate correction was used (Paper II). Analyses were performed using GraphPad Prism (Papers I and III), R (Paper II), and Statistica (Paper IV). Full methodological details for each analysis are provided under the corresponding paper below.

Study populations and settings

Papers I and II

Papers I and II included 19 age- and weight-matched women with severe obesity undergoing RYGB at Kalmar County Hospital: 9 normoglycaemic (NG) and 10 with type 2 diabetes (T2D). Inclusion criteria included female sex, age 30–55 years, BMI $>35 \text{ kg/m}^2$, and no preoperative incretin-based medication. Exclusion criteria included prior bariatric surgery and/or prior surgery to the upper gastrointestinal tract.

All patients adhered to a preoperative very-low-calorie diet (VLCD) of 858 kcal/day (Modifast: 110 g carbohydrate, 57 g protein, 18 g fat), targeting 5% weight loss to reduce liver size and minimise perioperative risk. Participants underwent four mixed-meal tolerance tests (MMTs): 4 weeks before surgery before starting the VLCD (MMT–4w), 1 day before surgery (MMT–1d), the morning of postoperative day 1 (MMT+1d), and 6 weeks after surgery (MMT+6w) (Fig. 6). The test meal was designed to be tolerable on the first postoperative day and consisted of 200 mL Modifast (220 kcal; 28 g carbohydrates, 14 g protein, 5 g fat) ingested over 20 minutes. Blood was sampled at –5, 0, 5, 10, 15, 30, 60, and 90 minutes relative to the start of meal ingestion (Fig. 6).

19 women with severe obesity undergoing RYGB (9 normoglycaemic, 10 type 2 diabetes)

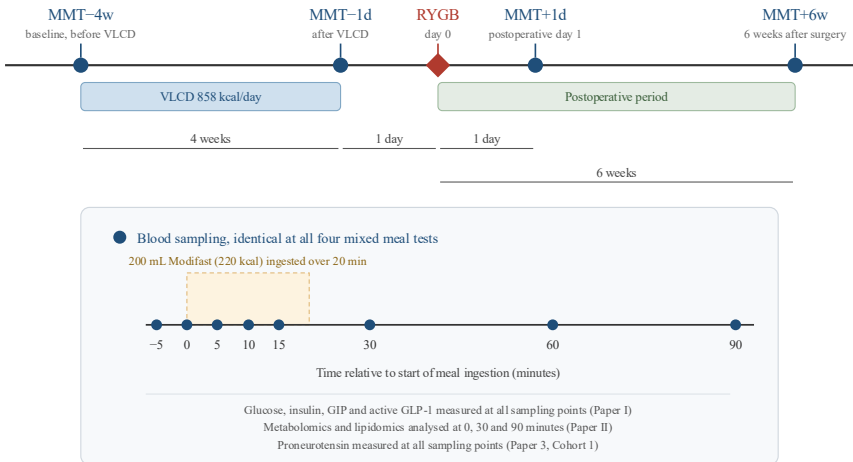


Figure 6: Study design for Papers I, II and III (Cohort 1).

Paper III

In Paper III we used samples from three previously published cohorts.

Cohort 1 (Kalmar; same as Papers I–II):

As described above, 19 women (9 NG, 10 T2D) undergoing RYGB with repeated MMTs at MMT–4w, MMT–1d, MMT+1d, and MMT+6w using 200 mL Modifast, with blood sampling at –5 to 90 minutes.

Cohort 2 (Turku, Finland):

This cohort included 10 patients with obesity and T2D undergoing RYGB (n=5) or vertical sleeve gastrectomy (SG; n=5), and 10 normal-weight NG control participants. The eligibility criteria for controls included a BMI of 18–27 kg/m², age 18–60 years, fasting plasma glucose <6.1 mmol/L, and a normal OGTT.

MMTs were performed before VLCD and at a median of 69 days postoperatively (range 55–97 days), using a 250-kcal liquid meal (NutriDrink; Nutricia Advanced Medical Nutrition: 40 g carbohydrates, 6 g fat, 9 g protein) ingested within 10 minutes. Blood sampling was at 0, 15, 30, 45, 60, and 90 minutes.

Within Cohort 2, additional GIP and GLP-1 infusion experiments were also performed as described in the original protocol (179).

Cohort 3 (Lund, fasting longitudinal cohort):

The cohort has been described previously (180). Of 167 individuals initially included, 148 subsequently qualified for RYGB during 2012 to 2019. All measurements were obtained after an overnight fast. Baseline sampling was performed at the first clinic visit, before the presurgical VLCD was introduced, on average approximately 2 months before surgery, and fasting samples were available in 161 individuals at baseline, 92 at 2 months and 118 at 12 months after RYGB.

Paper IV

Paper IV included all patients who underwent RYGB or SG at Kalmar County Hospital from 2010 onwards and who were consecutively registered in the Scandinavian Obesity Surgery Registry (SOREg). Patients were excluded if they (1) lived outside the region and therefore received postoperative follow-up elsewhere, (2) had undergone previous bariatric surgery, or (3) moved out of the region during the first postoperative year. Data were analysed retrospectively.

In response to reports in the early 2010s indicating increased risk of hazardous drinking and alcohol use disorder after bariatric surgery, Kalmar County Hospital implemented PEth screening as part of the routine pre- and postoperative follow-up aligned with SOReg-based follow-up practice. In 2022, data were extracted from SOReg and local hospital records. The initial database comprised 1,508 patients. After exclusions, the final study population included 1,238 patients.

Methods and statistics

Paper I

Methods

Using cohort 1 and the MMT protocol described above, plasma glucose, insulin, GIP, and active GLP-1 were measured at each of the four test occasions. Plasma glucose was measured using an enzymatic glucose oxidase/oxidase colorimetric assay and quantified spectrophotometrically against calibrators. Insulin was measured using a commercial sandwich ELISA. GIP and active GLP-1 were measured using ELISA from Millipore (Billerica, MA). For active GLP-1 and GIP, blood was collected with DPP-4 inhibition and plasma was processed on ice and stored frozen until analysis.

Statistics

Data were reported as mean $\pm$ standard error of the mean (SEM). The postprandial response for each analyte was summarised using four complementary measures: basal fasting values, to capture the baseline metabolic state across groups and occasions; 90-minute values, to reflect persistence of hyperglycaemia, sustained hormone response, or the rate of return towards baseline; area under the curve (AUC), to summarise the entire postprandial trajectory into a single value, and the peak-to-basal ratio, to quantify the magnitude of the meal-stimulated rise relative to each individual's or group's starting level. A one-way ANOVA with Bonferroni post hoc test was used to compare these summary measures across the four test occasions within each glycaemic group, and two-way ANOVA to test the effects of glycaemic status (T2D versus NG), test occasion, and their interaction. Insulin sensitivity was assessed using an insulin sensitivity index derived from fasting and meal-stimulated glucose and insulin, together with the Matsuda composite index, HOMA-IR, HOMA- β , and the insulinogenic index (IGI).

Paper II

Methods

Using the same cohort (Paper 1) and MMT protocol, metabolite and lipid profile were assessed in plasma samples collected at fasting (0 min) and postprandially at 30 and 90 minutes, capturing early and late meal-related responses. Metabolites were extracted and analysed by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC–QTOF-MS), and lipids were analysed on a separate UPLC platform. Metabolites and lipids were identified using tandem mass spectrometry. Samples were analysed in two analytical batches balanced for glycaemic status, with batch-to-batch variation corrected using ComBat. Data were log₂-transformed, and features with more than 15% missing values were excluded. Remaining missing values were imputed using k-nearest-neighbour averaging.

Statistics

Principal component analysis (PCA) was used to visualise the overall structure of the metabolomic data, providing an unsupervised overview of whether samples clustered by study occasion, glycaemic status, or both, before any formal hypothesis testing was applied.

For hypothesis testing, a linear mixed-effects model was fitted for each individual metabolite and lipid species. Because every patient was measured repeatedly across up to four occasions, the model included subject as a random intercept, effectively letting each patient serve as her own control and removing inter-individual variation from the analysis. The fixed effects in the model were study occasion, glycaemic status (T2D versus NG), and their interaction. The interaction term was included to detect whether a given metabolite responded differently to the intervention in T2D patients compared with normoglycaemic patients. Within this framework, four planned contrasts were defined to isolate specific effects: the effect of VLCD (–1d versus –4w), the immediate effect of surgery (+1d versus –1d), the 6-week postoperative state (+6w versus –1d), and the cumulative effect of the entire intervention (+6w versus –4w).

As hundreds of metabolites and lipids were tested simultaneously, a conventional $p < 0.05$ threshold would yield a large number of false positive findings by chance alone. Significance was therefore defined as $q < 0.05$ after false discovery rate (FDR) correction, which controls the expected proportion of false discoveries among all features declared significant rather than the probability of any single false positive. Associations between metabolite levels and clinical markers of glycaemic status (insulin, HOMA- β , Matsuda index) were assessed using linear models with empirical Bayes moderation, a technique that stabilises variance estimates for

individual metabolites by borrowing information across all features, particularly important when sample sizes are small and variance estimates for any single metabolite would otherwise be unreliable. Model-derived effects were summarised and visualised using hierarchical clustering and heatmaps.

Paper III

Methods

Three independent cohorts (179-181) were used to examine different aspects of the neurotensin response to diet and bariatric surgery. Since mature neurotensin is unstable, the stable N-terminal fragment pNT was measured. pNT is produced in stoichiometric amounts relative to mature neurotensin. Concentrations were quantified using a chemiluminometric sandwich immunoassay (SphingoTec GmbH). Cohort 1 (the same cohort as in Papers I and II) enabled differentiation between the effects of VLCD and surgery *per se* on fasting and postprandial pNT. Cohort 2 was used to replicate findings in cohort 1 and for comparing the effect of distinct types of surgery, and to examine whether the neurotensin response was influenced by increased incretin concentrations. The latter was investigated using GIP- and GLP-1-infusion experiments designed to mimic postprandial concentrations after RYGB. Cohort 3, a larger longitudinal cohort of fasting samples, was used to assess fasting pNT before and at 2 and 12 months after RYGB, providing longer-term validation of the observed effects.

Statistics

Data were presented as mean $\pm$ SEM or as median with range. Changes between two time points within the same cohort or within an MMT time course were assessed using *t* tests. For between-group comparisons involving more than two groups, one-way ANOVA was used, with post hoc testing performed using Dunnett's multiple comparisons test when the comparison was against a single prespecified reference group (typically controls or normoglycaemia). The ANOVA/Dunnett framework assumes similar variance across groups and uses a single pooled variance estimate, making it more efficient than all-pairs procedures when only comparisons to the reference are of interest. Pearson correlation coefficients were used to evaluate linear associations between continuous variables. Outliers were identified using ROUT (robust regression and outlier removal), a false discovery rate-based approach intended to reduce the influence of extreme values.

Paper IV

Methods

To optimise analyses given differences in available baseline PEth data and follow-up duration, the population was stratified into two cohorts:

Cohort 1: Patients with preoperative PEth measurements (surgery in 2015 or later), allowing follow-up of up to 5 years.

Cohort 2: Patients without preoperative PEth measurements, allowing postoperative follow-up from 1 year up to 10 years.

The primary rationale for this two-cohort approach was statistical: the cohort with baseline PEth enabled stronger within-individual inference across time, whereas the cohort without baseline PEth contributed longer-term postoperative estimates, albeit with less robust statistical inference.

Statistics

Baseline characteristics of the two cohorts were compared using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. The primary outcome, heavy drinking, was defined as PEth ≥ 0.30 $\mu\text{mol/L}$, while PEth ≥ 0.05 $\mu\text{mol/L}$ was used to identify at least moderate alcohol consumption. Both decision limits follow the national Swedish harmonisation of PEth 16:0/18:1 issued by the Equalis expert group for pharmaceuticals and toxicology (182). PEth values below the limit of quantification (<0.05 $\mu\text{mol/L}$) were set to 0.05 $\mu\text{mol/L}$ for calculation of means.

The association between baseline characteristics and heavy drinking at 5 years (dichotomised as PEth ≥ 0.30 $\mu\text{mol/L}$ versus <0.30 $\mu\text{mol/L}$) was assessed using multivariable logistic regression, adjusted for age. The proportions exceeding each threshold at 1, 2, 5, and 10 years were compared across time points using logistic regression models. Longitudinal changes in continuous PEth concentrations were evaluated using repeated-measures analysis of variance (ANOVA) with time as the within-subject factor. Post hoc pairwise comparisons between time points were performed with Tukey's adjustment for multiple testing.

Results and discussion

Metabolic mechanisms of gastric bypass: beyond caloric restriction

A central question in bariatric surgery research is to what extent the metabolic improvements observed after Roux-en-Y gastric bypass are driven by caloric restriction versus the surgical rearrangement of the gastrointestinal tract itself. Since the early observations in the 1990s that RYGB can induce remission of type 2 diabetes before substantial weight loss has occurred, the relative contributions of these two factors have remained debated (183, 184). The studies included in this thesis address this question from three complementary perspectives: hormonal responses to a mixed meal (Paper I), the broader metabolomic and lipidomic profile (Paper II), and changes in the gut hormone neurotensin (Paper III). Taken together, I conclude that caloric restriction and surgical rearrangement produce overlapping but distinct effects, operating at different biological levels and on different timescales.

The incretin and insulin response: evidence for surgery-specific effects

Paper I demonstrated that RYGB exerts powerful and immediate effects on insulin and incretin hormone responses to a standardised mixed meal, independently of the changes induced by preoperative caloric restriction. Caloric restriction, achieved through three to four weeks of VLCD, had limited effects: fasting and postprandial insulin levels were reduced in T2D patients, but glucose, GIP, and GLP-1 remained unaffected. In contrast, RYGB immediately increased plasma insulin and GIP, changes observed already at the very first postsurgical meal (MMT+1d), a time point not previously reported in the literature.

The finding that caloric restriction did not affect glucose levels stands in contrast to Lingvay et al., who argued that the major effect of RYGB on glycaemia is mediated via reduced food intake (185). However, in the Lingvay study patients had already been on VLCD for two days before baseline measurements, making it difficult to isolate the true pre-diet state, and their patients had a longer diabetes duration (7.4 versus 3.1 years in our cohort), which is known to negatively influence remission. Their dietary intervention was considerably more aggressive than ours, averaging roughly 250 kcal/day including near-fasting days, compared with the 858 kcal/day

VLCD used in our setting, hence a larger acute glycaemic effect would be expected. Furthermore, Lund et al. observed numerically higher GLP-1 and GIP responses to an oral glucose load after a low calorie diet, but did not test this effect statistically and attributed their significant incretin changes to surgery rather than to the diet (186). Their study also differed from ours in using a 25 g oral glucose load rather than a mixed meal ingested over 20 minutes, and a substantially longer diet of 64 days versus 21 to 28 days. Thus, a modest diet effect could have been present without being detected in either study. Sumithran et al. likewise reported increased postprandial GIP secretion after a ten-week very-low-energy diet, again with a longer intervention and a larger meal stimulus than ours (159). On the other hand, Isbell et al. similarly found no changes in GIP or GLP-1 after a shorter period of caloric restriction, supporting our findings and suggesting that the incretin response to caloric restriction may depend on both the duration and content of the dietary intervention and the type of stimulus used (187).

The GLP-1 response followed a different temporal pattern from that of GIP, and the two glycaemic groups diverged. In normoglycaemic patients the response rose steeply at the first postoperative meal and had fallen back by six weeks. In T2D patients the rise was delayed, reaching its maximum at six weeks, when they displayed markedly higher GLP-1 concentrations than their normoglycaemic counterparts. This delayed but robust GLP-1 response in T2D patients is notable and questions the role of GLP-1 as a mediator of the enhanced insulin response seen immediately after RYGB. It suggests that the enteroendocrine system may require a period of adaptation after anatomical rearrangement, possibly reflecting changes in L-cell density or sensitivity in the alimentary limb. Histological data from biopsies taken some ten months after RYGB show increased L-cell and K-cell density in the alimentary limb, consistent with such adaptation, although the time course over which it develops has not been established (188). The postprandial GIP increase seen in Paper I was transient, no longer significant by six weeks. This early rise is notable as it was captured at the first postoperative meal, a timepoint not previously examined, and it contrasts with the broader literature. Jørgensen et al. reported meal-stimulated GIP to be essentially unchanged in the first weeks after RYGB and reduced by one year, with only a transient increase in peak GIP in normoglycaemic patients at three months (151). Hindsø et al., studying participants without diabetes a median of about two years after surgery, found the postprandial GIP response to be lower after RYGB than after sleeve gastrectomy, with a similar but non-significant tendency compared with matched unoperated controls (150). Such reports are difficult to reconcile with one another, since the studies differ not only in postoperative timing but also in the size and composition of the test meal, in the assay used, and in whether the comparator was another procedure or an unoperated group. The finding that T2D patients exhibited a lower insulin response to the meal compared with normoglycaemic patients after RYGB, despite similar glucose levels, likely reflects underlying beta-cell dysfunction in the diabetic group. The fact that glucose levels remained unchanged throughout the study in both

groups was somewhat unexpected but may be explained by continued antidiabetic medication in T2D patients up until surgery.

The concept that RYGB has effects independent of caloric restriction is further supported by complementary experimental approaches. Lindqvist et al. compared meals delivered orally with those delivered through a gastrostomy in patients who had undergone gastric bypass and demonstrated markedly different incretin and insulin responses depending on the route of nutrient exposure. This provides direct evidence that the surgical anatomy per se drives the hormonal changes (189). Similarly, sham-operated pair-fed pigs did not exhibit the same improvements in glycaemia and insulin response as RYGB-operated animals (190). In a clinical setting, Courcoulas et al. randomised 61 participants with T2D to RYGB, adjustable gastric banding, or lifestyle intervention alone. At three years, partial or complete remission was achieved in 40% of the RYGB group and 29% in the banding group, but by none in the lifestyle group (191). Remission rate tracked the magnitude of weight loss across the three arms rather than the presence of intestinal rerouting, and in the RYGB group it declined from 60% at one year to 40% at three years, indicating that the surgical anatomy alone does not sustain remission.

In Paper we found that one of four measures of insulin sensitivity, the insulin sensitivity index, improved after RYGB but not after caloric restriction, while the Matsuda index, HOMA-IR and HOMA- β were unchanged by either intervention. This contrasts with Bojsen-Møller et al., who attributed early hepatic insulin sensitivity gains primarily to caloric restriction rather than to surgery-specific anatomical effects (192), although their study included no diet-matched control group. The discrepancy likely reflects methodological differences, including their inclusion of a 9% preoperative diet-induced weight loss before baseline and the use of a hyperinsulinaemic clamp rather than mixed-meal tests.

A further consideration cuts across all three studies, namely which tissue is being assessed. Campos et al. found that peripheral glucose uptake improved only once substantial weight loss had occurred, and in proportion to the amount of weight lost (193), while Bojsen-Møller et al. described hepatic insulin sensitivity as improving early and peripheral insulin sensitivity later (192). The indices used in Paper I are derived from a meal challenge and reflect whole-body insulin action, and do not separate the hepatic from the peripheral contribution. Based on the data in these two studies, the early improvement observed in Paper I is most plausibly hepatic in origin, with the peripheral component developing later as weight loss accumulates, although we cannot demonstrate this directly, due to study design. Read this way, these findings are consistent with rather than contrary to the interpretation advanced here, since they place the durable part of the gain in insulin sensitivity within the domain of sustained energy deficit.

Bojsen-Møller et al. are not alone with this concept. Two studies that replicated the postoperative intake in a control condition reported that caloric restriction

reproduced the early improvement in insulin sensitivity in full. Four days after RYGB, the fall in insulin resistance was no greater than after a matched period on the identical postoperative diet (187), and three days of caloric restriction matched to the intake after biliopancreatic diversion with duodenal switch improved insulin sensitivity and the disposition index to the same extent as surgery itself (165). Three differences may account for the divergent result in Paper I. Isbell et al. compared separate groups of individuals, rather than using each participant as its own control, whereas Plourde et al. studied the same individuals sequentially. Both replicated the postoperative intake with regimens far more severe than the clinically standard preoperative diet used here, a day of water only followed by 200 to 300 kcal daily in Isbell et al. and an intake matched to the first three days after biliopancreatic diversion in Plourde et al., so the restriction arm may have produced effects that a standard preoperative diet does not. And both assessed the immediate postoperative days, when the metabolic response to surgical stress is still unfolding, a limitation that both studies acknowledge and that they note could have masked a larger effect of surgery.

The metabolism perspective: caloric restriction as the dominant driver

In Paper II we extended the investigation from targeted hormone measurements to an untargeted metabolomic and lipidomic analysis, using the same cohort and study design as in Paper I. This allowed for a comprehensive assessment of whether the metabolic fingerprint observed after RYGB is attributable to surgery per se or to the preceding caloric restriction.

The results provided a more nuanced picture than the hormonal data alone would suggest. Principal component analysis separated the samples by study occasion, and the lipid profile was dominated by VLCD effects rather than by surgery effects, with the samples taken after VLCD, one day after RYGB and at six weeks clustering together and away from baseline samples. Out of 719 detected features (metabolites and lipids), 107 were affected by VLCD, whereas 26 showed an immediate effect of RYGB and 27 an effect of RYGB at six weeks. The cumulative effect of the entire intervention (baseline vs. six weeks post-RYGB) was associated with alterations in 58 features, of which 40 were governed by VLCD and only 7 by RYGB.

Acylcarnitine levels emerged as a particularly informative metabolite class. VLCD caused a pronounced increase in acylcarnitines, reflecting enhanced mitochondrial fatty acid oxidation during caloric restriction. This is a well-established metabolic adaptation to fasting (194). RYGB, both immediately and at six weeks, produced a smaller and opposite effect on short- and long-chain acylcarnitines, suggesting that the surgical state and the subsequent recovery period partially reverse the calorie-restriction-driven increase in fatty acid oxidation as food intake resumes. Changes in phospholipids and triglycerides followed a similar pattern, with the VLCD effect

dominating and the lipid profile after VLCD and after surgery clustering together on principal component analysis, distinct from the baseline profile.

These findings challenge the assumption that the metabolic improvements commonly attributed to RYGB in clinical practice are surgery-specific. Rather, the data indicate that many of the early changes in metabolism are a consequence of the caloric restriction that both precedes and follows surgery. This interpretation aligns with Lips et al., who concluded that caloric restriction is a major determinant of the short-term metabolic effects of gastric bypass in obese T2D patients, though their study used a between-group rather than a within-subject design (195). The observation that acylcarnitine levels rose during a 12-week hypocaloric weight loss intervention in a study by Schooneman et al., increasing at 28 days and partly declining again by 84 days, is also consistent with our findings, likely reflecting the transition from caloric restriction-driven fat oxidation to more normalised substrate handling as energy intake stabilises (196). However, our conclusions contrast with the prevailing narrative in much of the metabolomics literature, where studies examining short-term (4–15 days) and longer-term (1–12 months) consequences of RYGB and sleeve gastrectomy have generally reported metabolomic changes as effects of surgery without systematically accounting for the confounding effect of the mandatory preoperative diet. Since most studies assessed the combined effect of caloric restriction and RYGB rather than separating the two, the uniquely surgery-attributable metabolite signature has likely been overestimated. Our paired design, with tight sampling intervals bracketing both the VLCD and the surgical procedure, was specifically designed to disentangle these contributions.

This conclusion does not diminish the importance of RYGB. The hormonal changes documented in Paper I are clearly surgery-specific, but it does highlight that different analytical readouts can yield different answers to the same question. The metabolite profile is dominated by substrate flux (which tracks caloric intake), while incretin and insulin responses reflect anatomical rearrangement of the gut.

An important additional observation was that acylcarnitine and triglyceride profiles were associated with clinical markers of glycaemic status, including insulin levels, HOMA- β , and the Matsuda index. This raises the possibility that metabolomic profiling could serve as a tool for monitoring metabolic recovery after surgery, although this would require validation in larger cohorts.

A limitation of the study is that postprandial samples were analysed at only two time points, 30 and 90 minutes. Thus, we may have missed other temporal patterns in metabolites and lipids.

Proneurotensin: a novel marker with a distinct profile

Paper III extended the hormonal perspective from the incretins to proneurotensin, the stable circulating precursor of neurotensin. The effects of bariatric surgery on

neurotensin have been comparatively understudied, and the available human data derive from small cohorts that differ in surgical procedure, biliopancreatic limb length, and sampling time points (163, 164, 197).

Using three independent cohorts, the study revealed a temporal dissociation in the pNT response. Acutely, both VLCD and RYGB reduced fasting pNT levels to a similar extent, suggesting that the initial drop is driven by caloric restriction rather than by surgical anatomy. This aligns with the metabolomic findings in Paper II, where the acute postoperative metabolite profile was largely indistinguishable from that of the post-VLCD profile. However, at six weeks post-RYGB, fasting pNT levels rebounded to levels exceeding the pre-VLCD baseline levels. This increase was replicated in a second cohort at three months post-surgery and confirmed in a third cohort up to one year after RYGB, regardless of glycaemic status. The persistence of elevated fasting pNT for at least a year after surgery is consistent with Christ-Crain et al., who reported increased fasting pNT levels up to 24 months after RYGB but not after gastric banding, suggesting that the effect is specific to procedures that alter the anatomy of the small intestine rather than purely restrictive operations (163). This is likely related to the more rapid delivery of nutrients to the N-cell-rich distal small intestine after RYGB. Von Loeffelholz et al. similarly reported elevated proneurotensin levels after both RYGB and biliopancreatic diversion, and demonstrated a correlation between proneurotensin and insulin sensitivity, consistent with a role for this hormone in postoperative metabolic improvement (197).

Equally noteworthy was the finding that the postprandial pNT response was absent in both normal-weight controls and in patients with obesity prior to surgery. After RYGB or sleeve gastrectomy, however, a marked meal-stimulated rise in pNT emerged where none had been present before, and this response was greater after RYGB than after SG. This is consistent with Svane et al., who described enhanced neurotensin secretion after RYGB, with early postprandial neurotensin also elevated after sleeve gastrectomy (164). Holdstock et al. likewise found markedly higher proneurotensin concentrations in gastric bypass patients than in subjects with obesity or normal-weight controls, although in their cross-sectional study, performed a mean of 43 months after surgery with only two postprandial samples, concentrations did not rise in response to the meal (198). Notably, GIP infusion did not reproduce the pNT response, either in normal-weight controls or in patients before or after RYGB, and GLP-1 infusion, tested in normal-weight participants, was likewise without effect. This suggests that the postoperative pNT meal response is not merely a downstream consequence of incretin stimulation but likely reflects direct nutrient exposure to neurotensin-producing N-cells in the alimentary limb. The observation that the main trigger for pNT appears to be fat intake is supported by the small fat content of our mixed meal (only 5 g). In contrast, studies using larger fat loads, such as the 54 g load in the study by Fawad et al., demonstrated a clear pNT response even in non-surgical subjects (199). This suggests that the surgical rearrangement

lowers the threshold for nutrient-stimulated neurotensin release, possibly by delivering undigested fat more rapidly to distal N-cells. Patrício et al. provided further support for this interpretation by showing that gut hormone release after gastric bypass depends on the length of the biliopancreatic limb: a 200 cm limb produced higher fasting and postprandial neurotensin and GLP-1 than a classical limb, whereas the postprandial GIP response was lower (200).

Synthesis: overlapping but distinct mechanisms

Viewed together, Papers I–III paint a picture that is more nuanced than either a purely surgery-specific or purely diet-driven narrative. The immediate postoperative period is characterised by a dominant influence of caloric restriction on the metabolome, while the hormonal milieu is simultaneously reshaped by the anatomical rearrangement. Over time, surgery-specific effects become more apparent: GLP-1 responses increase progressively, particularly in T2D patients; fasting pNT levels rise above preoperative baseline levels; and a marked postprandial pNT response emerges where none existed before, independent of GIP and GLP-1.

The preoperative glycaemic state modulates many of these responses. T2D patients showed a more pronounced GLP-1 response at six weeks (Paper I), more subtle changes in medium- and long-chain acylcarnitines (Paper II), and pNT elevations comparable to those in normoglycaemic subjects (Paper III). This heterogeneity underscores that the metabolic effects of bariatric surgery cannot be reduced to a single mechanism and that the response to surgery reflects an interplay between the preexisting metabolic state, diet, and the surgical intervention itself.

That said, the overall picture emerging from these studies is that caloric restriction is a major contributor to the early metabolic improvement after RYGB, and quite plausibly the dominant driver of its longer-term maintenance. In our paired design study, the early fasting metabolome was altered far more by the VLCD than by surgery itself, and the lipid profiles after diet and after surgery clustered together, away from baseline. Most published studies do not separate the mandatory preoperative diet from the operation. Hence the metabolite changes they attribute to surgery likely include a substantial diet-driven component. Consequently, the uniquely surgery-attributable signature has probably been exaggerated. Paper II examined only the early postoperative period (up to six weeks), however, and therefore cannot by itself establish what drives improvement in glycaemia over the longer term. Whether the surgery-specific hormonal changes documented in Papers I and III are sufficient on their own to sustain metabolic remission without continued caloric restriction remains an open question, and the evidence bearing on it comes largely from longer-term work by other groups rather than from the present studies. Several lines of evidence suggest that hormonal alterations are not sufficient to explain the effect on metabolic remission. First, metabolic relapse is well

documented when patients regain weight and resume higher caloric intake, despite persistent surgical anatomy and presumably ongoing alterations in incretin secretion. This points to caloric balance as the decisive long-term factor. From our group, Rogova et al. recently demonstrated that metabolic alterations towards the pre-surgical state precedes possible weight regain, further supporting the idea that when caloric restriction wanes, metabolic benefits erode regardless of hormonal changes (180). Second, T2D remission rates decline over time, as shown in long-term follow-up studies such as the 10-year data from Mingrone et al. (201). This decline correlates more closely with gradual increases in food intake than with any demonstrated decline in incretin responses. Third, studies that hold caloric intake constant across the surgical and the dietary condition separate the hormonal from the metabolic response. Four days after RYGB the postprandial GLP-1 response had risen only after surgery and not after matched restriction, yet the improvement in insulin resistance was equivalent in the two conditions (187). The same dissociation was seen after biliopancreatic diversion with duodenal switch, where the improvement in beta-cell function usually attributed to the incretin response occurred in the absence of that response, and where the postprandial GIP response was reduced by caloric restriction alone, although a smaller test meal necessarily provides a weaker proximal stimulus irrespective of anatomy (165). The authors of the latter study proposed that gut-hormone signalling matters chiefly in sustaining the satiety that makes prolonged caloric restriction tolerable, an interpretation close to the one advanced here. Fourth, from a physiological standpoint, it is difficult to argue that the modest and pulsatile increases in endogenous GLP-1 after RYGB can sustain the same metabolic impact as the profound and continuous caloric deficit imposed by the surgery in its most effective phase.

This is illustrated by GLP-1 receptor agonists, which achieve sustained, supraphysiological receptor activation far exceeding the modest endogenous rises seen after RYGB, yet produce weight loss and glycaemic improvement largely by suppressing appetite and food intake, with the benefit dependent on continued treatment, and weight regain following discontinuation (49). If even maximal pharmacological GLP-1 agonism acts mainly through reduced caloric intake and erodes once treatment stops, it is difficult to attribute the durable effects of surgery to the much smaller endogenous GLP-1 changes acting independently of caloric balance.

This interpretation does not weaken the clinical value of RYGB; the surgery creates the anatomical conditions that make long-term caloric restriction more achievable through accelerated satiety, altered food preferences, and the hormonal changes that reinforce reduced intake. In this view, gut hormone changes may be better understood as facilitators of caloric restriction rather than as independent mediators of metabolic improvement. The distinction matters because it shifts the clinical focus toward ensuring that patients maintain reduced caloric intake over the long

term, and it has implications for understanding why some patients achieve lasting remission while others do not.

After a very-low-energy diet, the hormonal profile shifts in the direction that favours weight regain: peptide YY and cholecystokinin fall, ghrelin rises, and these changes are still detectable one year after the initial weight reduction (159). GIP moves in the opposite direction, with greater secretion in the first hour after a meal both at ten weeks and at one year, a change that points the same way, since GIP is thought to promote energy storage (159). Bariatric surgery moves peptide YY, cholecystokinin and ghrelin in the opposite direction, towards a profile that supports rather than opposes reduced intake. Yet when weight loss is matched between gastric bypass and a hypocaloric diet, fasting glucose, insulin and HOMA-IR decline to a similar extent in both, whereas the incretin response and postprandial glycaemic control improve only after surgery (160). Taken together, these observations suggest that at a given degree of energy deficit the two interventions converge in the fasting state but diverge postprandially, and that they differ most in how readily that deficit can be sustained. On this reading the postoperative gut hormone changes act primarily on adherence rather than on metabolism directly: hormonal changes make prolonged caloric restriction achievable, and it is the restriction that causes most of the metabolic improvement. The apparent paradox, that dietary weight loss produces a markedly less favourable hormonal profile than surgery while much of the early metabolic gain is comparable, is resolved once these two outcomes are kept separate.

Proneurotensin illustrates the same split within a single hormone. Fasting concentrations fall acutely after caloric restriction and after surgery alike, tracking energy balance rather than anatomy, whereas the postprandial response emerges only after surgery (Paper III). The two components of the same signal therefore follow the two different determinants.

The glycaemic dimension, and insulin resistance in particular, is where that correspondence breaks down, and it fits the framework rather than sitting outside it. In Paper I we showed that whole-body insulin sensitivity improved after RYGB but not after caloric restriction alone. Type 2 diabetes reflects a dual defect of impaired insulin secretion and insulin resistance, and RYGB appeared to act on both: the meal-stimulated insulin and incretin response was augmented at the first postoperative meal, and one measure of insulin sensitivity had improved by six weeks. Together these may contribute to a substantial part of the early improvement in glucose handling, although the finding on insulin sensitivity is not consistently reproduced when caloric intake is matched between conditions. Caloric restriction also lowered fasting and postprandial insulin in the T2D group, indicating that it contributed to the early glycaemic changes, though without the improvement in measured insulin sensitivity seen after surgery. What the long-term evidence adds is that this surgery-initiated improvement in insulin sensitivity and secretion is not, on its own, sufficient to maintain glycaemic remission: when caloric intake rises

and weight is regained, insulin resistance and hyperglycaemia return despite the surgical anatomy remaining in place. The early improvement in insulin resistance and glycaemia is therefore best understood as a surgery-initiated advantage that continued caloric restriction is required to sustain. The gut hormone changes contribute to that sustained restriction rather than substituting for it.

Alcohol use after bariatric surgery: a long-term concern

The metabolic benefits discussed above represent one side of the long-term outcomes after bariatric surgery. However, a comprehensive understanding of the consequences of surgery must also address potential adverse outcomes. In Paper IV we examined alcohol consumption patterns up to ten years after bariatric surgery, using the objective biomarker PEth, and revealed a sustained increase in heavy drinking among a clinically important subgroup of patients.

Biomarker-based evidence of increased alcohol consumption

Most patients undergoing bariatric surgery did not develop excessive alcohol consumption. Women, who constituted more than 75% of the study population, typically remained low-level consumers throughout follow-up. However, a substantial proportion of patients, particularly men, showed a progressive and sustained increase in alcohol intake. Five years after surgery, 37.5% of men had PEth concentrations above the clinical threshold for heavy drinking (≥ 0.30 $\mu\text{mol/L}$), compared with a low prevalence at baseline. Men had an eightfold higher risk of excessive drinking than women (OR 7.89; 95% CI 3.76–16.57). Sex was the only significant predictor in the multivariable analysis; age, type of surgery, quality-of-life measures, and degree of weight loss were not associated with heavy drinking. The second cohort, which lacked preoperative PEth measurements and therefore could not support within-individual comparisons, showed a similar pattern over a longer period: at ten years, 39.5% of men and 18.8% of women exceeded the heavy-drinking threshold. Within that cohort the proportion among women rose steadily, from 7.3% at one year to 14.5% at five years and 18.8% at ten years, suggesting that women, although at much lower risk than men, may develop heavy drinking later in the postoperative course. This finding is however based on the less robust cohort and should be interpreted cautiously.

These findings extend and complement earlier PEth-based studies. Walther et al. demonstrated increased PEth concentrations after gastric bypass, but noted that these patients still had lower PEth concentrations than blood donors. This raised the question of whether the increase represented a clinically significant problem or partly reflected altered alcohol pharmacokinetics after surgery (202). Siikaluoma et al. reported that 8.3% of patients had PEth concentrations above 0.30 $\mu\text{mol/L}$ at two

years, similar to the 9.3% observed at two years in our cohort with preoperative measurements (203).

Their study, however, had preoperative PEth results for only part of the cohort, which precluded within-individual comparison. Our finding of an eightfold higher risk among men points in the same direction as Siikaluoma et al., who reported a doubled risk in men in an unadjusted analysis (OR 2.14), although our estimate is considerably larger. Beyond these earlier reports, our study provides substantially longer follow-up (up to 10 years rather than 2 years), a larger longitudinal cohort, and a subcohort with baseline PEth measurements that permits more robust within-individual analysis.

The AUDIT-based literature provides a broader but methodologically different comparison. The landmark study by King et al. (2012) using the AUDIT in a US multicentre cohort found that the prevalence of AUD after RYGB rose from 7.0% in the year before surgery to 10.7% in the second postoperative year, whereas no significant change was seen after gastric banding (167). In a subsequent study with follow-up to seven years, King et al. (2017) found that incident AUD symptoms, illicit drug use and substance use disorder treatment were all about twice as common after RYGB as after gastric banding (166). The Swedish Obese Subjects (SOS) study, reported by Svensson et al., similarly found increased alcohol problems after gastric bypass and vertical banded gastroplasty, but not after banding (170). Östlund et al. showed increased hospital admissions for alcohol dependence specifically after gastric bypass compared with restrictive procedures (169). Our PEth-based findings are broadly consistent with these reports but suggest that the magnitude of the problem may be larger than self-report instruments indicate, particularly among men, a finding with direct implications for clinical screening practice.

The use of PEth as the primary endpoint is a methodological strength compared with most previous studies. PEth is formed exclusively in the presence of ethanol and is highly specific for alcohol consumption, thereby eliminating the reporting bias inherent in questionnaires. Such bias may be particularly pronounced before surgery, when patients know that alcohol use may affect their eligibility. An important consideration, however, is that PEth sensitivity is influenced by body size, with lower sensitivity at higher BMI (204). The presumed mechanism is that total body water determines the volume of distribution of ethanol; the same intake therefore produces a higher blood alcohol concentration, and consequently greater PEth formation, in a person with less total body water. As patients lose weight after surgery, the same alcohol intake could theoretically produce higher PEth concentrations. Walther et al. raised this as a possible partial explanation for the observed increase (202). Although this pharmacokinetic effect cannot be entirely excluded, the progressive and sustained increase over five to ten years, well beyond the period of active weight loss, argues against altered pharmacokinetics as the sole explanation.

No significant difference was observed between RYGB and sleeve gastrectomy, although the study was not specifically powered to detect such differences. This finding is noteworthy because the pharmacokinetic arguments for increased alcohol bioavailability after RYGB (reduced gastric alcohol dehydrogenase activity, rapid jejunal transit with accelerated absorption, higher peak blood alcohol concentration, prolonged elimination) have traditionally been emphasised primarily for this procedure. Earlier reports, including Östlund et al. and findings from the SOS study, suggested a lower risk after purely restrictive procedures such as gastric banding (169, 170). These analyses predate the widespread adoption of SG and included no sleeve gastrectomy patients, their restrictive comparison groups consisting of vertical banded gastroplasty and gastric banding, therefore they cannot speak to the risk after SG. More recent evidence, including studies by Maciejewski et al. and Wong et al., indicates that SG patients may also be at increased risk (205, 206). Taken together with our own findings, this suggests that increased alcohol consumption should not be regarded as a complication specific to gastric bypass. A risk that appears after two procedures with different anatomical consequences is difficult to attribute solely to the bypass-specific pharmacokinetic changes described above, and points instead towards a mechanism shared by both.

The GLP-1 paradox: connecting alcohol use with metabolic physiology

An intriguing aspect of the alcohol findings is their relationship to the gut hormone changes documented in Papers I and III. Pharmacological GLP-1 receptor agonists reduce alcohol intake and craving in both preclinical and clinical settings (173-175). The mechanistic basis lies in the mesolimbic dopamine reward system. GLP-1 receptors are expressed in key nodes of this pathway, including the ventral tegmental area and the nucleus accumbens, and GLP-1-producing neurons in the nucleus of the solitary tract project directly to these regions, where GLP-1R signalling modulates reward processing (207, 208). In preclinical models, GLP-1 receptor agonists attenuate alcohol-induced accumbal dopamine release, locomotor activation and conditioned place preference, and reduce alcohol intake and relapse-like behaviour (173, 174, 209). Complementary human imaging studies show that GLP-1R activation dampens activity in appetite- and reward-related brain areas in response to appetitive cues (210). GLP-1 signalling is thus not only a regulator of appetite and energy intake but a modulator of reward salience more broadly. Given that RYGB markedly amplifies the postprandial GLP-1 response, as demonstrated in Paper I, it might seem paradoxical that bariatric surgery is associated with higher, rather than lower, rates of problematic alcohol use.

It is tempting to assume that the rise in endogenous GLP-1 after surgery would exert effects equivalent to those of a pharmacological agonist, but several distinctions argue against this. Endogenous GLP-1 is released in brief postprandial pulses and is rapidly degraded by DPP-4, with a circulating half-life of only a few minutes,

whereas long-acting GLP-1RAs provide sustained, supraphysiological receptor activation over hours to days (106, 211). Endogenous GLP-1 also rises primarily after nutrient ingestion and not after alcohol intake. Acute alcohol ingestion in fact lowers circulating GLP-1 by roughly one third, to a similar degree after metabolic surgery (212). The short-lived, meal-triggered surges that follow RYGB are therefore unlikely to achieve the sustained engagement of central reward-modulating GLP-1 receptors that characterises pharmacological treatment, which offers a plausible explanation for why surgery does not reproduce the dampening of alcohol-related reward and craving reported with GLP-1RAs (213).

The findings of Paper IV are consistent with this interpretation. Alcohol consumption rose progressively over five to ten years in a substantial proportion of patients despite the amplified postprandial GLP-1 response that follows surgery, and no significant difference was seen between RYGB and SG. Whatever protective potential resides in the GLP-1 system, endogenous secretion after surgery does not appear to realise it, and the pharmacokinetic changes that accelerate alcohol absorption after surgery act in the opposite direction. This distinction has clinical relevance in an era when GLP-1RAs are increasingly used for T2D and weight management and, in emerging research, for alcohol use disorder (175). Surgery raises endogenous GLP-1 without protecting against problematic alcohol use, whereas pharmacological agonists appear to reduce it. The difference may lie less in how strongly the receptor is engaged than in when, meal-triggered surges on the one hand and continuous activation on the other.

Clinical implications for follow-up

The findings in Paper IV support the integration of objective alcohol biomarker testing, specifically PEth, into routine postoperative follow-up protocols. The strong male predominance of excessive drinking suggests that targeted screening of male patients may be particularly warranted, although the longer-term data from the second cohort indicate that women also show increasing prevalence of heavy drinking by ten years. Given that conventional questionnaire-based screening may underestimate the problem, especially in the preoperative setting, PEth provides a valuable complement that could enable earlier identification and intervention.

Strengths and limitations

A key strength of Papers I–III is the paired, within-subject study design using repeated mixed-meal tests at four time points spanning preoperative baseline through six weeks post-RYGB. By comparing responses before and after VLCD, and before and after surgery, in the same individuals, the design minimises confounding from interindividual variation and allows direct attribution of observed changes to either caloric restriction or the surgical procedure. The inclusion of the first postsurgical meal (MMT+1d) as a study occasion was a unique feature that captured the immediate effects of RYGB at the earliest possible time point. Furthermore, the multi-cohort design in Paper III, which replicated the pNT findings across three independent patient groups, adds robustness to the conclusions regarding neurotensin.

However, the cohort used in Papers I and II is small ($n=19$) and limited to women, which restricts generalisability to male patients and reduces statistical power for detecting smaller effect sizes, particularly in subgroup analyses by glycaemic status. The metabolomic findings in Paper II, while internally consistent, would benefit from replication in larger and more diverse cohorts, including patients undergoing sleeve gastrectomy. Additionally, the six-week follow-up in Papers I and II captures early postoperative effects but does not address whether the observed hormonal and metabolomic patterns persist or evolve over longer time frames. Longer-term data from our group partly address this. Paper III followed pNT for up to one year, and Rogova et al. followed the fasting metabolome and lipidome in a separate cohort to twelve months, finding that most of the early changes had reversed by then (180). Whether the meal-stimulated responses studied here follow the same course remains unknown. A further limitation stems from the within-subject design of Papers I and II. Because each participant underwent VLCD immediately before surgery and served as her own control, the metabolic state induced by the diet may carry over into the early postoperative period. Therefore, the isolated effect of RYGB cannot be fully separated from a residual effect of the preceding caloric restriction. This design increases sensitivity by removing inter-individual variation, but it cannot exclude that the VLCD period itself modifies the subsequent response to surgery. A related consideration applies to MMT+1d specifically. On the first postoperative day, participants remained under the influence of the surgical stress response and of drugs given peri-operatively, including anaesthetic and analgesic agents, all of which can affect glucose metabolism, insulin secretion and gastrointestinal motility.

Paper IV benefits from a large cohort ($n=1,238$), objective biomarker measurement, and follow-up extending to ten years, the longest PEth-based follow-up reported in a bariatric-surgery population. However, the absence of preoperative PEth measurements in the larger second cohort limits the statistical inferences that can be drawn from that group. Nevertheless, the similarity between the two cohorts in baseline characteristics and postoperative PEth concentrations supports the assumption that they originate from the same population. The lack of a general-population comparison group makes it difficult to determine whether the observed increases in PEth reflect a surgery-specific phenomenon or parallel trends in the broader population, although Swedish national monitoring data show a decline in overall alcohol consumption during the study period. Furthermore, PEth reflects the level of consumption but does not distinguish heavy drinking from clinical alcohol use disorder.

Conclusions

This thesis demonstrates that the metabolic effects of Roux-en-Y gastric bypass arise from an interplay between caloric restriction and surgery-specific mechanisms, but that caloric restriction is likely the primary driver of long-term maintenance of these benefits, including glycaemic control, insulin sensitivity and weight loss. While RYGB induces real and measurable changes in gut hormone physiology, including altered incretin responses and elevated proneurotensin, the broader metabolomic evidence suggests that these hormonal changes may serve more as facilitators of the caloric restriction that surgery imposes than as independent mediators of long-term T2D remission.

Beyond metabolic physiology, this work provides biomarker-based evidence that a significant proportion of patients, particularly men, develop sustained heavy alcohol consumption after bariatric surgery. This trend persists for up to ten years and is not limited to RYGB, with no significant difference between RYGB and sleeve gastrectomy.

Based on these findings, the following conclusions can be drawn:

- RYGB produces immediate, surgery-specific changes in insulin and incretin secretion that are independent of caloric restriction, but these act mainly to reinforce reduced caloric intake rather than to drive long-term remission on their own. (Paper I)
- The early metabolic response is explained by caloric restriction, with only a small surgery-specific component, indicating that studies not separating the effect of preoperative diet from that of the operation overstate the surgical signature. (Paper II)
- Proneurotensin shows a distinct, surgery-specific postoperative pattern independent of incretin signalling, though its functional role relative to caloric restriction remains unresolved. (Paper III)
- Alcohol consumption rises significantly after surgery, with a strong male predominance, supporting objective PEth screening in long-term follow-up. (Paper IV)

Future research

The findings presented in this thesis suggest several avenues for future research. If caloric restriction is indeed the dominant mechanism underlying long-term metabolic improvement, a critical question is what determines whether individual patients maintain the reduction in caloric intake that sustains both weight loss and glycaemic remission. Longitudinal studies combining metabolomics, gut-hormone measurements, appetite assessments, and detailed dietary monitoring could clarify whether patients who relapse, through weight regain or recurrent hyperglycaemia, do so primarily because of increased caloric intake and the extent to which persistent hormonal changes, such as elevated GLP-1 or pNT, modify this trajectory. The hormonal and metabolomic findings would also benefit from replication in larger, mixed-sex cohorts that include both RYGB and sleeve gastrectomy and have follow-up beyond six weeks.

The role of proneurotensin as a potential mediator of postoperative metabolic changes, rather than merely a marker, warrants mechanistic investigation. In particular, whether neurotensin directly influences appetite, lipid handling, or insulin sensitivity in the postoperative setting, and whether its sustained elevation contributes to the caloric restriction that the surgery facilitates, are questions that could be addressed through targeted intervention studies.

The observation that different analytical approaches (targeted hormone measurement *vs.* untargeted metabolomics) yield different conclusions regarding the relative contributions of caloric restriction and surgery raises the question of which level of biological organisation that best predicts long-term clinical outcomes. This is not merely an academic question, as it has implications for the design of therapies, the development of pharmacological alternatives to surgery, and the identification of patients at risk for metabolic relapse.

Regarding alcohol use, future studies should include general-population comparisons to contextualise the observed increases in PEth and should investigate the mechanisms underlying the strong sex difference in postoperative drinking patterns. The relationship between endogenous GLP-1 levels and alcohol consumption after surgery deserves direct investigation: Do patients with the highest postprandial GLP-1 responses show any degree of protection against increased drinking, or is the pulsatile nature of endogenous GLP-1 insufficient regardless of magnitude? Finally, the emerging evidence for GLP-1 receptor

agonists as potential treatments for alcohol use disorder, including the recent randomised trial by Hendershot et al. reporting reduced craving and drinking quantity with semaglutide, raises the question of whether such agents could have a role in managing problematic alcohol use in the post-bariatric surgery population specifically (175).

Ethical and societal implications

All studies in this thesis were approved by ethical review boards and conducted in accordance with the Declaration of Helsinki. Beyond formal approval, several ethical dimensions warrant discussion.

In Papers I–III, participants underwent repeated blood sampling and mixed-meal tests at four time points. Since the protocol was designed to capture the immediate effects of surgery, one mixed-meal test was performed less than a day after major abdominal surgery. Although the meal size was specifically adapted to be tolerable, the procedure raises questions about participant burden. All patients provided written informed consent before enrolment. Nevertheless, the relationship between surgeon and patient in the preoperative setting may create pressure to participate, a well-recognised concern in research (214).

Paper IV raises a distinct set of ethical considerations. PEth testing was introduced at Kalmar County Hospital as a mandatory component of the routine pre- and postoperative follow-up programme for bariatric surgery patients, not as a research-specific intervention. Patients were informed of this requirement at the time of surgical acceptance. The retrospective use of these clinically collected data for research purposes was approved by the ethical review board without requiring additional individual consent. This complies with Swedish legislation, and Ludvigsson et al. argued that this benefits both patients and society (215).

The mandatory nature of PEth testing in the clinical programme nonetheless deserves reflection. Although routine alcohol biomarker screening after bariatric surgery can be justified on clinical grounds, it limits patient autonomy regarding what information is generated about their behaviour. On the other hand, the primary purpose of screening was to identify patients in need of support and intervention at an early stage. Patients with elevated PEth levels were offered counselling and follow-up rather than being subjected to punitive measures, and those with elevated values prior to surgery were given the opportunity to demonstrate sustained low consumption before being reconsidered for the procedure.

Taken together, the potential benefits of the research, improving the understanding of metabolic mechanisms after bariatric surgery and informing strategies for early detection of postoperative alcohol misuse, were judged to outweigh the risks and burdens imposed on participants. The findings from these studies have the

potential to benefit the much larger population of patients undergoing bariatric surgery in the future.

The use of AI

During the preparation of this work, the author used Claude and Copilot to improve readability and grammar. After using these tools, I have reviewed and edited the content as needed and take full responsibility for the final publication.

Populärvetenskaplig sammanfattning

Fetma (obesitas) är ett av de största folkhälsoproblemen i världen och förekomsten har ökat stadigt under de senaste decennierna. Fetma ökar risken för en rad allvarliga sjukdomar, däribland typ 2-diabetes, hjärt-kärlsjukdom, metabolt syndrom och vissa cancerformer. Metabolt syndrom är ett samlingsbegrepp för en kombination av riskfaktorer – bland annat bukfetma, högt blodtryck, höga blodfetter och förhöjt blodsocker – som tillsammans kraftigt ökar risken för hjärt-kärlsjukdom och diabetes. En central mekanism bakom dessa sjukdomstillstånd är insulinresistens, det vill säga att kroppens celler blir mindre känsliga för hormonet insulin, som normalt hjälper cellerna att ta upp socker från blodet.

Fetmakirurgi (bariatrik kirurgi), och framför allt Roux-en-Y gastrisk bypass (RYGB), är den mest effektiva behandlingen vid svår fetma. Ingreppet leder inte bara till betydande vikttnedgång utan förbättrar eller botar ofta typ 2-diabetes, ibland redan innan patienten hunnit gå ned nämnvärt i vikt. De bakomliggande mekanismerna till denna snabba förbättring är inte fullt klarlagda. En teori är att den kirurgiska ombyggnaden av mag-tarmkanalen i sig förändrar frisättningen av tarmhormoner, så kallade inkretiner, som påverkar insulinfrisättning och blodsockerreglering. En annan förklaring är att förbättringen helt enkelt beror på minskat kaloriintag. Under de senaste åren har även behandling med GLP-1-receptoragonister vuxit fram som ett alternativ till kirurgi, men långtidsresultat saknas ännu och kirurgi kvarstår som en viktig behandlingsform.

Trots de påtagliga fördelarna med fetmakirurgi har flera studier pekat på att en del patienter ökar sin alkoholkonsumtion efter operationen. Mekanismerna bakom detta är inte helt klarlagda men inkluderar förändrad alkoholmetabolism efter kirurgi, med snabbare upptag och högre toppkoncentrationer av alkohol i blodet. De flesta tidigare studier har dock förlitat sig på självskattningsformulär, vilka tenderar att underskatta den faktiska konsumtionen. En objektiv biomarkör för alkoholkonsumtion, fosfatidyletanol (PEth), erbjuder ett mer tillförlitligt mått.

Denna avhandling omfattar fyra delarbeten som utifrån olika infallsvinklar undersöker metabola och hormonella effekter av fetmakirurgi samt risken för ökad alkoholkonsumtion efter ingreppet.

I den första studien undersökte vi de omedelbara effekterna av RYGB på insulin- och inkretinsvar (GLP-1 och GIP) jämfört med effekterna av kalori restriktion. Nitton kvinnor med svår fetma, varav tio med typ 2-diabetes, genomgick

standardiserade måltidstester vid fyra tillfällen: före en lågkaloridiet, efter dieten men före operationen, morgonen efter operationen (den allra första måltiden efter ingreppet), samt sex veckor postoperativt. Vi fann att kalori restriktion hade begränsad effekt på de studerade hormonerna, medan RYGB omedelbart ökade insulin- och GIP-svaren. GLP-1-svaret var fördröjt jämfört med GIP-svaret men markant förhöjt sex veckor efter operationen, särskilt hos patienter med typ 2-diabetes. Insulinkänsligheten förbättrades efter RYGB men inte av kalori restriktion. Studien talar starkt för att de tidiga metabola förbättringarna efter RYGB drivs av själva operationen snarare än av minskat kaloriintag.

I den andra studien använde vi samma patientgrupp och mättillfällen men analyserade nu det bredare metabola fingeravtrycket i blodet med hjälp av metabolomik och lipidomik. Vi fann att kalori restriktion framför allt påverkade acylkarnitiner och ketonkroppar – ett mönster som speglar förbränning av kroppsfett vid svält – samt minskade nivåer av vissa blodfetter. Den omedelbara effekten av RYGB var delvis motsatt, med förändrade nivåer av medel- och kortkedjiga acylkarnitiner, aminosyror och puriner. Sex veckor efter operationen liknade det samlade metabola mönstret mest det som sågs efter kalori restriktion, med förändringar i acylkarnitiner och blodfetter. Sammantaget visar resultaten att många av de metabola förändringar som tidigare tillskrivits RYGB i huvudsak kan förklaras av minskat kaloriintag, medan den förändrade hormonfrisättningen (inkretiner) tycks vara en direkt effekt av den kirurgiska ombyggnaden.

I den tredje studien undersökte vi tarmhormonet neurotensin, mätt som dess stabila prohormon proneurotensin (pNT), i tre patientkohorter. Neurotensin frisätts från tunntarmen och har kopplats till fettmetabolism och insulinkänslighet. Vi fann att fastande pNT-nivåer sjönk akut efter både en lågkaloridiet och RYGB, vilket talar för att den initiala sänkningen beror på energirestriktion. Däremot var fastande pNT-nivåer förhöjda från sex veckor och upp till ett år efter RYGB, oberoende av om patienten hade normal glukostolerans, prediabetes eller typ 2-diabetes. Dessutom uppvisade opererade patienter, men inte icke-opererade kontroller, ett tydligt måltidssvar i pNT, ett svar som inte kunde förklaras av ökade GLP-1 eller GIP-nivåer, eftersom infusion av dessa inkretiner inte framkallade samma stegring. Detta tyder på att det kirurgiska ingreppet i sig skapar förutsättningar för ett förändrat neurotensinsvar.

I den fjärde studien undersökte vi långsiktiga förändringar i alkoholkonsumtion efter fetmakirurgi med hjälp av alkoholbiomarkören PETH. Studien omfattade 1 238 patienter som genomgått RYGB eller sleeve-gastrektomi vid Länssjukhuset i Kalmar under åren 2010–2020, med uppföljningstid upp till tio år. Vi fann att PETH-nivåerna ökade signifikant över tid, särskilt hos män. Fem år efter operationen hade drygt en tredjedel av männen PETH-värden över gränsen för hög alkoholkonsumtion, jämfört med cirka fem procent av kvinnorna. Risken att överskrida gränsvärdet var nära åtta gånger högre för män än för kvinnor. Mönstret kvarstod vid tioårsuppföljningen och ingen tydlig skillnad sågs mellan RYGB och sleeve-

gastrektomi. Majoriteten av patienterna, framför allt kvinnorna, bibehöll dock en låg alkoholkonsumtion.

Sammanfattningsvis visar denna avhandling att RYGB har omedelbara och kraftfulla effekter på tarmhormoner och insulinsvar som inte kan förklaras enbart av minskat kaloriintag, medan de bredare metabola förändringarna till stor del drivs av energirestriktionen. Vidare påvisas att neurotensin uppvisar ett distinkt mönster efter bariatrisk kirurgi som inte beror på inkretinerna GLP-1 och GIP. Slutligen visar avhandlingen att en betydande andel patienter, särskilt män, ökar sin alkoholkonsumtion efter fetmakirurgi, vilket stödjer rutinmässig screening med PEth-provtagning i den långsiktiga postoperativa uppföljningen.

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Paper I



Original article

Roux-en-Y gastric bypass versus calorie restriction: support for surgery *per se* as the direct contributor to altered responses of insulin and incretins to a mixed meal

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Abstract

Objective: To study the immediate effects of Roux-en-Y gastric bypass (RYGB) on glucose homeostasis, insulin, and incretin responses to mixed-meal tests compared with the effects of calorie restriction (CR).

Setting: University-affiliated bariatric surgery clinic.

Background: RYGB induces remission of type 2 diabetes (T2D) long before significant weight loss occurs. The time course and underlying mechanisms of this remission remain enigmatic. A prevailing theory is that secretory patterns of incretin hormones are altered due to rearrangement of the gastrointestinal tract. To what extent reduced calorie intake contributes to the remission of T2D is unknown.

Methods: Nine normoglycemic patients and 10 T2D patients were subjected to mixed-meal tests (MMT) 4 weeks before surgery before initiation of a very low calorie diet regimen (MMT_{-4 w}), 1 day before surgery on a very low calorie diet regimen (MMT_{-1 d}), on the morning of the first day after surgery (MMT_{+1 d}; first postoperative meal), and 6 weeks after surgery (MMT_{+6 w}). Insulin, glucose, active glucagon-like peptide 1 (GLP-1), and glucose-dependent insulintropic polypeptide (GIP) were measured.

Results: CR lowered insulin in T2D patients, whereas glucose, GIP, and GLP-1 were unaffected. RYGB immediately increased plasma insulin and GIP. The GLP-1 response was delayed compared with the GIP response. T2D patients exhibited lower insulin responses after RYGB compared with normoglycemic patients. GIP responses were similar in both groups at all occasions, whereas T2D patients displayed markedly elevated GLP-1 responses 6 weeks after RYGB. Glucose was unaffected by CR and RYGB in both groups. Insulin sensitivity was unaffected by CR but improved with RYGB.

Conclusion: RYGB exerts powerful and immediate effects on insulin and incretin responses to food, independently of changes caused by CR. (Surg Obes Relat Dis 2017;13:234–242.) © 2017 American Society for Metabolic and Bariatric Surgery. All rights reserved.

Keywords: Bariatric surgery; Gastric bypass; Incretins; Calorie restriction

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Roux-en-Y gastric bypass (RYGB) is currently the most commonly used method for treating morbid obesity. Even before significant weight loss occurs, most type 2 diabetes (T2D) patients who undergo RYGB experience remission. The underlying mechanisms, however, remain elusive. It is established that the early effect of RYGB is associated with

increased insulin secretion and improved insulin action [1–4]. A number of factors have been proposed to explain the rapid RYGB-induced remission of T2D. These factors include improved hepatic insulin sensitivity [1], altered bile acid composition [5,6], altered gut microbiota [7,8], and increased β -cell mass [9]. In addition, altered circulating levels of the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) that have been observed after RYGB likely contribute. GLP-1 is secreted from L-cells present mainly in the distal parts of the intestine, whereas GIP is secreted from K-cells predominantly located in the proximal parts of the intestine. Circulating levels of GLP-1 are elevated after RYGB in both humans [10] and rats [11], and the effects on GIP are more unclear [12]. Although the aforementioned early effects of RYGB have been reported, no data are available on the immediate effects of RYGB (i.e. the effects of the first meal after surgery) on insulin and incretin responses.

RYGB has recently been shown to induce increased densities of L-cells and K-cells in the upper jejunum in humans [13,14], and similar results in pigs have been reported [15]. A pressing issue regarding T2D remission is the influence of the subsequently reduced food intake. It is not known whether the acute effect of RYGB on insulin, insulin sensitivity, and incretins is a consequence of reduced food intake or can be attributed to surgery *per se*. A few reports have compared the effect of caloric restriction (CR) with RYGB on glycemia [16–19]. Most studies [16–19] compared a group of patients who underwent surgery with another group who underwent CR, with the exception of Lingvay et al. [18], who examined a group of patients before and after surgery. In the study presented here, we compared the effect of CR with the immediate effect of RYGB on glycemia, insulin, and incretin responses in both diabetic and nondiabetic patients. To this end, mixed-meal tests (MMTs) were performed before and after presurgery CR and before and after RYGB. The design of the study also allowed us to investigate, for the first time, the immediate effect of RYGB.

Patients and Methods

Patients

Inclusion criteria were female sex, 30–55 years of age, body mass index (BMI) > 35, Caucasian, and no incretin-based

preoperative treatment. Exclusion criteria were prior bariatric surgery and/or surgery to the upper gastrointestinal tract. Nineteen age- and weight-matched Caucasian women with an average age of 43 ± 1.7 years and BMI of $37.5 \pm .9$ kg/m² were included (Table 1). Nine patients were normoglycemic (NG) and 10 had been diagnosed with T2D. Patients were regarded as NG if their glycated hemoglobin was < 42.1 mmol/mol (6%) and their fasting plasma glucose was < 6.1 mmol/L without antidiabetic treatment. Of the T2D patients, 2 patients were treated with insulin alone, 3 with diet alone, 2 with metformin alone, 2 with insulin and metformin, and 1 with insulin, metformin, and glimepiride. Hypoglycemic medications/insulin were held the night before surgery and on the first postoperative day. Mean duration of T2D was 3.1 ± 0.9 years. At a preoperative visit, all patients received counseling for weight reduction, with a target weight loss of 5% to reduce liver size. This was accomplished by a 3- to 4-week preoperative period of low calorie diet (VLDC) consisting of 4 portions of Modifast® per day, adding up to a total of 858 kcal/day and consisting 18 g fat, 110 g carbohydrates and 57 g protein. The study was approved by the Human Ethical Committee in Lund, Sweden, and adhered to the Declaration of Helsinki.

Surgery

RYGB was performed as previously described [20]. A small 15- to 20 mL pouch was created using linear cutters. The alimentary limb, placed antecolic and antegastric, measured 150 cm. The biliopancreatic limb (50 cm) was anastomosed to the jejunum in a side-to-side fashion, finalizing the RYGB. All procedures were performed laparoscopically.

Mixed-meal tests

Overnight-fasted patients arrived to the clinic and basal blood samples were drawn using a cubital vein catheter. The volume of the mixed meal was designed to be tolerable to the patient at 1 day after surgery (MMT_{+1 d}; the first postsurgical meal). Previous studies from our laboratory have shown that 200 mL of Modifast (Modifast, Täby, Sweden) (220 kcal, 14 g protein, 28 g carbohydrates, 5 g fat) given over a 20-minute period is tolerable [21,22]. Blood was collected at 0, 5, 10, 15, 30, 60, and 90 minutes after meal ingestion in chilled EDTA tubes

Table 1
Patient information

	Normoglycemic (NG)				Type 2 diabetes (T2D)			
	MMT _{-4 w}	MMT _{-1 d}	MMT _{+1 d}	MMT _{+6 w}	MMT _{-4 w}	MMT _{-1 d}	MMT _{+1 d}	MMT _{+6 w}
BW (kg)	114.2 ± 3.7	106.5 ± 3.2	106.5 ± 3.2	95.1 ± 3.5	111.6 ± 4.3	103.4 ± 4.1	103.4 ± 4.1	93.7 ± 3.9
BMI (kg/m ²)	40.2 ± 1.2	37.5 ± 1.0	37.5 ± 1.0	33.5 ± 1.1	39.4 ± 1.1	36.5 ± 1.0	36.5 ± 1.0	33.1 ± 1.0
EBMIL (%)	-	17.8 ± 1.9	17.8 ± 1.9	45.6 ± 4.1	-	20.5 ± 1.2	20.5 ± 1.2	46.3 ± 3.7

BMI = body mass index; BW = weight; EBMIL = excess body mass index loss.

EBMIL% = (preopBMI – current BMI) × 100/(preopBMI – 25).

(0.1 mmol/L diprotin A, 500 KIU/mL aprotinin). Plasma was stored at -80°C until analysis. Care was taken to ensure that patients tolerated the MMTs at all time points. Thus, the patients were given the exact same volume at the same rate at all MMTs [21]. MMTs were performed 4 weeks before surgery (MMT_{-4 w}) before initiation of a VLCD, 1 day before surgery (MMT_{-1 d}), on the morning of the first day after surgery (MMT_{+1 d}), and 6 weeks after surgery (MMT_{+6 w}) when patients had returned to eating normal food. This study design allowed for the following comparisons: the effect of a VLCD (MMT_{-4 w} versus MMT_{-1 d}), the immediate effect of surgery (MMT_{-1 d} versus MMT_{+1 d}), and the effect of RYGB in a longer perspective (MMT_{-1 d} versus MMT_{+6 w}). In addition, it was possible to analyze pattern differences between NG and T2D patients.

Hormone analyses

The following variables were measured: plasma glucose using the glucose oxidase method (Infinity Glucose Oxidate Kit; Thermo Fisher Scientific, Waltham, MA); insulin using a human enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden); and GIP and active GLP-1 using enzyme-linked immunosorbent assays from Millipore (Billerica, MA), all according to the manufacturer's instructions.

Calculations and statistical analyses

Basal, 90-minute, and area under the curve (AUC) values are given. Incremental values were calculated as peak values divided by basal values and presented as a ratio. All data are presented as mean $\pm$ standard error of the mean. Statistical significance was calculated using one-way or 2-way analysis of variance with Bonferroni's test post hoc where appropriate, using Graphpad Prism 6 (Graphpad Software, San Diego, CA). A P value $< .05$ was considered statistically significant.

Results

Weight, BMI, and excess BMI loss

There were no significant differences between groups (T2D versus NG) in weight, BMI, or excess BMI loss (see Table 1).

Fasted state

Fasting levels of glucose were similar at all MMTs in both groups of patients (Table 2). CR (MMT_{-1 d} versus MMT_{-4 w}) had no effect on fasting insulin levels in the NG group but lowered fasting insulin levels in T2D patients (see Table 2). CR had no effect on fasting GIP levels; however, after RYGB lower GIP levels were demonstrated in T2D but not in NG patients. CR had no effect on fasting GLP-1 levels, but at MMT_{+6 w} fasting GLP-1 levels were increased compared with MMT_{-4 w} levels. Data are summarized in Table 2. No differences in basal levels of

glucose, insulin, GIP, or GLP-1 were observed between NG and T2D patients at any MMT.

MMT response

Glucose The glucose response to the MMTs was similar on all occasions and was unaffected by either CR or RYGB (Fig. 1, A–D). Both groups of patients responded similarly to the MMTs at all time points (Fig. 3, A–D). Ninety-minute glucose and AUC_{glucose} were unaffected by CR and RYGB in both groups (see Table 2).

Insulin CR had no effect on the insulin response during MMT in NG patients (Fig. 1, E). However, in T2D patients, decreased insulin responses were observed after CR ($P < .01$; Fig. 1, G). NG patients responded to surgery with increased insulin levels ($P < .001$; see Fig. 1, E). Incremental insulin values were increased in NG patients as a consequence of RYGB ($P < .05$; Fig. 1, F). Furthermore, AUC_{insulin} was increased in both groups of patients after surgery (see Table 2). After RYGB, NG patients had greater insulin responses than did T2D patients ($P < .01$ for both MMTs; Fig. 3, G and H, respectively).

GIP CR had no effect on the GIP response to the MMT in either group (Fig. 2, A and C, respectively). Whereas RYGB had no effect on the GIP response over the duration of the MMTs (see Fig. 2, A and C, respectively), incremental values were increased after MMT_{+1 d} compared with MMT_{-1 d} in NG patients (Fig. 2, B). Also, AUC_{GIP} was greater at MMT_{+1 d} compared with MMT_{-1 d} in NG patients (see Table 2). NG and T2D patients displayed similar GIP responses at all test occasions (Fig. 4, A–D).

GLP-1 CR had no effect on the GLP-1 response (Fig. 2, E and G, respectively). After RYGB (MMT_{+1 d}) NG patients displayed an increased GLP-1 response compared with MMT_{-1 d} ($p < .001$; see Fig. 2, E). No differences were observed in incremental GLP-1 levels between MMT_{-1 d} and MMT_{+1 d} in either group (Fig. 2, F and 2, H, respectively), but at MMT_{+6 w} both groups of patients demonstrated an increased incremental response compared with MMT_{-1 d} (see Fig. 2, F and H, respectively). At MMT_{-1 d}, T2D patients demonstrated moderately higher GLP-1 levels than did NG patients ($P < .001$; Fig. 4, F), but at MMT_{+1 d} the situation was reversed ($P < .05$; Fig. 4, G). Finally, at MMT_{+6 w}, T2D patients again responded with markedly higher GLP-1 levels than did the NG patients ($P < .001$; Fig. 4, H).

Insulin sensitivity and secretion indices The insulin sensitivity index was increased after RYGB in both groups (see Table 2), whereas CR lacked effect on the insulin sensitivity index. The homeostatic model assessment-insulin resistance, homeostatic model assessment-beta, insulinogenic index, and the Matsuda index were unaffected by CR and RYGB in both groups (see Table 2).

Table 2
Indices, fasting, and 90-minute values for parameters included in the study

	Normoglycemic (NG)				Type 2 diabetes (T2D)			
	MMT _{-4 w}	MMT _{-1 d}	MMT _{+1 d}	MMT _{+6 w}	MMT _{-4 w}	MMT _{-1 d}	MMT _{+1 d}	MMT _{+6 w}
Fasting glucose (mmol/L)	5.1 ± .40	5.1 ± .28	5.4 ± .49	5.5 ± .39	7.3 ± 1.18	6.5 ± .68	6.5 ± .47	6.7 ± .83
90-min glucose (mmol/L)	7.1 ± .93	7.3 ± .76	7.7 ± 1.1	5.6 ± .43	8.9 ± 1.29	7.4 ± .93	7.4 ± .86	7.4 ± .66
AUC glucose (mmol/L × min)	735 ± 79.3	703 ± 60	755 ± 75	651 ± 27	793 ± 93.6	728 ± 87.2	774 ± 75.5	738 ± 60.6
Fasting insulin (uIU/mL)	23.2 ± 4.7	16 ± 2.39	18 ± 1.85	18.9 ± 3.88	25 ± 5.99**	18.7 ± 5.79	20.6 ± 4.76	14.5 ± 1.5
90-min insulin (uIU/mL)	44.2 ± 11.23	40.6 ± 11.35 [†]	70.4 ± 11.5 [‡]	25.3 ± 3.71	42.1 ± 12.87	35.3 ± 10.47	44.1 ± 8.76	23.7 ± 2.76
AUC insulin (uIU/mL)	4201 ± 572	3107 ± 389 [†]	6820 ± 1073.2	5490 ± 979	4244 ± 916.3**	3143 ± 913 [†]	4222 ± 615	3933 ± 326
Fasting GIP (pg/mL)	169.5 ± 20.43	133.6 ± 16.03	112.1 ± 15.56	135.6 ± 14.66	194.8 ± 19.31	180.6 ± 17.13 [†]	123 ± 17.85	147.9 ± 37.72
AUC GIP (pg/mL × min)	32,006 ± 5738 [§]	36,441 ± 3217 [†]	47,325 ± 3202 ^{‡‡}	29,335 ± 3382	29,598 ± 4115 ^{§§}	34,681 ± 3735	41,712 ± 2697	33,90 ± 3769
fasting GLP-1 (pM)	4.0 ± .27	3.9 ± 2.17	11.8 ± 4.14	4.5 ± .33	4.4 ± .25 [¶]	4.3 ± .31	7.5 ± 2.64	5 ± 0.23
AUC GLP-1 (pM × min)	416 ± 46.7	867 ± 242 [†]	2779 ± 521	1151 ± 371	464 ± 26.2 [‡]	472 ± 30.7	607 ± 28	1894 ± 482.2
ISI (uIU/nmol)	6.4 ± 1.08	4.8 ± .76 [†]	10.1 ± 1.82	8.3 ± 1.38	6 ± 1.27	4.3 ± .75 [†]	6.1 ± .79	5.6 ± .53
HOMA-IR [(uIU/mL) × (mmol/L)]	7.3 ± 1.96	4.5 ± .98	5.1 ± .46	5 ± 1.12	7 ± 1.59	5.4 ± 2.18	5.4 ± 1.48	4.2 ± .9
HOMA-beta (%)	10.7 ± 2.84	8.7 ± 2.28	18.8 ± 8.86	14.8 ± 4.13	12.5 ± 4.21	8.4 ± 3.79	12.9 ± 6.28	9.6 ± 3.51
IGI (uIU/nmol)	23.9 ± 7.27	32.7 ± 17.7	25.4 ± 9.18	24.3 ± 8.7	6.6 ± 1.64	4.9 ± 1.92	5.2 ± 1.44	7.1 ± 3.87
Matsuda index[(dL × mL)/(ng × uIU)]	22.4 ± 4.53	32.2 ± 6.56	19.3 ± 2.04	21.7 ± 4.07	18.7 ± 2.92	30.0 ± 4.35	20.7 ± 2.71	27.0 ± 4.37

AUC = area under the curve; GIP = glucose-dependent insulinotropic polypeptide; GLP-1 = glucagon-like peptide 1; HOMA = homeostatic model assessment; IGI = insulinogenic index; ISI = insulin sensitivity index; MMT = mixed-meal test.

^{†–‡‡}Comparisons within the same glycemic group.

One footnote symbol indicates $P < .05$.

Two identical footnote symbols indicate $P < .01$.

[§]MMT_{-4 w} versus MMT_{-1 d}.

[†]MMT_{-1 d} versus MMT_{+1 d}.

[‡]MMT_{+1 d} versus MMT_{+6 w}.

[§]MMT_{-4 w} versus MMT_{+6 w}.

[¶]MMT_{-4 w} versus MMT_{+1 d}.

^{‡‡}MMT_{-1 d} versus MMT_{+6 w}.

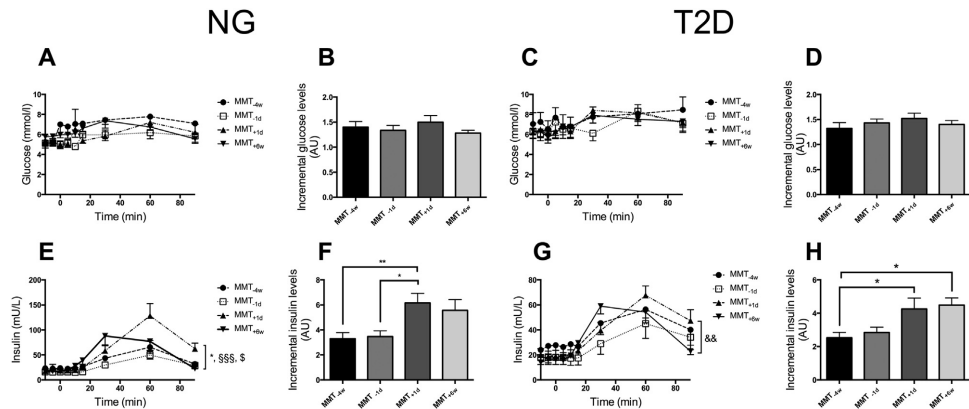


Fig. 1. No effects were observed on glucose neither in NG-patients nor in T2D-patients (A–D). In NG-patients MMT_{+1d} increased insulin response to the MMT compared MMT_{-1d} and MMT_{-4w} (E). Also, at MMT_{+6w} NG-patients responded with increased insulin compared with MMT_{-1d} (E). Incremental insulin values in NG-patients were increased at MMT_{+1d} compared with MMT_{-1d} and MMT_{-4w} (F). T2D-patients responded with more insulin at MMT_{-4w} than they did at MMT_{-1d} (G). In T2D-patients incremental insulin values were higher at MMT_{+1d} and MMT_{+6w} compared with MMT_{-4w} (H). * indicates comparison between MMT_{-4w} and MMT_{+1d}, § indicates comparison between MMT_{-1d} and MMT_{+1d}, # indicates comparison between MMT_{+1d} and MMT_{+6w}, & indicates comparison between MMT_{-4w} and MMT_{-1d} and ° indicates comparison between MMT_{-4w} and MMT_{+6w}. Number of symbols indicates level of significance; one symbol, $p < .05$; two symbols, $p < .01$; three symbols, $p < .001$. AU = arbitrary units.

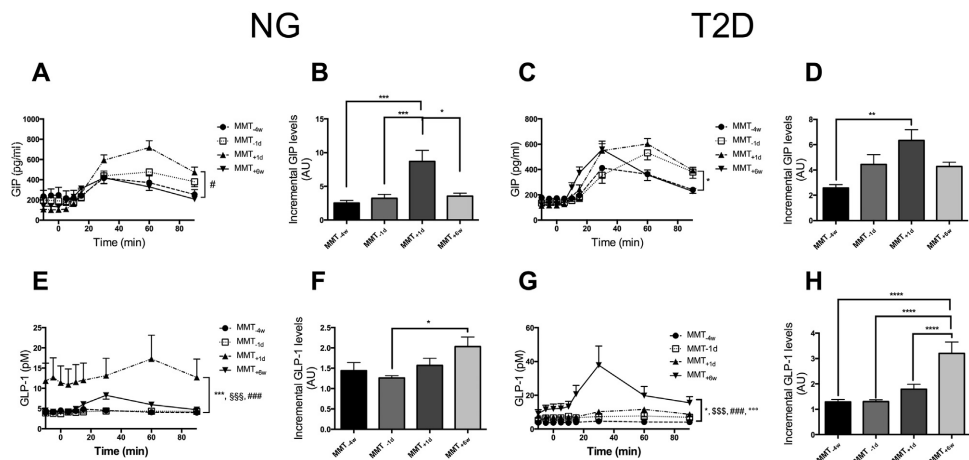


Fig. 2. At MMT_{+1d} NG-patients responded with increased GIP compared with at MMT_{+6w} (A) and in T2D-patients MMT_{+1d} provoked greater GIP response than did MMT_{-4w} (C). Incremental GIP levels in NG-patients were increased at MMT_{+1d} compared with MMT_{-1d} and MMT_{-4w} (B). In T2D-patients incremental GIP levels were higher at MMT_{+1d} compared with MMT_{-4w} (D). NG-patients responded to MMT_{+1d} with increased active GLP-1 compared with all other MMTs (E). In T2D-patients MMT_{+6w} provoked increased active GLP-1 compared with the other MMTs (G). MMT_{+6w} also provoked higher incremental active GLP-1 compared with MMT_{-1d} and MMT_{-4w} (H). * indicates comparison between MMT_{-4w} and MMT_{+1d}, § indicates comparison between MMT_{-1d} and MMT_{+1d}, # indicates comparison between MMT_{+1d} and MMT_{+6w}, & indicates comparison between MMT_{-4w} and MMT_{-1d} and ° indicates comparison between MMT_{-4w} and MMT_{+6w}. Number of symbols indicates level of significance; one symbol, $p < .05$; two symbols, $p < .01$; three symbols, $p < .001$. AU = arbitrary units.

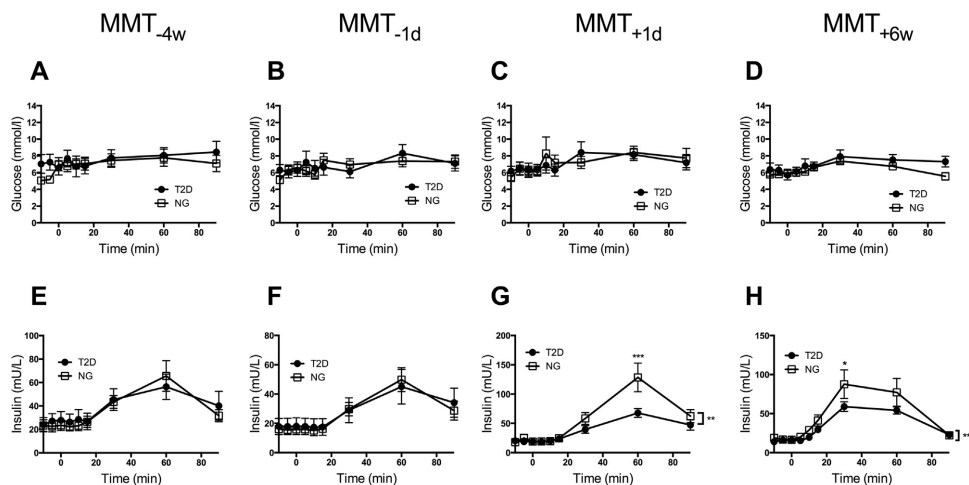


Fig. 3. Glucose response to the MMT was unaffected at all time points studied (A–D). At MMT_{+1d} and MMT_{+6w} NG-patients responded with more insulin than T2D-patients (G and H, respectively). * with brackets indicate statistical significance over the duration of the entire MMT. **, $p < .01$.

Discussion

It is now an accepted fact that RYGB induces a rapid remission of T2D [4]. The precise mechanisms accounting for this phenomenon are still not fully understood. The surgery results in an enhanced release of incretin hormones and insulin in response to food intake, but it is unclear to what extent this is attributable to the surgery-

induced reduction in food intake. Here, we compared the effect of RYGB with that of CR on glycemia and hormone release in the same patients. We show that preoperative CR resulted in reduced insulin secretion but had no effect on glucose, GIP, and GLP-1. On the other hand, RYGB was a primary cause of the increased responses in insulin and incretin hormones.

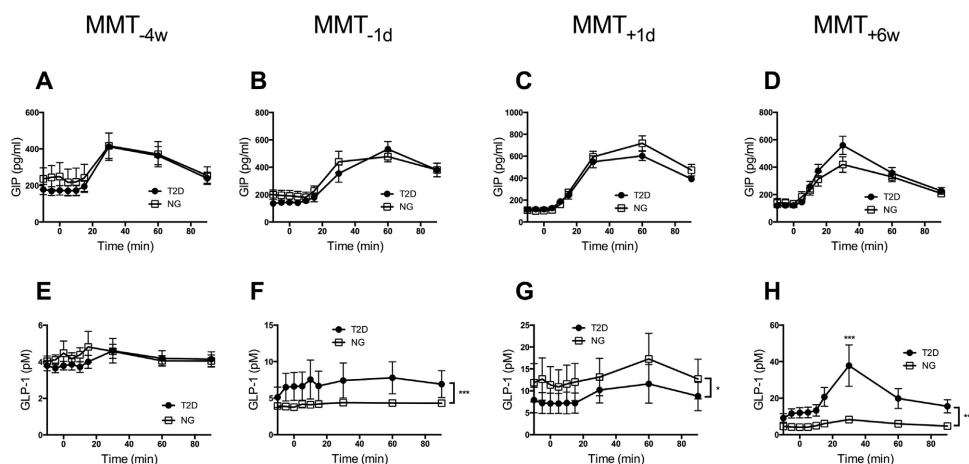


Fig. 4. GIP response to MMT was unaffected by glycemic state of the patients (A–D). At MMT_{-1d} and MMT_{+6w} , T2D-patients responded with more active GLP-1 than did NG-patients (F and H, respectively). Conversely, at MMT_{+1d} , T2D-patients responded with less than did NG-patients (G). * with brackets indicate statistical significance over the duration of the entire MMT. *, $p < .05$; ***, $p < .001$.

One recent study suggested that short-term dietary intervention is equally effective as RYGB for glycemic control [18], arguing that the major effect of RYGB on glycemia is mediated via reduced food intake. In contrast Courcoulas et al. [23], who compared bariatric surgery and lifestyle intervention and investigated remission of T2D, found that 40% of the patients who underwent RYGB experienced partial or complete remission after 3 years. In contrast, the patients who underwent lifestyle intervention alone failed to achieve T2D remission [23]. Furthermore, studies using a gastrostomy tube, allowing nutrient exposure selectively to the bypassed stomach or via the transoral route, provide further support that the effect of RYGB on insulin and incretin hormone release is independent of diet and weight loss [21,24,25]. This notion is corroborated by the fact that sham-operated pair-fed pigs do not exhibit the same improvements in glycemia and increase in insulin to an intravenous glucose test as do RYGB-pigs [9].

Assessment of the effect of CR on glucose, insulin, and incretin responses was performed by comparing the responses before (MMT_{-4 w}) and after introduction of a VLCD regimen (MMT_{-1 d}). CR exerted minor influence on the parameters studied. The only effects were lower fasting and postprandial insulin levels in T2D patients. Lower fasting insulin levels would imply improved insulin sensitivity or less stimulation of insulin attributable to less carbohydrate in the diet. However, all insulin sensitivity indices calculated failed to show effect of CR. Furthermore, CR had no effect on the release of incretin hormones, which contrasts with the findings of Lund et al. [26], whose study indicates increases in GLP-1 and GIP responses to an oral glucose tolerance test in T2D patients as a consequence of CR. It should be noted that the oral glucose tolerance test used by Lund et al. [26] provided patients with 25 g glucose, whereas the MMTs in the study presented here contained 7.3 g glucose. Also, the presurgery VLCD regimen in the study by Lund et al. [26] was 64 ± 8 days; the VLCD regimen in the study presented here lasted 21–28 days. On the other hand, Isbell et al. [17] were unable to observe changes in GIP and GLP-1 levels after CR for 4 ± 0.5 days.

Our data demonstrate that CR did not affect glucose levels in response to the MMTs. This is in contrast to data from recent studies [16,18]. It is important, however, to acknowledge that there are differences in design of this and the aforementioned studies. Campos et al. [16] studied nondiabetic patients, whereas we studied both nondiabetic and diabetic patients. In the study by Lingvay et al. [18], the patients had been on a VLCD regimen for 2 days before baseline data were obtained. Furthermore, their patients had a longer diabetes duration (7.4 years versus 3.1 years in the study presented here). A longer disease duration influences diabetes remission negatively [27]. Thus, it seems probable that at least some of the results in the study by Lingvay et al. [18] could be explained by differences in disease duration (compared with the study presented here).

To study the immediate effect of RYGB, we compared MMT responses 1 day before surgery (MMT_{-1 d}) with those on the first postsurgical day (MMT_{+1 d}). This was the first meal the patients received after surgery and allowed for isolation of surgery as the only factor differing between the MMTs. Weight loss was not a factor in this setting because the time between the 2 MMTs was approximately 36 hours, and no differences in weight change, BMI, or excess BMI loss were observed. In contrast to the insulin-reducing effect of CR, RYGB markedly improved the insulin and GIP responses, most clearly in the NG patients. This increase in the GIP response is of interest because the putative main source of GIP is bypassed after RYGB and thereby does not receive stimuli from luminal content. The reason for increased GIP levels has not been clearly determined, but a secondary source of the hormone may exist (e.g., K-cells in more distal gut regions contributing to the secretion of GIP in response to the MMT after RYGB). Support for this comes from increased K-cell density in the alimentary limb in RYGB patients after surgery [13–15] and recently in rats [28]. Alternatively, neural or other signaling factors may contribute to stimulate GIP from the bypassed upper intestine. Nonetheless, the increased GIP response could potentially contribute to increased insulin release. This increase in GIP seemed transient because no difference between MMT_{-1 d} and MMT_{+6 w} was observed. Jørgensen et al. [3] also observed a transient rise in GIP. Another factor that could explain the rise in insulin secretion after RYGB is increased GLP-1, as previously proposed in multiple studies [2]. Although an increase in plasma levels was seen in NG patients at MMT_{+1 d}, the incremental GLP-1 response was delayed compared with that of GIP. We also demonstrated that 6 weeks after surgery T2D patients responded to the MMT with increased GLP-1 levels compared with NG patients. The lower GLP-1 response in NG patients warrants further investigation. In contrast to CR and despite the fact that glucose levels remained unaffected, RYGB improved insulin sensitivity in both groups, which is in line with previous observations [1].

To our knowledge, this is the first study reporting the very immediate effects of surgery on insulin and incretin responses to an MMT. A potential limitation would be a possible postsurgical inflammatory response immediately after surgery (MMT_{+1 d}). Recently, it was reported that tumor necrosis factor- α infusion to healthy, lean male patients results in blunting of the GLP-1 effect on endogenous glucose production [29]. However, all surgeries were without complications and all patients left the hospital on postoperative day 1, immediately after MMT_{+1 d}.

Our study design also enabled comparison of the responses between T2D and NG patients. Glucose levels were similar in both groups. A possible explanation may be that the T2D patients were medicated until surgery. After RYGB, T2D patients responded with markedly less insulin than did the NG patients, a finding similar to what was

reported by Lund et al. [26] and Lips et al. [19]. In the study presented here, both groups of patients exhibited similar effects of RYGB on insulin sensitivity. GIP did not differ between the 2 groups at any time point at any test occasion, which is in line with the findings Lips et al. [19]. However, T2D patients responded with higher GLP-1 levels at MMT_{-1 d} and at MMT_{+6 w}. Three weeks after surgery, Lips et al. [19] did not observe any difference in GLP-1 response to an MMT in T2D patients and NG patients. At MMT_{+1 d}, our T2D patients responded with less GLP-1 than did NG patients. Because the incretins have been implicated in the remission of T2D after RYGB, one would hypothesize that levels of incretins would be affected in a more uniform manner than was observed in the study presented here. However, an explanation for this could be that K-cells and L-cells respond differently to the alteration of the gastrointestinal tract. RYGB is likely to result in the majority of K-cells being excluded from luminal stimuli and therefore being placed in a dormant state. This is evident in studies in which differences in GIP response are dependent on administration routes of a meal [21] and from massive GIP responses to a mixed meal being observed 2 months after RYGB reversal [22], suggesting hypersecretion of GIP as a response to a renewed exposure to nutrients.

A possible limitation of the study may be that, for safety reasons, we allowed the postsurgical volume of the pouch to determine the size of all MMTs. Thus, we may have provided the patients with an insufficient amount of carbohydrates preoperatively to elicit a response in insulin and incretins. However, this seems unlikely because insulin and GIP in both NG and T2D patients were responsive at MMT_{-4 w}, when the patients had an intact anatomy. Another limitation may be that differences in glucose levels may be confounded by the medications administered to the T2D patients. However, it is to be noted that hypoglycemic medications/insulin were held the night before surgery and on the first postoperative day.

Conclusion

In this study, RYGB-associated alterations in insulin and incretin responses to an MMT dominate over the effects of CR alone. Notably, the effect of RYGB on insulin and GIP release are immediate and evident within hours after the surgery. Also, the preoperative glycemic state of the patient is of importance to the response to a postsurgical meal.

Disclosures

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Paper II





Metabolic Effects of Gastric Bypass Surgery: Is It All About Calories?

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Bariatric surgery is an efficient method to induce weight loss and also, frequently, remission of type 2 diabetes (T2D). Unpaired studies have shown bariatric surgery and dietary interventions to differentially affect multiple hormonal and metabolic parameters, suggesting that bariatric surgery causes T2D remission at least partially via unique mechanisms. In the current study, plasma metabolite profiling was conducted in patients with ($n = 10$) and without T2D ($n = 9$) subjected to Roux-en-Y gastric bypass surgery (RYGB). Mixed-meal tests were conducted at baseline, after the presurgical very-low-calorie diet (VLCD) intervention, immediately after RYGB, and after a 6-week recovery period. Thereby, we could compare fasted and postprandial metabolic consequences of RYGB and VLCD in the same patients. VLCD yielded a pronounced increase in fasting acylcarnitine levels, whereas RYGB, both immediately and after a recovery period, resulted in a smaller but opposite effect. Furthermore, we observed profound changes in lipid metabolism following VLCD but not in response to RYGB. Most changes previously associated with RYGB were found to be consequences of the presurgical dietary intervention. Overall, our results question previous findings of unique metabolic effects of RYGB and suggest that the effect of RYGB on the metabolite profile is mainly attributed to caloric restriction.

Obesity presents a major challenge to our society, due to comorbidities such as type 2 diabetes (T2D), cardiovascular disease, and nonalcoholic fatty liver disease. Bariatric

surgery efficiently induces rapid and sustainable weight loss, also resulting in a rapid weight loss-independent remission of T2D (1) and improvements in cardiovascular disease risk factors (2) and nonalcoholic fatty liver disease (3). In the long term, T2D remission is likely a result of massive weight loss; however, the exact mechanism is not completely understood. Various factors may underlie the beneficial effects of bariatric surgery, including improved insulin secretion and action (4), increased β -cell mass (5), altered blood flow (6), bile acid (7), and gut microbiota composition (8). Despite the fact that reduced caloric intake results in T2D remission (9), we and others have shown that this may not be the only factor underlying bariatric surgery-elicited T2D remission (10,11).

While the majority of studies on T2D remission have focused on hormonal changes (12), a few studies investigated the effects on metabolism using metabolomics (13–21). Metabolomics enables a more comprehensive description of metabolism as compared with measurements of the one single metabolite glucose, which is assessed in most studies. Metabolomic studies have investigated both short-term (4–15 days) (19,20) and long-term (1–12 months) (13–19) consequences of Roux-en-Y gastric bypass surgery (RYGB) and sleeve gastrectomy. Collectively, these studies show effects on metabolism of all macronutrients, but do not resolve whether bariatric surgery elicits effects that differ from those observed after caloric restriction.

Very-low-calorie diet (VLCD) regimens for only 8 weeks induce T2D remission, which is then sustained for several

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weeks (22,23). This shared feature of bariatric surgery and VLCD suggests that the effect of RYGB on T2D remission could be caused by caloric restriction. In support of this, two recent studies found similar effects of RYGB and VLCD on weight loss, β -cell function, and insulin sensitivity (24,25). Despite these similarities, RYGB causes a more rapid T2D remission when compared with VLCD (26), as well as having different effects on the plasma metabolome (27). The lack of consensus may be explained by relatively small sample sizes in relation to the interindividual variability (21). A potential confounder in these studies is that dietary interventions tend to suffer from poor compliance (28), which is less expected after RYGB, in which food intake is physically limited. Hence, studies taking the interindividual variation into account are needed to resolve whether RYGB elicits unique effects or if it is just an extreme form of caloric restriction.

In the current study, we aimed to assess the immediate metabolic response to RYGB and compare that with that of caloric restriction (VLCD) in the same patients, thereby eliminating interindividual variation.

RESEARCH DESIGN AND METHODS

Study Participants and Study Setup

The study design has been described previously (11) and is summarized in Fig. 1A. Briefly, 19 age- and weight-

matched Caucasian women with a BMI >35 kg/m² were enrolled in the study. Nine subjects were normoglycemic (NG; HbA_{1c} $<6.0\%$, fasting glucose <6.1 mmol/L), and 10 were diagnosed with T2D at baseline (Supplementary Tables 1 and 2). Prior to surgery, patients were given weight-loss counseling and followed a VLCD regimen (858 kcal/day), aiming at losing 5% of body weight. Average age was 43 ± 6.3 years, BMI 39.8 ± 3.3 kg/m², and median HbA_{1c} 5.9% (interquartile range 3.5%). Median duration of T2D was 2.0 (interquartile range 2.75) years. RYGB was performed in a laparoscopic fashion, standardized to create a small (15–30 mL) gastric pouch, a 150-cm-long Roux-limb, and a 60-cm biliopancreatic limb. After surgery, patients were allowed sipping liquids and received 1,500–2,000 mL Ringer solution over 18–20 h. The patients were on a liquid diet for 2 weeks, followed by 2 weeks of purée, and a gradual introduction of solid foods. Expected caloric intake following surgery is 300 kcal/day the first few days, increasing to $\sim 1,500$ kcal/day after 6 weeks.

Mixed-meal tests (MMTs; 220 kcal, 5 g fat, 28 g carbohydrates, and 14 g protein) (Modifast) were performed at each study occasion (i.e., before the intervention) (–4 weeks, baseline), after 4 weeks of VLCD (1 day prior to surgery; –1 day, diet), 1 day postsurgery (+1 day, surgery), and 6 weeks after RYGB (+6 weeks, recovery) (Fig. 1A). Blood

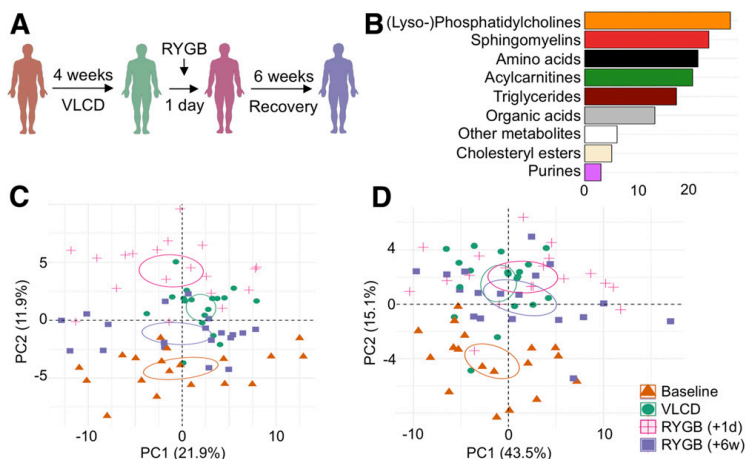


Figure 1—Multiplatform metabolomics of plasma samples from individuals subjected to a VLCD followed by gastric bypass surgery. **A:** Overview of the study design. Nine NG subjects and ten individuals with T2D were enrolled in the study and subjected to a VLCD, followed by RYGB. Before the intervention (–4 weeks, baseline, red), 4 weeks after VLCD (–1 day, diet, green), as well as 1 day (+1 day, surgery, pink) and 6 weeks after RYGB (+6 weeks, recovery, purple), the participants underwent a frequently sampled MMT (samples taken at fasting, 30 min, and 90 min after food intake were analyzed in the current study). **B:** Bar plot indicating the distribution of detected metabolites within various metabolite classes. Plot indicates percentage of total ($n = 212$) identified metabolite species per class. Score scatter plot from the PCA conducted on metabolite (**C**) and lipid (**D**) profiling data from fasting plasma samples. The first two principal components (PC1 and PC2) are shown, and the percentage of explained variance by the components is indicated in parentheses. Dotted lines show the 95% confidence regions of the indicated study occasions. Samples clustered according to the study occasion on PC2 (vertical axis). Fasting plasma samples obtained at baseline (–4 weeks) are depicted as orange triangles, samples obtained after the VLCD (–1 day) are depicted as green circles, samples obtained immediately after surgery (+1 day [+1d]) are depicted as pink crosses, and samples obtained after a 6-week recovery period (+6 weeks [+6w]) are depicted as purple squares.

samples were collected in prechilled EDTA tubes at several time points between 0 and 90 min after mixed-meal intake using a cubical vein catheter. Plasma was stored at -80°C until analysis. The study was approved by the Human Ethical Committee in Lund, Sweden, and adhered to the Declaration of Helsinki.

Metabolomics and Lipidomics

Metabolomics and lipidomics were conducted in plasma samples collected at 0, 30, and 90 min, reflecting the largest variation in glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1, during the MMT (11). Metabolites were extracted from 40 μL plasma (29) and analyzed on an Agilent 1,290 infinity UPLC connected to an Agilent 6550 iFunnel Q-TOF (Agilent Technologies, Santa Clara, CA), operated in positive and negative electrospray ionization mode. Metabolites of intermediate polarity were analyzed as previously described (29). Polar metabolites were separated on an Acquity BEH Amide column (1.7 μm , 2.1×100 mm) (Waters Corporation, Milford, MA), with an Acquity BEH Amide precolumn (1.7 μm , 2.1×5 mm) (Waters Corporation). Mobile phase A was 5 mmol/L ammonium formate and 0.1% formic acid, and B was acetonitrile/water (9:1; v/v) with 5 mmol/L ammonium formate and 0.1% formic acid, with the gradient: 0–2 min, 100% B; 2–5 min, 100–80% B; 5–9 min, 80–70% B; 9–11 min, 70–40% B; 11–14 min, 40% B; and 14–15 min, 40–95% B.

Lipids were extracted from 40 μL plasma with 150 μL methanol and 750 μL methyl tertbutyl ether (29,30). Following evaporation, the upper phase was reconstituted in 50 μL isopropanol/acetonitrile (90:10; v/v). Four microliters of sample was injected on an Acquity UPLC (Waters Corporation), equipped with an Acquity CSH C18 column (1.7 μm , 2.1×100 mm) (Waters Corporation) and an Acquity CSH C18 precolumn (1.7 μm , 2.1×5 mm) (Waters Corporation). Mobile phase A was acetonitrile/water (60:40; v/v), and B was isopropanol/acetonitrile (90:10; v/v), both with 10 mmol/L ammonium formate with the gradient: 0–13 min, 40–100% B; and 13–15 min, 100% B. Detection was performed in positive electrospray ionization on a Xevo 2G QToF MS (Waters Corporation).

Samples were analyzed in two batches, matched for glycemic status, using constrained randomization (31). Hence, samples from the same individual were analyzed in blocks, with randomization within and between blocks. Quality control samples, created by mixing aliquots from all samples, were analyzed eight times prior to the first injection and then every 10th injection. Metabolites were identified by tandem mass spectrometry using in-house libraries and the MassHunter METLIN Metabolite PCDL (Agilent Technologies). Leucine and isoleucine eluted as a single peak and were quantified as a single signal. Metabolites were integrated and manually confirmed in MassHunter Profinder B.06.00 (Agilent Technologies). Lipids were identified by tandem mass spectrometry using in-house libraries and integrated using MZmine 2.2.3 (32).

Statistical Analysis and Data Visualization

When the same metabolite was detected by multiple methods, data were kept from the analysis showing the lowest percent coefficient of variation of indicated metabolite in the quality control samples. Statistical analyses were conducted in R3.3.3. Metabolite and lipid values were log2-transformed, and missing data ($\leq 15\%$) were imputed using k-nearest-neighbor averaging (`impute.knn`, *impute*); metabolites with $>15\%$ missing data were excluded from further analysis. Common variance compensation (`ComBat`, *SVA*) was used to remove batch-to-batch variation. For principal component analysis (PCA; `prcomp`, *stats*), data were mean centered and scaled to unit variance. Linear mixed-effect models (LMMs), using the individual as blocking factor and including the interaction between study occasion and glycemic state, were fitted to the data via REML (`lmer`, *lme4*), followed by general linear hypotheses and multiple comparisons for parametric models (`glht`, *multcomp*). Outcomes of the LMMs were visualized in heat maps using hierarchical clustering (`heatmap.2`, *gplots*). The effect of VLCD was defined as differences between samples collected after 4 weeks of VLCD (1 day before RYGB; -1 day) versus baseline (-4 weeks). The immediate effect of RYGB was defined as the difference between samples obtained at first meal after surgery ($+1$ day) versus 1 day before RYGB (-1 day), whereas the 6-week effect of RYGB was defined as the difference between samples collected at 6 weeks' recovery after RYGB ($+6$ weeks) versus 1 day before RYGB (-1 day). The cumulative effect of the intervention (i.e., the collective effect of VLCD and RYGB) was defined as the difference between samples obtained at 6 weeks' recovery after RYGB ($+6$ weeks) versus baseline (-4 weeks) (Fig. 1A). A Tukey test was used post hoc to evaluate the significance of study occasion and glycemic state. We evaluated associations between metabolite data and clinical markers assessing an individual's glycemic status by building linear models on the scaled data (`lmFit`, *limma*) using empirical Bayes statistics for differential expression (`ebayes`, *limma*). Significance was defined as $q < 0.05$ using multiple testing adjustments according to the false discovery rate method.

Data and Resource Availability

The data sets generated and analyzed during this study are available for download from the MetaBioLights data repository.

RESULTS

Distinctive Effects of VLCD and RYGB on Weight, Hormones, and Glycemia

Anthropometric and clinical data for the patients included in this study have been reported previously using an unpaired analysis design (11). HbA_{1c} levels were lower in NG compared with T2D (Supplementary Table 1). VLCD resulted in significantly reduced weight (Supplementary Fig. 1) and BM, and improved HOMA of β -cell function (HOMA- β) (Supplementary Table 3). Six weeks after

surgery, BMI and weight decreased further, but no additional effect on HOMA- β was observed (Supplementary Fig. 1 and Supplementary Table 3). Fasting GIP levels increased immediately after surgery. The cumulative effect of the intervention elicited a decrease in fasting GIP levels, weight, and BMI (Supplementary Fig. 1 and Supplementary Table 3). All 10 patients with T2D were in a state of remission, as judged from normal HbA_{1c} levels and absence of medication, for 4 years after RYGB, after which medication was restarted in 1 patient.

Different Effects of VLCD and RYGB on the Fasting Plasma Metabolite Profile

We determined relative levels of 104 plasma metabolites using two complementary platforms (Fig. 1B and Supplementary Table 4). First, we applied PCA to obtain an overview of the metabolite profiles in the fasting state. The score scatter plot revealed samples to cluster according to study occasion along principal component 2 (Fig. 1C and Supplementary Fig. 2). Samples collected 1 day after RYGB (+1 day) were most distant from those collected at baseline (−4 weeks), followed by samples collected after VLCD (−1 day) and samples collected after 6 weeks of recovery (+6 weeks) (Supplementary Table 5).

The PCA loading plot revealed substantial alterations in multiple metabolite classes, including lipids (Supplementary Fig. 2). Therefore, we investigated lipids in more detail using a dedicated platform, yielding data on relative levels of 72 lipids, including phosphatidylcholines (PCs), lysophospholipids, cholesteryl esters, sphingomyelins (SMs), and triglycerides (TGs). A PCA calculated on the lipid profiles in the fasting state revealed that samples collected at baseline (−4 weeks) clustered far from all other occasions along principal component 2 (Fig. 1D and Supplementary Fig. 2). Samples collected after VLCD (−1 day), 1 day after RYGB (+1 day), and after 6 weeks of recovery (+6 weeks) clustered together (Supplementary Table 5), indicating that lipid profiles were predominantly affected by VLCD, but not by RYGB.

VLCD and RYGB Alter Acylcarnitine and Phospholipid Levels

Next, we examined alterations in fasting metabolite and lipid levels elicited by VLCD and RYGB using LMMs. VLCD resulted in a dramatic increase in levels of acylcarnitines, essential amino acids, and the ketone body 3-hydroxybutyrate and reduced levels of basic amino acids (Fig. 2A, Supplementary Fig. 3, and Supplementary Table 6). All changes remained significant after adjustment for insulin levels and the majority also after adjustment for weight loss (Supplementary Table 6). Notably, PCs and TGs with shorter fatty-acyl chain length were decreased and those with longer chain length increased ($P < 0.05$) (Fig. 2A and Supplementary Table 6). The majority of changes in lipids remained significant after adjustment for insulin levels, whereas those showing increased levels were significant also after adjustment for weight loss (Supplementary Table 6).

The 6-week effect of RYGB was associated with reduced levels of 6 out of the 20 detected acylcarnitines and 3-hydroxybutyrate (Fig. 2A and Supplementary Table 6). Changes in other polar metabolite classes were less systematic, and levels of very few lipids were affected (Fig. 2A and Supplementary Table 6). All effects remained significant after adjustment for insulin levels and most also after adjustment for weight loss (Supplementary Table 6). Hence, the 6-week effect of RYGB was associated with alterations in levels of acylcarnitines and 3-hydroxybutyrate, as well as SM 36:0, which were opposite to those observed after VLCD.

As RYGB can induce remission of T2D within days after surgery (1), we additionally examined the immediate effect of RYGB. The immediate response to RYGB elicited reduced levels of medium-chain acylcarnitines, whereas levels of the short-chain acylcarnitine 2:0 and carnitine were increased (Fig. 2A and Supplementary Table 6). Levels of most amino acids and 3-hydroxybutyrate were increased, purines were decreased, and few lipids were altered (Fig. 2A and Supplementary Table 6). All effects remained significant after adjustment for insulin levels and the majority also after adjustment for weight loss. Thus, the immediate effect of surgery was opposite to that of VLCD for several metabolites, mainly acylcarnitines, whereas the effect was similar regarding others, such as 3-hydroxybutyrate.

Finally, we investigated the cumulative effect of the intervention, which is similar to the comparisons examined in the majority of previous studies (21). Levels of medium- and long-chain acylcarnitines were increased, whereas those of the short-chain acylcarnitine 3:0 and carnitine were decreased (Fig. 2A and Supplementary Table 6). In addition, levels of 27 out of 72 detected phospholipids and TGs were altered (Fig. 2A and Supplementary Table 6). Overall, the cumulative effect was similar to the effect of the VLCD, including the reduction in PCs and TGs with shorter fatty-acyl chain length, and an increase in those with longer chain length ($P < 0.05$). The majority of changes remained significant after adjustment for insulin levels, whereas changes in levels of long-chain acylcarnitines and 11 lipids remained significant also after adjustment for weight loss (Supplementary Table 6).

As changes in acylcarnitines were a predominant feature in all comparisons, we investigated this class of metabolites in more detail. VLCD increased levels of total acylcarnitine, with the effect being more pronounced for medium- and long-chain compared with short-chain and free carnitine (Fig. 2B). Conversely, total acylcarnitine levels were decreased as an immediate and 6-week effect of RYGB, which was mainly driven by changes in short- and long-chain acylcarnitines (Fig. 2B and Supplementary Table 6). The VLCD and cumulative effects on acylcarnitine levels were subtle, in particular for medium- and long-chain acylcarnitines, in T2D as compared with NG (Fig. 2B and Supplementary Table 7). The immediate and 6-week effects of surgery were similar in both groups (Fig. 2B).

Both the VLCD and cumulative effect of the intervention on levels of the branched-chain amino acid (BCAA)

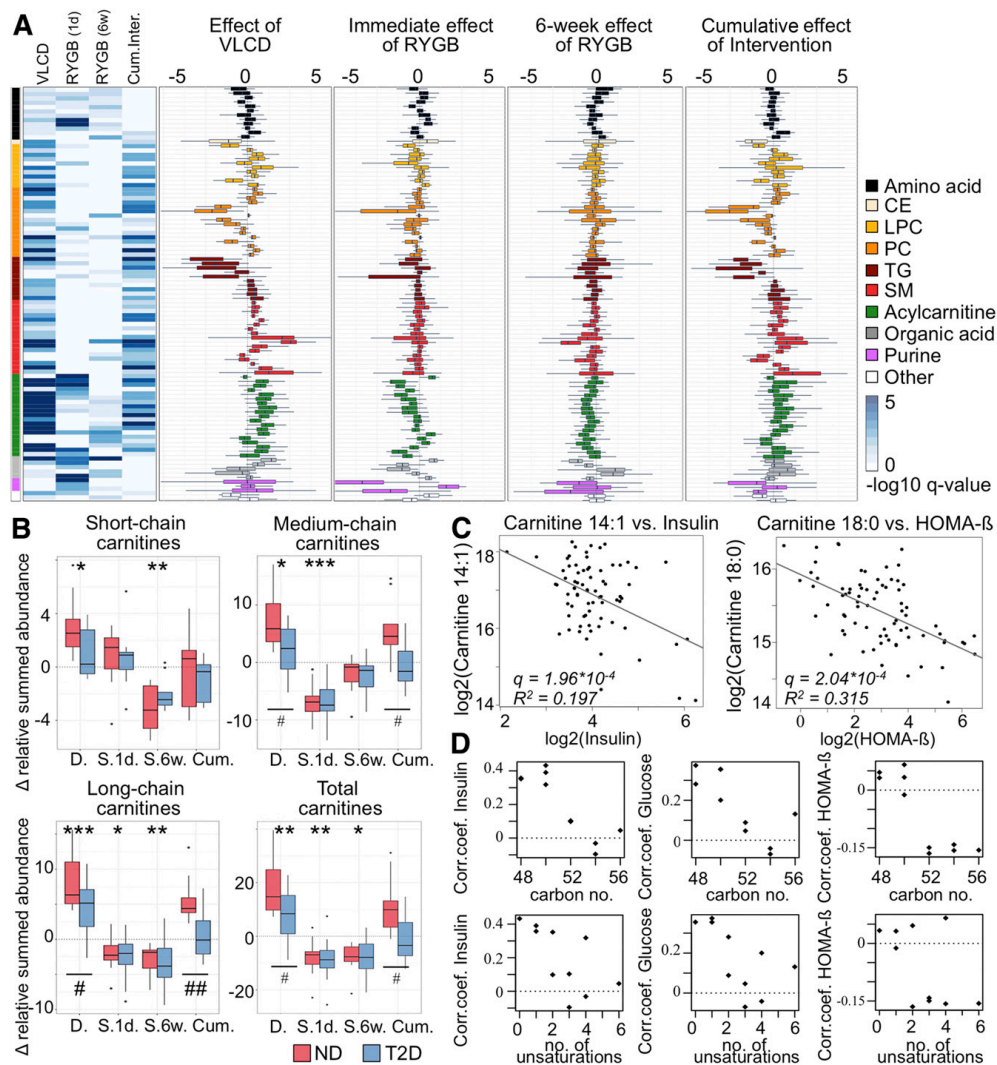


Figure 2—The effect of diet and surgery on metabolite and lipid levels. **A**: Metabolites with significant differences in response to the VLCD, the immediate effect of RYGB (1d), the 6-week effect of RYGB (6w), and the cumulative effect of the intervention (Cum.Inter.) as determined by LMMs are displayed in a heatmap (left panel). Color intensity reflects the $-\log_{10}$ q values of the LMMs. In the right panels, box-and-whisker plots indicate the relative changes in metabolite levels, displayed separately for the effect of VLCD, RYGB (immediately and after 6 weeks), and the cumulative intervention. Colors of the box plots indicate metabolite classes. For an enlarged graph, see Supplementary Fig. 3. **B**: Box-and-whisker plots indicating the deltas of summed relative levels of acylcarnitine species divided by their chain length, as an effect of diet (D.), the immediate effect of surgery (S.1d.), the 6-week effect of surgery (S.6w.), and the cumulative effect of the intervention (Cum.). Short-chain acylcarnitines were defined as ranging from C3 to C7, medium-chain acylcarnitines as ranging from C8 to C13, and long-chain acylcarnitines as ranging from C14 to C20. Red bars represent fasting plasma samples obtained from ND (ND) individuals, and blue bars represent samples from individuals with T2D. Asterisks indicate significant effects as determined by LMMs: * $q < 0.05$; ** $q < 0.01$; *** $q < 0.001$. Hashtags indicate significant differences between individuals without diabetes (ND) and with T2D for the indicated effect: # $P < 0.05$; ## $P < 0.01$. **C**: Scatter plots displaying associations between metabolites and clinical markers; all metabolites and clinical parameters are presented in logarithmic scale. Linear regression lines, q values, and R^2 of the linear regression models are displayed. Data for all metabolites and their associations with clinical variables are found in Supplementary Table 9. **D**: Scatter plots displaying associations between TG species and measures of glycemic status. Carbon number (top panels) and number of unsaturations (bottom panels) of all determined

(iso)leucine and lipids depended on the glycemic status (Supplementary Table 7). In particular, the increase of highly unsaturated lipids was less pronounced in T2D as compared with NG (Supplementary Table 7).

The targeted data analysis reveals that most changes occur as an effect of VLCD. Similar results were observed in an untargeted analysis (Supplementary Fig. 4 and Supplementary Table 8). Out of 719 detected features, 107 were affected by VLCD, 26 as an immediate effect of RYGB and 27 as an effect of RYGB. The cumulative effect of the intervention was associated with alterations in 58 features, out of which 40 were governed by VLCD and only 7 by RYGB.

Levels of Acylcarnitines and Lipids Associate With Measures of Glycemic Control

Having established that diet and surgery elicit unique changes in the metabolite and lipid profiles, we investigated whether these changes are associated with clinical variables for assessment of glycemic status using fasting metabolite levels from all occasions in linear models adjusted for age and BMI. Insulin levels were negatively associated with levels of several acylcarnitines, HOMA- β was negatively associated and glucose was positively associated with levels of long-chain acylcarnitines, and the Matsuda index was positively associated with short- and medium-chain acylcarnitines (Fig. 2C and Supplementary Table 9). TGs with shorter acyl chains and few unsaturations showed positive associations with HOMA- β , insulin, GIP, and glucose levels and negative associations with glucagon-like peptide 1 and the Matsuda index, whereas those with longer acyl chains and higher degree of unsaturation were not correlated (Fig. 2D and Supplementary Table 9), suggesting that TGs harbor detailed information on glycemic state. Associations for all variables, except for HOMA- β , remained after adjustment for weight loss.

VLCD and RYGB Affect the Response of Acylcarnitine Levels to an MMT

Finally, we evaluated the response to an MMT for each metabolite, estimated as the incremental area under the curve (iAUC). VLCD elicited decreased iAUC for the majority of acylcarnitines and several phospholipids (Fig. 3A and B and Supplementary Table 10). All changes remained significant after adjustment for insulin levels, whereas mainly acylcarnitines remained significant after adjustment for weight loss (Supplementary Table 10).

The iAUCs for the majority of metabolites and lipids remained largely unchanged as an effect of RYGB. Only the iAUC of acylcarnitine 3:0 was increased, an effect remaining significant after additional adjustment for weight loss or insulin levels (Supplementary Table 10).

The immediate effect of RYGB elicited increased iAUCs of dietary purines, as well as short- and medium-chain acylcarnitines, and reduced iAUCs for several amino acids (Fig. 3A and Supplementary Table 10). The majority of alterations remained significant after adjustment for insulin levels and changes in purines and medium-chain acylcarnitines also after adjustment for weight loss (Supplementary Table 10).

The cumulative effect of the intervention resulted in significantly decreased iAUCs for several acylcarnitines and lipids (Fig. 3A and B and Supplementary Table 10). The changes in iAUCs for most acylcarnitines remained significant after adjustment for insulin levels or weight loss (Supplementary Table 10).

None of the observed effects differed significantly between NG and T2D (Supplementary Table 11). At a nominal level, however, alterations in the iAUC for several acylcarnitines, BCAAs, aromatic amino acids (lipo)leucine, phenylalanine, and tyrosine, and phospholipids elicited by the VLCD and the cumulative effect of the intervention were significantly different between T2D and NG (Fig. 3B and Supplementary Table 11).

DISCUSSION

It is well established that both VLCD and RYGB result in improved glycemia (4). RYGB has gained enormous attention for its ability to induce rapid T2D remission (1), which was observed in all patients with T2D in this study. VLCD has a transient effect on T2D with respect to improved fasting plasma glucose, HbA_{1c}, and β -cell function (9,22,23,33). As presurgical caloric restriction is a safety prerequisite for undergoing RYGB, most studies assessing the impact of RYGB on various end points have in fact studied the combined effect of caloric restriction and RYGB (24,25). In this study, we assessed the impact of both types of interventions separately by using tight sampling intervals. Furthermore, the paired study design allowed us to eliminate interindividual variation. Our data show that the initial caloric restriction achieved by VLCD resulted in very distinct alterations in the metabolome, as compared with those later elicited by RYGB.

VLCD resulted in a marked increase in acylcarnitine levels, whereas RYGB, both immediately and after a 6-week recovery period, resulted in a smaller, but opposite, effect. Consequently, acylcarnitine levels were still above basal levels in the postoperative phase. This is in line with a previous study showing acylcarnitine levels to be elevated 15 days postsurgery, which were diminished to preoperative levels after 90 days (20). To some extent, these changes may be due to increased caloric and carbohydrate intake over time. However, whereas these changes have previously been

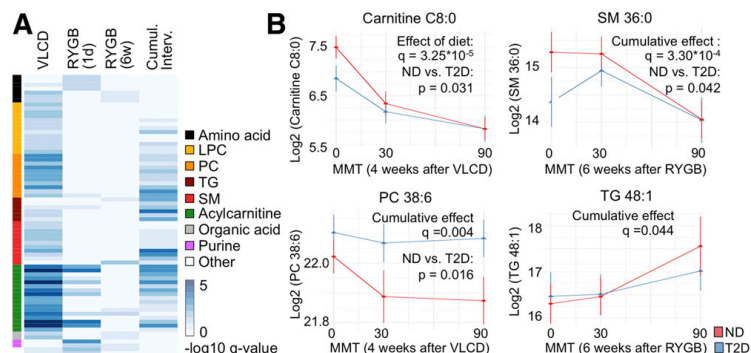


Figure 3—The effect of diet and surgery on the postprandial response to an MMT. **A:** Heat map indicating metabolites for which the iAUCs were significantly altered as an effect of diet, the immediate effect of surgery (1d), the 6-week effect of surgery (6w), and the cumulative effect of the intervention (Cumul.Interv.). Color intensity reflects the $-\log_{10}$ q values as determined by LMM. **B:** Line plots showing levels of carnitine 8:0 and PC 38:6 during the MMT at -1 day diet (left panels) and levels of SM 36:0 and TG 48:1 during the MMT at $+6$ weeks' recovery (right panels). Red lines indicate samples derived from NG individuals (ND), and blue lines indicate samples from individuals with T2D. The q values of significant effects as determined by LMMs as well as P values for significant differences between those without diabetes (ND) and individuals with T2D are shown. Data are presented as mean $\pm$ SE. For details, see Supplementary Table 10. LPC, lysophosphatidylcholine.

attributed to RYGB, our data rather suggest that they result from the preoperative caloric restriction. An early study comparing RYGB with VLCD revealed both RYGB and VLCD to increase total acylcarnitine levels, whereas short (C3 and C5) acylcarnitines decreased only after RYGB (27). These results are in line with the effects of VLCD and the cumulative effect of the intervention observed in this study. Presumably, increased levels of acylcarnitines are caused by increased lipolysis in combination with excretion of acylcarnitines to avoid CoA trapping (34) and accumulation of toxic fatty acids (35). The reduction in acylcarnitine levels observed as an effect of RYGB may reflect a stress response (36), in line with observations of acylcarnitines to be elevated after the preoperative dietary restriction and then to decrease after induction of anesthesia (37). In addition, we found that the immediate effect of RYGB was associated with increased levels of 3-hydroxybutyrate, which has also been previously reported as an effect of anesthesia (38), but also may be caused by the preoperative fasting. Whereas anesthesia previously has been shown to reduce levels of most amino acids (38), our data reveal a more complex pattern. It must be noted that our samples are collected after and not during anesthesia. Hence, some of the immediate effects of surgery are likely to be associated with surgical stress, fasting, and anesthesia, whereas others are likely the result of the RYGB per se.

The VLCD-induced increase in acylcarnitine levels was less pronounced in patients with T2D when compared with NG individuals. Multiple studies have shown levels of acylcarnitines to be elevated in T2D and insulin resistance (39). Hence, a starvation-associated increase in acylcarnitines could blunt the reduction of these intermediates after normalization of insulin signaling, leading to normalization

in levels of the metabolites. This is further supported by our findings of acylcarnitine levels being inversely associated with the Matsuda index. The lack of association with HOMA of insulin resistance implicates an effect on postprandial glucose disposal, which is perturbed in obesity, rather than on fasting insulin resistance (40,41). This notwithstanding, both indices may have shortcomings when applied on RYGB and during a negative energy balance.

We observed profound changes in lipid metabolism following VLCD, but less pronounced changes in response to RYGB. Notably, PCs and TGs with shorter fatty-acyl chain lengths were decreased after VLCD, whereas lipids with longer fatty-acyl chains were increased. These findings could explain some of the improvement in glucose homeostasis, as it is coherent with several previous findings in lipid metabolism associated with glycemic control and future risk of T2D (42–44). With respect to specific lipids, TG 48:1 and 50:0, which previously have been associated with an increased future risk of T2D (45), were positively correlated with insulin and glucose levels.

Our findings contradict previous studies in which similar changes have been observed but attributed to the RYGB (16–20) instead of the preoperative VLCD. Moreover, our finding of carbon number and unsaturation-dependent alterations in lipids contradict previous studies finding a coherent decrease in PC and phosphatidylethanolamine levels after RYGB (17–19). Our observation of decreased levels of long saturated SMs and increased levels of long polyunsaturated SMs is in agreement with a previous study (17).

We found a few weak ($0.01 < q < 0.05$), but unique, immediate, and 6-week effects of RYGB on the metabolite profile. However, these alterations, involving an

unsystematic response in BCAAs and aromatic amino acids, elevated levels of arginine and taurine, and changes in presumably diet-derived metabolites such as caffeine and *trans*-cinnamic acid, do not support a beneficial effect on glycemia (45–49). Nonetheless, a coordinated decrease in levels of amino acid–derived short-chain acylcarnitines (C3–C5) may suggest a shift in substrate preference for oxidative metabolism.

Some limitations of the study should be mentioned. Firstly, patients were followed for a relatively short period of time, precluding prediction of long-term effects and potentially introducing carryover effects. Secondly, all samples except for baseline samples were taken very close to the interventions, with patients in a catabolic state, which was initiated during the VLCD and maintained throughout the subsequent interventions. The catabolic state is expected to remain for at least 12 months, as patients continue to lose weight in the first 12 months after surgery (50). Retesting at steady state would be interesting and will be attempted in the future, but then metabolic regulation occurring during T2D remission will be concealed. Finally, the number of individuals included in this study was fairly small, all were women, and all were in a state of T2D remission, precluding analyses stratified for remission. However, by combining constrained randomization with dependent analysis of effects, we could minimize the impact of individual and technical variation, thereby improving the power of our analysis (31).

Overall, our data suggest that the preoperative dietary restriction elicits most of the metabolic changes that have previously been attributed to RYGB. Our data are in concordance with more recent studies showing that weight loss either by surgery or VLCD elicits similar effects on glucose homeostasis, despite documented changes of incretin/insulin levels after RYGB (24,25). Moreover, some of the metabolic changes that associated with the immediate effect of surgery are likely to result from the surgical procedure and not RYGB per se.

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Author Contributions. K.H., J.B., M.A.M., and C.B.A. performed experiments. K.H. analyzed the data and wrote the first draft of the manuscript. J.H., L.G., N.W., and P.S. planned and conceived the study. All authors contributed in writing the final version of the manuscript. P.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Paper III



The Effects of Calorie Restriction and Bariatric Surgery on Circulating Proneurotensin Levels

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Abstract

Context: Proneurotensin (pNT) is associated with obesity and type 2 diabetes (T2D), but the effects of Roux-en-Y gastric bypass (RYGB) on postprandial pNT levels are not well studied.

Objective: This work aimed to assess the effects of RYGB vs a very low-energy diet (VLED) on pNT levels in response to mixed-meal tests (MMTs), and long-term effects of RYGB on fasting pNT.

Methods: Cohort 1: Nine normoglycemic (NG) and 10 T2D patients underwent MMT before and after VLED, immediately post RYGB and 6 weeks post RYGB. Cohort 2: Ten controls with normal weight and 10 patients with obesity and T2D, who underwent RYGB or vertical sleeve gastrectomy (VSG), underwent MMTs and glucose-dependent insulintropic polypeptide (GIP) infusions pre surgery and 3 months post surgery. Glucagon-like peptide-1 (GLP-1) infusions were performed in normal-weight participants. Cohort 3: Fasting pNT was assessed pre RYGB (n = 161), 2 months post RYGB (n = 92), and 1 year post RYGB (n = 118) in NG and T2D patients. pNT levels were measured using enzyme-linked immunosorbent assay.

Results: Reduced fasting and postprandial pNT were evident after VLED and immediately following RYGB. Reintroduction of solid food post RYGB increased fasting and postprandial pNT. Prior to RYGB, all patients lacked a meal response in pNT, but this was evident post RYGB/VSG. GIP or GLP-1 infusion had no effect on pNT levels. Fasting pNTs were higher 1 year post RYGB regardless of glycemic status.

Conclusion: RYGB causes a transient reduction in pNT as a consequence of caloric restriction. The RYGB/VSG-induced rise in postprandial pNT is independent of GIP and GLP-1, and higher fasting pNTs are maintained 1 year post surgically.

Key Words: calorie restriction, RYGB, pNT, gastric bypass

Abbreviations: AUC, area under the curve; BMI, body mass index; BPD, biliopancreatic diversion; GI, gastrointestinal; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; MMT, mixed-meal test; NG, normoglycemic; NT, neurotensin; pNT, proneurotensin; RYGB, Roux-en-Y gastric bypass; T2D, type 2 diabetes; VLED, very low-energy diet; VSG, vertical sleeve gastrectomy.

Roux-en-Y gastric bypass (RYGB) is the most effective weight loss treatment for severe and complex obesity. Furthermore, the majority of people with type 2 diabetes (T2D) who undergo RYGB experience T2D remission, independently of weight loss (1). The mechanisms through which T2D remission is achieved are not yet fully understood. Indeed, RYGB is associated with exaggerated secretion of glucagon-like peptide-1 (GLP-1) (1, 2) as well as an increased number of L cells post RYGB (3). Our previous studies, however, suggest that the improvement in glycemia precedes the response in GLP-1 after RYGB (1). Furthermore, the immediate effect of RYGB on the metabolome seems mainly related to caloric restriction, rather than the rerouting of the gastrointestinal (GI) tract (4). Thus, there is a need for continued investigations on

RYGB-associated alterations to shed light on the mechanisms behind the effect on glycemia as well as sustained weight loss.

The gut hormone neurotensin (NT) is mainly expressed in the distal small intestine (5) and has been shown to be positively correlated with increased T2D incidence in a Nordic population-based study (6). Furthermore, NT reduces food intake (7), gastric emptying, and gastric acid secretion (8); and increases blood flow to the small intestine (9), hepatic bile acid secretion (10), and uptake (11), as well as pancreatic exocrine activity (12). Gene targeting or inhibition of NT results in reduced fat absorption as well as a protective effect against high-fat diet-mediated obesity and insulin resistance (13). It is not fully understood how NT is affected by RYGB. However, a number of studies have tried to address this. First, in a study

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by Jorsal et al (2), fasting NT levels were shown to be increased 1 week and 3 months after RYGB. NT levels were also increased in response to a mixed-meal 1 week post surgery with a reduction toward presurgical levels being observed 3 months post surgery, although an increase in peak levels of NT was also observed at this time point (2). Another study (14) reported increased fasting proneurotensin (pNT) levels in patients who underwent RYGB compared to both age-matched patients that were either normal weight or had obesity; furthermore, patients who underwent RYGB had a blunted response in pNT levels after a mixed-meal compared with patients that were either normal weight or had obesity. Increased pNT levels in patients with obesity has also been shown at 24 months after adjustable gastric banding or RYGB (15). This response was shown to be affected by biliopancreatic limb length, with a longer limb resulting in higher fasting NT levels as well as higher levels in response to a mixed meal (16). In rats, *Nts* gene expression was increased in the alimentary limb in response to RYGB along with increased plasma NT levels (7). It has also been shown that fasting plasma pNT levels increase more robustly after biliopancreatic diversion (BPD) than after RYGB (17). The same study identified a positive correlation between pNT levels and insulin sensitivity, and a negative correlation with fasting glucose, suggesting positive effects of NT on glucose homeostasis (17). In our lean porcine model of RYGB, a substantial increase in duodenal NT cell density was identified post RYGB (18). On the other hand, we have shown that NT cell density was unaffected in human jejunal samples 1 year post RYGB (3).

To gain further insight into potential RYGB-induced alterations in pNT plasma levels, we took advantage of our study design with mixed-meal tests (MMTs) performed with tight intervals during the RYGB treatment regimen. This design enabled a direct comparison of the effect of caloric restriction vs that of RYGB. Thus, we performed MMTs both in patients with normoglycemia (NG) and T2D without underlying comorbidities, before and after a very low-energy diet (VLED) regimen, acutely post operation as well as 6 weeks post surgically when solid food had been reintroduced to the patients. We used another cohort of patients who had undergone RYGB or vertical sleeve gastrectomy (VSG) for replication and also studied the long-term effects of RYGB on plasma pNT levels in a third cohort. Finally, we addressed the potential influence of incretin hormones on pNT levels in infusion experiments.

Materials and Methods

Patients

In the present study, we used samples from 3 previously published patient cohorts (1, 19, 20).

Cohort 1

The characteristics of the patients used in this study have been described previously (1). Briefly, 19 age- and weight-matched White women who had undergone RYGB were studied. Patients had a mean age of 43 ± 1.7 years and a body mass index (BMI) of 37.5 ± 0.9 . Inclusion criteria consisted of female sex, age 30 to 55 years, BMI greater than 35, and no preoperative incretin-based medication. Exclusion criteria was prior bariatric surgery or previous surgery to the upper GI tract. Nine patients were NG (glycated hemoglobin $A_{1c} < 42.1$ mmol/mol and

fasting plasma glucose < 6.1 mmol/L without antidiabetic intervention) while 10 patients had T2D. Average duration of T2D was 3.1 ± 0.9 years with 2 patients being treated solely with insulin; 2 patients being treated with insulin alongside metformin; 2 patients with only metformin; 1 patient with insulin, metformin, and glimepiride; and 3 patients controlling their T2D through diet and lifestyle. Antidiabetic medications were withheld the night prior to surgery and the first day post operation. Patients received preoperative counseling to try to reduce liver size by targeting 5% weight loss. Weight loss was accomplished by way of a very low-energy diet (VLED). This diet consisted of 4 servings of Modifast (Modifast) per day, totaling 858 kcal/day (110 g carbohydrate, 57 g protein, and 18 g fat). All participants provided written informed consent, and the study was approved by the human ethical committee in Lund, Sweden, and adhered to the Declaration of Helsinki.

Mixed-meal tests. MMTs were performed as previously described (1). Patients fasted overnight prior to arrival to the clinic. Basal blood samples were collected using a cubital vein catheter. The volume of the mixed-meal was decided by the volume that was tolerable to the patients 1 day post operation (MMT_{+1d}). We have previously shown this volume to be 200 mL of Modifast ingested over 20 minutes (21, 22) consisting of 28 g carbohydrates, 14 g protein, and 5 g fat resulting in a total of 220 kcal. The meal was ingested within 20 minutes. Blood samples were collected in chilled EDTA tubes coated with 0.1 mmol/L diprotin A and 500 KIU/mL aprotinin at $-5, 0, 5, 10, 15, 30, 60$, and 90 minutes post meal ingestion. Plasma samples were stored at -80°C until analyses. Patients were given the same volume and at the same rate during all MMTs as described previously (21). Four MMTs were performed: (1) before starting the VLED 4 weeks before the operation (MMT_{-4w}); (2) 1 day prior to surgery (MMT_{-1d}); (3) the morning of the first day post operation (MMT_{+1d}); and (4) 6 weeks after the operation (MMT_{+6w}).

Cohort 2

The characteristics of the patients used in this study have been described previously by Honka et al (19). In brief, the study included 10 patients with obesity and T2D who underwent RYGB ($n = 5$) or VSG ($n = 5$), as well as 10 normal-weight and NG control participants. The inclusion criteria for the control population were BMI of 18 to 27, age 18 to 60 years, fasting plasma glucose less than 6.1 mmol/L, and normal oral glucose tolerance test. All participants provided written informed consent and the ethics committee of the Hospital District of Southwest Finland (Turku, Finland) approved the study.

Mixed-meal tests. Before the introduction of VLED and 69 days (range, 55–97 days) after surgery, patients had MMTs consisting of a 250-kcal liquid meal (NutriDrink; Nutricia Advanced Medical Nutrition) containing 40 g carbohydrates, 6 g fat, and 9 g protein, which was ingested within 10 minutes. Blood samples were taken at 0, 15, 30, 45, 60, and 90 minutes postmeal ingestion and handled as in cohort 1.

Gastric inhibitory polypeptide and glucagon-like peptide-1 infusions (cohort 2). The glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) infusions have been described previously (19, 23). GIP infusions were performed on patients with T2D who underwent RYGB ($n = 5$) or VSG ($n = 5$) and control patients who were

normal weight ($n = 10$). GLP-1 infusions were performed only on patients who were normal weight ($n = 10$). Briefly, GIP₁₋₄₂ and GLP-1₇₋₃₆ (Bachem Holding AG) were dissolved in 2% human serum albumin in sterile water and administered via a cannula with syringe pumps into the peripheral vein. For both, a constant 75-minute infusion was performed following collection of baseline parameters. GIP₁₋₄₂ was infused at a rate of 4.0 pmol/kg/min for 15 minutes and thereafter at a rate of 2.0 pmol/kg/min. This was to reproduce GIP concentrations in the circulation after a meal. Infusion was performed pre surgery and 80 days post surgery on average (range, 47-92 days). GLP-1 was infused at a rate of 0.75 pmol/kg/min for the duration of the test. Blood samples were collected as per the MMT for cohort 2 and handled in the same manner.

Cohort 3

This cohort has been described previously (20). The study involved an initial cohort of 167 individuals, of whom 148 qualified for RYGB during the years 2012 to 2019 in Sweden. All measurements were performed after an overnight fast. Baseline clinical parameters and blood samples were obtained at the first visit to the clinic, before introduction of the presurgical VLED, which was on average 2 months pre surgery. The day of the operation, only body weight was measured. Blood was sampled at baseline ($n = 161$ out of which $n = 27$ were diagnosed with T2D), then at 2 ($n = 92$) and 12 months post RYGB ($n = 118$). We classified patients as having prediabetes if they had a glycated hemoglobin A_{1c} of 39 mmol/mol or greater as per the American Diabetes Association guidelines (24). The study was approved by the human ethical committee in Lund, Sweden, and adhered to the standards of the Declaration of Helsinki. All patients provided written informed consent.

Proneurotensin Analysis

Due to the in vivo and in vitro instability of mature neurotensin, we measured a stable 117-amino acid fragment from the N-terminal of the proneurotensin/neuromedin precursor prohormone (pNT) as previously reported (6, 25). Furthermore, the production of this hormone has been proven to be in stoichiometric amounts relative to mature NT (26). To quantify pNT levels, a chemiluminometric sandwich enzyme-linked immunosorbent assay (SphingoTec GmbH; RRID:AB_3086809) was performed as has been described previously (26).

Statistical Analyses

All data are presented as mean $\pm$ SEM or as median minimum to maximum. Statistical significance was calculated between time points using the *t* test and between groups using one-way analysis of variance with the Dunnett multiple comparisons test, with a single pooled variance. Pearson correlation coefficients were calculated to perform correlation analyses. Outliers were identified using ROUT. This method adopts a false discovery rate for non-linear regression approach to identify outliers (27). All analyses were performed using Prism 9.2.0 (GraphPad Software).

Results

Effect of a Very Low-Energy Diet and Roux-en-Y Gastric Bypass on Fasting Plasma Levels of Proneurotensin (Cohort 1)

Fasting samples were collected prior to commencing MMTs to assess any differences in basal pNT levels (MMT_{-4w}) which

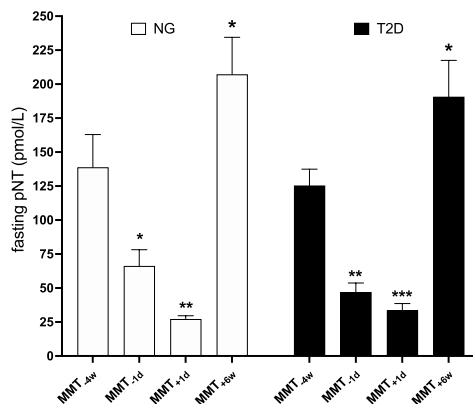


Figure 1. Fasting proneurotensin (pNT) levels 4 weeks pre-RYGB (MMT_{-4w}), immediately pre-RYGB (MMT_{-1d}), immediately post-RYGB (MMT_{+1d}), and 6 weeks post-RYGB (MMT_{+6w}) in patients with obesity and normoglycemia (NG) or type 2 diabetes (T2D). **P* less than .05, ***P* less than .01, and ****P* less than .001 compared with MMT_{-4w} in patients with the same glycemic status.

may be due to VLED (MMT_{-1d}), RYGB (MMT_{+1d}), or the re-introduction of solid food (MMT_{+6w}). There were no differences in fasting pNT levels between patients who had NG or patients with T2D at any time points (Fig. 1). VLED resulted in a 52% reduction in fasting pNT levels in patients with NG ($P < .05$; see Fig. 1). RYGB (MMT_{+1d}) further reduced fasting pNT levels by 59% in patients with NG ($P < .01$). However, at MMT_{+6w}, fasting pNT levels in patients with NG were increased 7.6-fold ($P < .001$) compared with MMT_{+1d}.

In patients with T2D, VLED resulted in a 63% reduction in fasting pNT levels ($P < .001$). There was no significant further acute effect of RYGB on fasting levels of pNT in patients with T2D. Fasting pNT increased 5.7-fold between MMT_{+1d} and MMT_{+6w} in patients with T2D ($P < .001$; see Fig. 1). Thus, both in participants with T2D and NG, caloric restriction reduced fasting levels of pNT, whereas pNT levels rose to above presurgery levels after reintroduction of solid food.

Effect of Very Low-Energy Diet and Roux-en-Y Gastric Bypass on Postprandial Plasma Levels of Proneurotensin (Cohort 1)

To assess the effects of VLED, RYGB, and the reintroduction of solid food post RYGB on postprandial pNT responses, MMTs were performed before VLED (MMT_{-4w}), the day prior to RYGB (MMT_{-1d}), the morning after RYGB (MMT_{+1d}), and 6 weeks post RYGB (MMT_{+6w}). Notably, before the intervention neither of the groups showed a meal response in pNT levels (Fig. 2A and 2C). In line with the fasting data, patients with NG (Fig. 2A and 2B) and T2D (Fig. 2C and 2D) demonstrated reduced pNT levels in response to VLED (MMT_{-1d}), as well as acutely in response to RYGB (MMT_{+1d}) compared with starting levels (MMT_{-4w}).

Interestingly, acutely after RYGB (MMT_{+1d}) both groups displayed a meal-response in pNT, with 3.9-fold increases being observed at 60 to 90 minutes compared with 0 minutes in patients with NG ($P < .001$; see Fig. 2A) and a 4.0-fold increase at

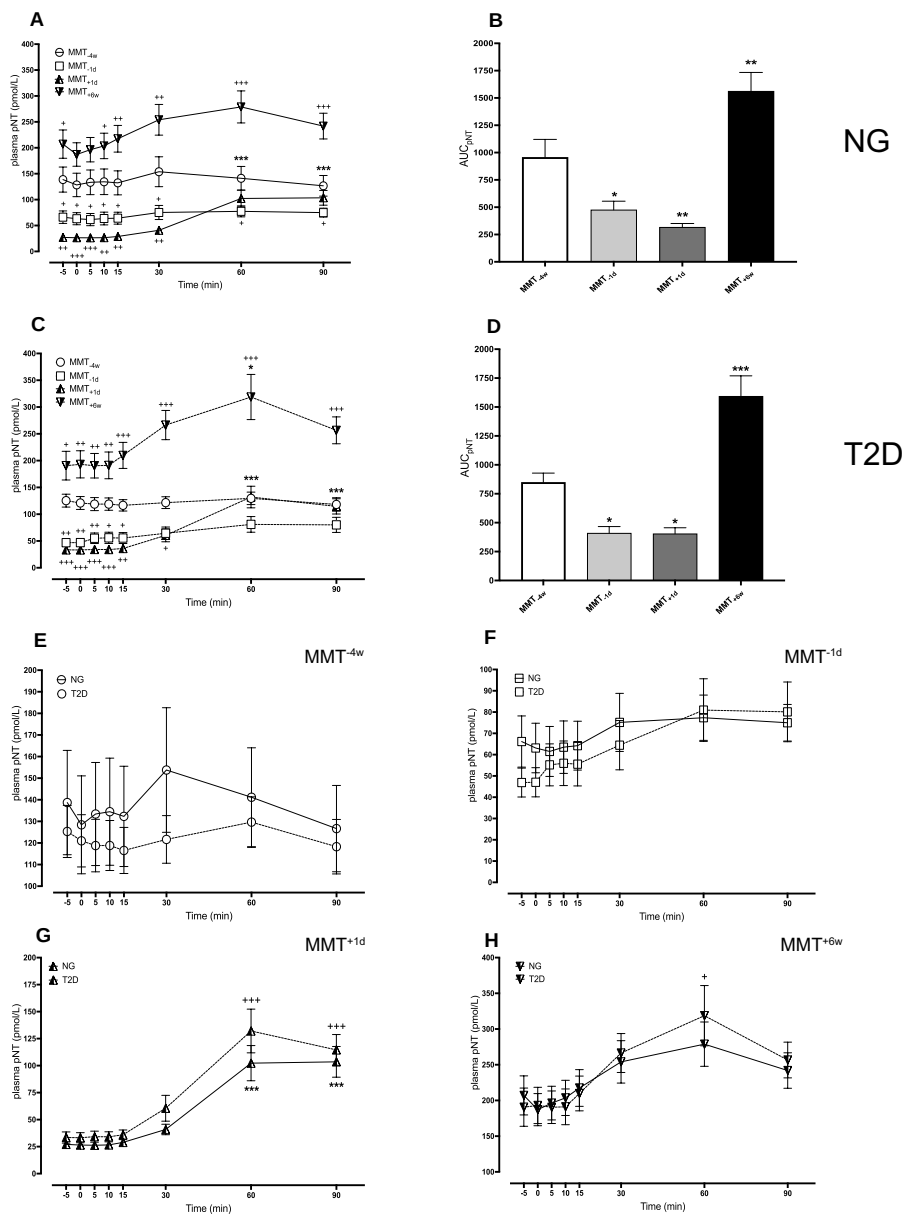


Figure 2. pNT levels following MMT in patients with obesity and NG (A) at MMT^{-4w}, MMT^{-1d}, MMT^{+1d} and MMT^{+6w} and corresponding AUC (B). pNT levels following MMT in patients with obesity and T2D (C) at the same time points with corresponding AUC (D). pNT levels in response to MMT in patients with obesity and NG versus patients with obesity and T2D at MMT^{-4w} (E), MMT^{-1d} (F), MMT^{+1d} (G) and MMT^{+6w} (H). Patients with T2D are represented with dashed lines. For A and C, * $P < .05$ and *** $P < .001$ compared with 0 mins for same MMT and + $P < .05$, ++ $P < .01$ and +++ $P < .001$ compared with MMT^{-4w} at the same time point. For B and D, * $P < .05$, ** $P < .01$ and *** $P < .001$ compared with MMT^{-4w}. For G, *** $P < .001$ for patients with NG compared with 0 mins and +++ $P < .001$ for patients with T2D compared with 0 mins. For H, + $P < .05$ for patients with T2D compared with 0 mins. NG - participants with normoglycaemia; T2D - participants with type 2 diabetes.

60 minutes and a 3.4-fold increase at 90 minutes compared with 0 minutes being observed in patients with T2D ($P < .001$; see Fig. 2C). The reintroduction of solid food caused a marked increase in postprandial pNT levels, and 1.6-fold higher levels were seen at MMT_{+6w} compared with MMT_{+1d} in patients with NG ($P < .01$; see Fig. 2B), while patients with T2D demonstrated a 1.9-fold increase at MMT_{+6w} compared with MMT_{+1d} based off area under the curve (AUC) ($P < .001$; see Fig. 2D). A meal response in pNT levels was seen in both groups but reached significance only in patients with T2D at 60 minutes postmeal ingestion with a 1.7-fold increase being observed ($P < .05$; Fig. 2C). There were no statistically significant differences in postprandial responses between patients with NG and T2D (Fig. 2E–2H). Thus, our data show that participants with severe obesity lack a meal response in pNT, but after RYGB such a response is evident.

Replication in a Type 2 Diabetes Cohort and Control Participants Who Were Normal Weight (Cohort 2)

We next sought to replicate our findings in another cohort composed of patients both with obesity and T2D who had undergone RYGB or VSG surgery, as well as control, normal-weight participants (19). The RYGB/VSG patients had MMTs before the introduction of VLED and within 3 months post RYGB on average. Similar effects to that observed in cohort 1 of RYGB/VSG on fasting, as well as postprandial pNT levels, were also observed in this cohort (Fig. 3A). Notably, participants with obesity pre RYGB/VSG as well as control participants who were normal weight lacked a meal response in NT, but RYGB/VSG caused a marked meal response in pNT (see Fig. 3A). When analyzing RYGB and VSG patients separately, VSG had higher overall levels of pNT pre surgery based off AUC ($P < .05$; Fig. 3C) but RYGB was found to cause a larger postprandial response in pNT than that of VSG (Fig. 3B), with levels being 1.6-fold higher than those observed for VSG post surgery ($P < .05$; see Fig. 3C).

Influence of Glucose-Dependent Insulinotropic Polypeptide or Glucagon-like Peptide-1 Infusion on Proneurotensin Plasma Levels (Cohort 2)

As pNT levels in the post-RYGB situation were increased concurrently with the incretin hormones GIP and GLP-1 (1), we next investigated the possibility that the pNT response is secondary to the rise in incretin levels. To this end we used available samples from experiments with GIP or GLP-1 infusion (19, 23), infused to reach circulating concentrations comparable with those seen postprandially after RYGB. GIP infusion had no effect on pNT levels in control participants of normal weight or in participants with obesity before or after RYGB (Fig. 3D). GLP-1 infusion, as assessed in control participants of normal weight, did not affect pNT levels (Fig. 3E). Further to this, we failed to find any significant correlations (using our previously published data from cohort 1 (1)) between plasma levels of pNT and GIP or pNT and GLP-1 in any patient group at any time point (data not shown). Thus, the observed increase in postprandial pNT seen after RYGB is unlikely secondary to the concomitant increases in GLP-1 and GIP levels.

Long-term Effect of Roux-en-Y Gastric Bypass on Fasting Proneurotensin Plasma Levels (Cohort 3)

Having established that pNT levels are affected in the short term by RYGB, we next assessed long-term effects of RYGB on pNT

levels. To this end we assessed fasting pNT levels in pre- and post-RYGB samples from 27 patients with T2D and 35 patients with prediabetes and 99 patients who were NG but also had obesity (20). All 3 groups of patients had higher fasting pNT levels 1 year post RYGB while patients with NG also had increased fasting pNT levels 2 months post RYGB, compared with pre RYGB (Fig. 4). At 2 months post surgery, patients with NG demonstrated a 1.7-fold increase in fasting pNT levels ($P < .001$), while patients classified as having prediabetes or T2D did not demonstrate a significant increase at this time point. Twelve months post RYGB, patients with NG demonstrated a 2.5-fold increase in fasting pNT ($P < .001$), while increases of 2.0-fold ($P < .001$) and 1.5-fold ($P < .01$) were observed for patients classified as having prediabetes or T2D. Patients diagnosed with T2D pre RYGB had a 1.5-fold increased fasting pNT levels compared with patients with NG ($P < .001$) pre surgery.

Discussion

RYGB frequently results in immediate remission of T2D prior to weight loss (1), as well as sustained improved glycemic control several years after the operation (28, 29). Although numerous studies have been reported and mechanistic insight has been demonstrated (30, 31), there are many questions remaining on the exact details underlying RYGB-induced remission of T2D.

To try to address a gap in this knowledge, we assessed RYGB-related alterations in plasma levels of the somewhat neglected gut hormone NT in 3 cohorts of RYGB patients. Here we show that RYGB, as well as energy restriction, caused reduced fasting levels of pNT in the short term. In the longer term, patients who had undergone RYGB had elevated fasting levels of pNT. Furthermore, control participants who were normal weight, as well as patients with obesity before surgery, lacked a meal response in pNT. However, in patients who had undergone RYGB or VSG a meal response in pNT was evident.

Our study design in cohort 1 allowed for comparisons between the effects of energy restriction vs the short-term effects of RYGB. Immediately after RYGB, fasting pNT levels were markedly reduced, and this was similar to the observed effect of VLED. The period around surgery is an intense period of energy restriction in a bid to reduce presurgery liver size. As there were no differences observed between pNT levels following VLED and acutely post RYGB, we propose that energy restriction explains the initial drop in fasting pNT levels. However, 6 weeks post RYGB we observed a marked increase in fasting levels of pNT compared with before both VLED and RYGB. To strengthen this finding, we replicated these results in a second cohort that we have previously described (19). This confirmed the observed increased fasting levels of pNT longer term post surgery as MMTs were performed 3 months post surgery on average. Increased fasting pNT in response to RYGB was further confirmed in a third cohort, up to 1 year post RYGB. This is in agreement with a study showing RYGB to increase fasting pNT levels up to 24 months post RYGB (15).

We have previously shown that RYGB acutely causes a number of alterations in the metabolite profile that are explained by reduced food intake (4). Notably, these alterations could not explain the parallel increases in GLP-1 or GIP (1, 4). Thus, our data suggest a more diet-dependent behavior of pNT acutely in conjunction with RYGB compared with that of the incretin hormones. Nevertheless, in the long term, fasting pNT levels remained elevated 1 year after RYGB in patients without diabetes, patients with prediabetes, as well as patients with T2D.

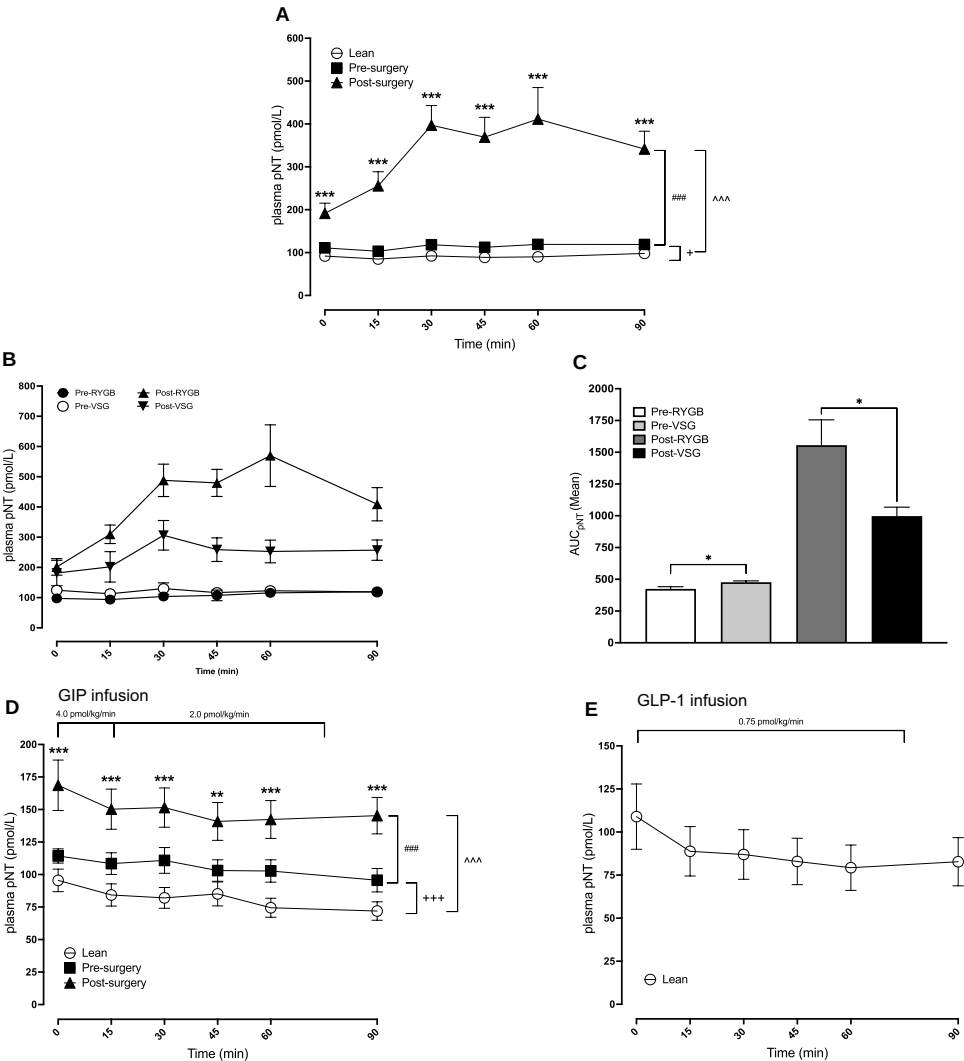


Figure 3. A, Proneurotensin (pNT) levels in response to mixed-meal tests (MMT) in patients undergoing either Roux-en-Y gastric bypass (RYGB) or vertical sleeve gastrectomy (VSG) pre and post surgery as well as control participants who were normal weight. B, pNT levels in response to MMT in patients with obesity undergoing RYGB or VSG with C, corresponding area under the curve (AUC). D, pNT levels in response to GIP infusion (4.0 pmol/kg/min and 2.0 pmol/kg/min as indicated) in patients undergoing RYGB or VSG pre and post surgery as well as control participants who were normal weight. E, pNT levels in response to GLP-1 infusion (0.75 pmol/kg/min) in control participants who were normal weight. For A, $^{***}P$ less than .001 between postsurgery and control participants who were normal weight, ^{+}P less than .05 between presurgery values and control participants who were normal weight, and $^{###}P$ less than .001 between presurgical and postsurgical values in patients with obesity. For B, $^{*}P$ less than .05 between patients undergoing RYGB vs VSG at the same time point. For C, $^{*}P$ less than .05 between patients undergoing RYGB and to a lesser extent, VSG, triggered a mixed-meal response in pNT both

Participants with obesity planned for RYGB and control participants who were normal weight both lacked an MMT response in pNT. The MMT included 5 g of fat. Of note, the main trigger of pNT secretion is fat intake, rather than

carbohydrates and proteins, and in normal participants who ingested 54 g of fat (oil or cream) there was a clear pNT response (32). Nevertheless, we found that RYGB, and to a lesser extent, VSG, triggered a mixed-meal response in pNT both

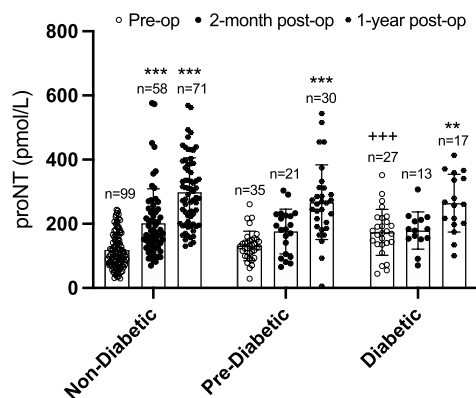


Figure 4. Pre, 2 months post, and 1-year post-RYGB fasting proneurotensin (pNT) levels in patients with obesity who had normoglycemia (nondiabetic), impaired fasting glucose (prediabetic), or were diagnosed with diabetes (diabetic). ***P* less than .01 and ****P* less than .001 compared with presurgical levels in patients with the same glycemic status. +++*P* less than .001 compared with patients with normoglycemia at the same time point.

acutely and longer term both in cohort 1 and cohort 2. The observation that this meal response is evident at both MMT_{+1d} when fasting levels dropped and at MMT_{+6w} when fasting pNT are increased, speak in favor that this effect is not related to energy restriction, but rather to the rearrangement of the GI tract. Supporting this notion, even though VLED caused reduced fasting pNT levels, VLED had no effect on the postprandial response in pNT. This is line with what is reported in a number of other studies that have addressed the effect of bariatric surgery on NT plasma levels both in humans (2, 15–17, 33–39) and rats (7). A handful of studies have also addressed the expression of NT in intestinal cells (3, 40–42).

A previous study assessing biliopancreatic limb length showed that a longer biliopancreatic limb results in increased fasting and postprandial levels of pNT alongside GLP-1 (16). Meanwhile, postprandial GIP levels were reduced in patients with a longer biliopancreatic limb. This could suggest a negative correlation between GIP and pNT levels postprandially. However, we found no effect of GIP infusion on pNT levels and no correlation between GIP and pNT levels in response to a mixed-meal. The work by Patrício et al (16) may also suggest a positive correlation between GLP-1 and pNT levels. Although performed only in control participants who were normal weight, our GLP-1 infusion data do not suggest any effect of GLP-1 on pNT levels. Furthermore, we could not find any correlation between pNT and GLP-1 levels. Therefore, we propose that the observed postprandial rise in pNT levels is unlikely secondary to the parallel increase in GIP or GLP-1 levels.

Another study showed that BPD resulted in a greater increase in fasting pNT levels compared with RYGB (17). We observed no difference in fasting pNT levels between RYGB and VSG but did observe a larger increase in meal response after RYGB. This in agreement with a previous study (33). Although the exact mechanisms remain to be established, these 2 studies taken in tandem suggest that bypassing the intestine clearly affects pNT levels. Further studies are needed to

understand whether the observed effect of bariatric surgery on pNT levels are related to alterations in bile acids, which have been shown to regulate pNT levels (35, 36), or alterations in, for example, the enteric nervous system or in food composition. It should be mentioned that previous studies in humans demonstrated increased pNT levels in response to fat (32, 37), in particular long-chain fatty acids (34).

In cohort 3 we found pNT levels to be higher in patients with T2D. This finding gains support from a previous study reporting higher NT levels in women with gestational diabetes (43). Nevertheless, our data do not suggest any major influence of glycemic status on fasting, or postprandial pNT levels following bariatric surgery.

Limitations

Our study design in cohort 1 allowed for comparisons of VLED and RYGB as well as the immediate effect of RYGB. The volume of the meal in the MMTs had to be limited to a volume tolerable the day after surgery. A potential limitation is that this low volume may not be enough to trigger a response preoperatively. In cohort 2, we could test the potential influence of GIP infusion on pNT levels in patients before and after RYGB, but for GLP-1 infusion we had access to samples only from control participants.

Conclusion

pNT plasma samples were reduced by caloric restriction and immediately after RYGB, however, also likely as a consequence of caloric restriction. On the contrary, after the introduction of solid food post RYGB, pNT plasma levels were elevated and maintained at a higher level for 12 months. RYGB and to a lesser extent VSG triggered a meal response in pNT that is likely independent of the rise in GIP or GLP-1. Fasting pNT levels were higher in patients with T2D preoperatively, but otherwise the responses in pNT levels were largely unaffected by the glycemic status of the patients. Even though experimental studies have shown that NT has a dual effect on insulin secretion (44, 45) and NT knockout mice have improved insulin sensitivity (13), it is not well known how NT affects insulin secretion or insulin sensitivity in humans. It is not inconceivable that the observed increase in pNT levels post RYGB/VSG is a consequence of weight loss and part of a compensatory mechanism trying to increase intestinal fat absorption. Thus, our study has provided detailed insight into the effect of bariatric surgery on plasma levels of pNT. Further studies are needed to understand whether alterations in pNT levels play a role in RYGB-induced weight-loss, improved glycemia, or alteration of long term cardiometabolic morbidity and mortality.

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N.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosures

The authors have nothing to declare.

Data Availability

Original data generated and analyzed during this study are included in this published article or in the data repositories listed in “References.”

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Paper IV





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SURGERY FOR OBESITY
AND RELATED DISEASES

Original article

Alcohol consumption patterns up to 10 years after bariatric surgery: using the highly specific biomarker phosphatidylethanol

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Abstract

Background: The increasing prevalence of obesity has led to a rise in bariatric surgery as an effective intervention for weight loss and associated co-morbidities. However, concerns persist regarding increased alcohol consumption after surgery.

Objectives: To objectively assess long-term changes in alcohol use after bariatric surgery, we used the highly specific alcohol biomarker phosphatidylethanol (PEth).

Setting: Kalmar County Hospital, Sweden.

Methods: This longitudinal cohort study included 1238 patients who underwent Roux-en-Y gastric bypass or sleeve gastrectomy at Kalmar County Hospital from 2010 to 2020. Patients were divided into 2 cohorts based on preoperative PEth sampling ($n = 327$ with, $n = 911$ without). PEth levels were measured before surgery and at intervals up to 10 years postoperatively, using $.30 \mu\text{mol/L}$ (approximately 210 ng/mL) as the threshold for heavy alcohol use.

Results: Mean PEth concentrations increased from $.07$ to $.38 \mu\text{mol/L}$ ($P < .001$) in men and from $.06$ to $.11 \mu\text{mol/L}$ nonsignificantly in women. The proportion of patients that postoperatively exceeded the heavy drinking threshold rose significantly over time. At 5 years, 37.5% of men and 4.8% of women had PEth values above $.30 \mu\text{mol/L}$ (both $P < .001$). Men were at significantly higher risk (odds ratio: 7.89, 95% confidence interval: 3.76–16.57; $P < .001$). This pattern remained consistent at the 10-year follow-up.

Conclusions: Alcohol consumption, as measured by PEth, increased significantly after bariatric surgery, particularly in men. These findings support routine postoperative alcohol screening and suggest incorporating PEth testing into long-term follow-up protocols. (Surg Obes Relat Dis 2026;22:619–627.) © 2026 American Society for Metabolic and Bariatric Surgery. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Keywords:

Alcohol after bariatric surgery; PEth; Alcohol; RYGB; SG; Gastric bypass; Sleeve gastrectomy

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Obesity has consistently increased throughout the 20th and 21st centuries, leading to a rise in obesity-related health issues such as type 2 diabetes, heart diseases, stroke, metabolic syndrome, and hypertension worldwide. Bariatric surgery has been extensively proven to not only achieve weight loss but also to reduce obesity-related diseases and extend life expectancy in previously overweight individuals [1–3]. In the last decade, pharmacological treatment has emerged as a promising alternative to surgery [4,5]. However, it has not yet demonstrated the same effect on weight loss, and long-term results are still lacking [6]. Thus, bariatric surgery remains a valid method for patients with morbid obesity, with more than 500,000 procedures performed worldwide in 2021 [7].

Although bariatric surgery offers significant benefits for many obesity-related conditions, there is also evidence of potential adverse effects. Some of these complications arise from the physiological changes after surgery, including impaired nutrient absorption, alterations in endocrine function, and reductions in bone mineral density [8–12]. Furthermore, several studies have identified an increased risk of postoperative alcohol abuse and higher rates of alcohol consumption after both Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) procedures, as well as an elevated risk of substance abuse [13–18].

The mechanism of possible alcohol use disorder (AUD) after bariatric surgery remains unclear, with both physiological and psychological models suggested. Gastric bypass (RYGB), the most common procedure, may increase alcohol potency due to gastrointestinal tract rearrangement, eliciting physiological changes in alcohol uptake and first pass metabolism [19–21]. This effect is less consistently observed in SG and other types of bariatric procedures [22,23]. Neurohormonal alterations impacting signaling pathways and reward mechanisms have been suggested [24], alongside the concept of addiction transfer from food to alcohol [25]. Notably, certain foods and substances of abuse activate overlapping reward circuits within the brain. As a result, some researchers have posited the existence of an addictive pathology among bariatric patients [26]. However, there is currently no conclusive evidence establishing a link between obesity or the degree of weight loss and the prevalence of AUD.

Most previous studies have used self-report forms, for example, the Alcohol Use Disorders Identification Test (AUDIT-C), or have used records of registered in-patient care for alcohol-related conditions [14,15,27]. However, both methods are likely to underestimate actual use, as self-reported data often underrepresent true consumption levels, and many individuals with AUD do not seek health-care services [28,29].

Phosphatidylethanol (PEth) is a highly sensitive and specific alcohol biomarker [30]. It is widely recognized as an indicator of alcohol consumption in both research and forensic contexts. PEth reflects alcohol intake over the

past 2–4 weeks and is used to differentiate between various levels of alcohol use.

In the present study, we used PEth to elucidate the long-term (up to 10 years) use of alcohol after RYGB and SG. This approach addresses some of the shortcomings of previous studies regarding alcohol use.

Methods

Study design

This study was longitudinal with all data sequentially entered into the Swedish Obesity Registry (SOREG) and originating from the Kalmar County hospital database. All data collected adhered to the specifications detailed in the SOREG protocol [31] with the addition of PEth once it was put into clinical practice in 2015. Data extraction was made in 2022.

At the time of surgical acceptance, all patients were informed about the postoperative follow-up program, which includes routine anthropometric assessments and blood-based monitoring. Participation in all components of the follow-up, including PEth testing, was mandatory.

Patients presenting with PEth levels above the .30 $\mu\text{mol/L}$ threshold at the first clinical appointment were deemed ineligible for surgery. However, they could be reconsidered for surgical eligibility if they subsequently provided documentation of 1 year of consecutive PEth measurements consistently below the threshold.

Study population

We identified a total of 1508 patients who underwent gastric bypass or gastric sleeve surgery at Kalmar County Hospital from 2010 to 2020 in SOREG. Excluded from the study were patients refusing participation, with previous bariatric surgery, referred from other regions, or those who migrated within the first year, totaling 262 exclusions.

The remaining 1238 patients were divided into 2 cohorts. The first cohort ($n = 327$; RYGB $n = 219$, SG $n = 108$) included patients with baseline PEth measurements and postoperative follow-up extending beyond 1 year and up to 5 years. The second cohort ($n = 911$; RYGB $n = 754$, SG $n = 157$) consisted of patients operated before PEth was implemented in clinical practice; for this group, pre- and postoperative anthropometric data, quality-of-life measures, and postoperative PEth values were available for up to 10 years of follow-up. The rationale for separating the cohorts was methodological: the first cohort enabled more statistically robust analyses of preoperative alcohol use, whereas the larger second cohort allowed us to describe longer-term postoperative trajectories.

Both cohorts were screened at intervals as suggested by SOREG, preoperatively, 1, 2, 5, and 10 years after surgery. Postoperative follow-up after bariatric surgery included

structured monitoring for micronutrient deficiencies as well as alcohol use through PEth (from 2015). Throughout the study period, the follow-up rates, anthropometric and/or PEth, were 93% at 1 year, 91% at 2 years, 82% at 5 years, and 65% at 10 years.

PEth values exceeding .30 $\mu\text{mol/L}$ at any point during the study were reported to the participant, who was advised to initiate contact with an appropriate healthcare unit for further evaluation.

The study was approved by the regional ethical board in Linköping, Sweden, and adhered to the 1964 Declaration of Helsinki and its amendments.

Phosphatidylethanol

PEth is formed in the cell membrane of red blood cells when alcohol reacts with phosphatidylcholine, creating several homologues that can be measured in whole blood. The PEth 16:0/18:1 homologue is the most abundant form and has been used in both research and clinical settings in recent years. PEth 16:0/18:1 has a half-life ranging from 3.7 to 10.4 days [32]. It reflects alcohol use over the past 2–4 weeks. PEth 16:0/18:1 was used consistently in this study. A PEth level of $<.05 \mu\text{mol/L}$ ($<35 \text{ ng/mL}$) was considered to indicate no or low drinking, a PEth level of $.05\text{--}.30 \mu\text{mol/L}$ ($36\text{--}210 \text{ ng/mL}$) suggested moderate drinking, and a PEth level of $>.30 \mu\text{mol/L}$ ($>210 \text{ ng/mL}$) corresponded to excessive alcohol intake [32].

Translating a PEth value to a specific alcohol intake is complex. Research indicates that a daily consumption of more than 60 g for men and 40 g for women over an extended period may result in PEth levels exceeding .30 $\mu\text{mol/L}$ [33,34]. This corresponds to approximately 4.3–5 units per day for men and 2.85–3.3 units per day for women (depending on the definition of a unit, 12–14 g alcohol). The formation of PEth may differ based on patterns of drinking, such as binge drinking versus regular drinking, as well as factors such as body mass index (BMI) and other biological individual differences. These caveats acknowledged, PEth still correlates very well with consumption [34].

Whole blood concentration of PEth 16:0/18:1 in this study was measured by liquid chromatography tandem-mass spectrometry essentially as described [35].

Statistical methods

Baseline characteristics are presented in Table 1 for the 2 cohorts and were compared using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. The association between baseline characteristics and PEth $>.30 \mu\text{mol/L}$ at 5 years (dichotomized outcome) was assessed using multivariable logistic regression (Table 2). The proportions with PEth $>.30 \mu\text{mol/L}$ and $>.05 \mu\text{mol/L}$ at 1, 2, 5, and 10 years are presented as counts and percentages (Tables 3 and 4), and logistic regression models were used to test differences across time points. Longitudinal

changes in continuous PEth over time were evaluated using repeated-measures analysis of variance with time as the within-subject factor. Post hoc pairwise comparisons between time points were performed with Tukey adjustment for multiple testing. Values below the limit of quantification ($<.05 \mu\text{mol/L}$) were set to .05 $\mu\text{mol/L}$ for calculation of means. All analyses were carried out using Statistica (version 12; StatSoft Inc.).

Results

Baseline characteristics of the study cohorts with and without baseline PEth are presented in Table 1. Females make up over 75% of the total. Two-thirds underwent RYGB, whereas one-third underwent SG.

In cohort 1 at baseline, the prevalence of moderate drinking was 14% (PEth, $.06\text{--}.29 \mu\text{mol/L}$), whereas 86% were classified below the threshold (PEth, $<.05 \mu\text{mol/L}$) and thus placed in the low or nondrinking group. Mean PEth concentrations increased over time in both sexes. At 5 years, mean PEth increased from .07 $\mu\text{mol/L}$ at baseline to .39 $\mu\text{mol/L}$ in men, and from .06 $\mu\text{mol/L}$ to .11 $\mu\text{mol/L}$ in women (Fig. 1). Tukey-adjusted pairwise comparisons demonstrated no significant changes across time points in women. In contrast, men exhibited significantly higher PEth concentrations at 1, 2, and 5 years compared with baseline (adjusted $P = .015$, $P < .001$, and $P < .001$, respectively), with additional significant differences between all consecutive postoperative time points (all $P < .05$).

No sex differences were observed preoperatively ($P > .99$); however, significant differences emerged at 1, 2, and 5 years ($P = .001$, $P < .0001$, and $P < .0001$, respectively). Across follow-up, the proportion of patients with elevated PEth levels increased relative to baseline in both sexes (Fig. 2A), with a progressively higher prevalence of heavy drinking observed over time, particularly among men (Fig. 2B). Notably, by year 5, 37.5% of men had PEth levels $\geq .30 \mu\text{mol/L}$, in contrast to 4.7% of women, with both sexes demonstrating statistically significant increases from baseline ($P < .001$; Tables 3 and 4). At the 5-year follow-up, 62.5% of men exhibited PEth concentrations exceeding .05 $\mu\text{mol/L}$, compared with 25.6% at baseline ($P < .001$). Among women, the corresponding increase was from 10.8% to 21.4% ($P < .001$).

Logistic regression indicated that males had significantly higher odds (odds ratio: 7.89, 95% confidence interval: 3.76–16.57, $P < .001$) of excessive drinking (PEth $>.30 \mu\text{mol/L}$) compared with females. No other factors, including age, type of surgery, physical or mental status (as measured by Short Form-36 and Obesity Problem-9), weight loss (expected nadir—2 years), or weight regain (as measured at 5 years), were significant in multi- or univariate analyses (Table 2).

The second cohort analyzed comprised individuals for whom baseline PEth levels were unavailable, that is, before

Table 1

Patient details separated into those with preoperative phosphatidylethanol values (cohort 1) and those without (cohort 2)

	Group 1	Group 2	Difference (<i>P</i>)	Total
N	327	911		1238
Age (yr)				
Mean (SD)	40.8 (11.3)	41.5 (10.7)		41.3 (10.8)
Median (range)	41 (17–63)	42 (18–68)	.522	42 (17–68)
Age categories (yr), n (%)				
<30	82 (25.1)	165 (18.1)		247 (20.0)
30–40	74 (22.6)	243 (26.7)		317 (25.6)
40–50	89 (27.2)	310 (34.0)		399 (32.2)
>50	82 (25.1)	193 (21.2)	–	275 (22.2)
Gender				
Male	78 (23.9)	208 (22.8)		286 (23.1)
Female	249 (76.1)	703 (77.2)	.765	952 (76.9)
Height (cm)				
Mean (SD)	169.6 (8.9)	169.8 (8.9)		169.7 (8.9)
Median (range)	169 (147–207)	168 (147–203)	.938	168 (147–207)
Weight (kg)				
Mean (SD)	118.3 (21.2)	119.3 (19.7)		119.0 (20.1)
Median (range)	114 (85–225)	116 (77–224)	.132	116 (77–225)
BMI (kg/m ²)				
Mean (SD)	41.0 (5.3)	41.2 (5.2)		41.2 (5.2)
Median (range)	40 (32–73)	40 (29–66)	.129	40 (29–73)
Method, n (%)				
GBP	219 (67.0)	754 (82.8)		973 (78.6)
SG	108 (33.0)	157 (17.2)	<.001	265 (21.4)
PEth_preoperative				
Mean (SD)	.06 (.04)	–		.06 (.04)
Median (range)	.1 (.1–.3)	–	–	.1 (.1–.3)
Hypertension, n (%)				
No	257 (78.6)	709 (77.8)		966 (78.0)
Yes	70 (21.4)	202 (22.2)	.834	272 (22.0)
Diabetes, n (%)				
No	302 (92.4)	811 (89.0)		1113 (89.9)
Yes	25 (7.6)	100 (11.0)	.108	125 (10.1)
Glucose				
Mean (SD)	6.4 (2.0)	6.2 (1.8)		6.3 (1.9)
Median (range)	5.9 (4.6–20.0)	5.7 (3.7–17.4)	<.001	5.8 (3.7–20.0)
HbA1c				
Mean (SD)	37.2 (9.9)	39.8 (10.6)		38.9 (10.4)
Median (range)	35 (3–86)	37 (3–104)	<.001	36 (3–104)
BMI at surgery				
Mean (SD)	38.1 (4.7)	38.8 (4.8)		38.6 (4.7)
Median (range)	37 (21–56)	38 (29–60)	.074	38 (21–60)

BMI = body mass index; GBP = gastric bypass; SD = standard deviation; SG = sleeve gastrectomy.

Differences analyzed using nonparametric the Mann-Whitney *U* test for continuous variables and χ^2 for categorical ones.

the implementation of PEth testing in clinical practice. Although a non-AUD status before surgery has consistently been a prerequisite, there has been no objective method for reliably determining preoperative alcohol consumption (i.e., via PEth). This cohort was assessed based on postoperative PEth levels and other characteristics, including sex, weight, and weight loss. Except for method of surgery (RYGB/SG) and glucose/HbA1c levels, no significant differences were found compared with the first cohort. Among these individuals, both men and women demonstrated an overall increase

in PEth levels across the 10-year follow-up period when considering mean values, as well as when grouped into different drinking categories (Tables 3 and 4).

Over the 10 year follow-up men and women continuously increased the proportion of the heavy drinking category. At the 10-year follow-up the proportion of patients with PEth above 0.3 $\mu\text{mol/L}$ significantly rose to 39.5% ($P < .05$) and 18.8% ($P < .001$) for men and women, respectively.

The large difference between men and women was thus evident in both cohorts.

Table 2

Results from logistic regression at 5 years including all patients having preoperative phosphatidylethanol (PEth) values <.3

Variable	Total	PEth >.3	Percentage	Univariate logistic regression		Multivariate logistic regression	
				OR (95% CI)	P	OR (95% CI)	P
Age (yr)							
<30	82	4	5	1.00		1.00	
30–40	74	11	15	1.24 (.91–1.69)		1.18 (.84–1.65)	
40–50	89	12	13	1.53 (.82–2.87)		1.40 (.71–2.74)	
>50	82	10	12	1.90 (.74–4.87)	.180	1.65 (.60–4.52)	.331
Sex							
Female	249	13	5	1.00		1.00	
Male	78	24	31	8.07 (3.85–16.9)	<.001	7.89 (3.76–16.57)	<.001
Surgical method							
GBP	219	28	13	1.00		–	
SG	108	9	8	.62 (.28–1.37)	.236	–	–
Physical pre Q							
<25	42	6	14	1.00		–	
25–32	39	3	8	.87 (.55–1.37)		–	–
33–42	48	4	8	.75 (.30–1.88)		–	
>42	40	4	10	.65 (.16–2.59)	.541	–	
Mental pre Q							
<32	38	4	11	1.00		–	
32–43	47	6	13	.91 (.57–1.45)		–	–
43–52	43	3	7	.82 (.32–2.09)		–	
>52	41	4	10	.75 (.19–3.02)	.682	–	
OP9 pre Q							
<54	30	4	13	1.00		–	
55–75	57	3	5	1.06 (.65–1.73)		–	–
76–85	36	4	11	1.13 (.43–3.00)		–	
>85	44	5	11	1.20 (.28–5.18)	.806	–	
Percent EBMIL 2-year Q							
<76	76	3	4	1.00		–	
7–90	77	14	18	1.03 (.73–1.44)		–	–
91–108	82	9	11	1.05 (.54–2.07)		–	
>108	72	5	7	1.08 (.39–2.97)	.883	–	
Percent EBMIL 5-year Q							
<60	38	1	3	1.00		–	
60–81	38	3	8	1.35 (.80–2.29)		–	
81–98	38	6	16	1.82 (.63–5.24)		–	
>98	39	3	8	2.46 (.50–11.99)	.265	–	

CI = confidence interval; EBMIL = excess body mass index loss; GBP = gastric bypass; OP9 = obesity problem-9; OR = odds ratio; SG = sleeve gastrectomy.

PEth >.3 is defined as those having measured PEth values >.3 at any occasion during the follow-up period until 5 years. Univariate to the left and multivariate to the right adjusted for age.

Discussion

This is a study in a single-center large set of patients followed for up to 10 years. Our data show a steady increase in the prevalence of both moderate alcohol consumption and heavy drinking (PEth >.30 $\mu\text{mol/L}$) over time, with 37.5% of men classified within this category at the 5-year mark. Furthermore, when analyzing the second cohort (i.e., without preop PEth), this trend seems to stabilize at a high level after 10 years, especially for male patients. Our 10-year data suggest that all patients were drawn from the same population because anthropometrics and postoperative PEth values did not differ significantly between cohorts. Notably, we found an 8-fold higher risk in men compared

with women. However, the data collected over 10 years also raise significant concerns regarding women's alcohol consumption. There are currently no long-term follow-up studies of PEth in general population settings, either in Sweden or internationally. Existing research has primarily focused on clinical, forensic, or otherwise selected high-risk groups, leaving a gap in knowledge regarding long-term PEth trajectories in the general population. During the study period, overall alcohol consumption in Sweden has shown a steady decline since 2001, according to national monitoring data [36]. Consequently, the observed increases in PEth levels may be more closely linked to factors such as obesity or the effects of obesity treatment, as has been increasingly suggested in literature.

Table 3
Results from simple logistic regression

	N	PEth >0.05μmol/L		Logistic regression	P value
		n	%	OR (–95% to 95% CI)	
Cohort 1					
Baseline preoperative cohort: female					
Pre	249	27	10.8	1.00	.002
1 yr	229	33	14.4	1.3 (1.1–1.6)	
2 yr	232	44	19.0	1.8 (1.2–2.5)	
5 yr	126	27	21.4	2.3 (1.4–4.0)	
10 yr	0	0	–	–	
Baseline preoperative cohort: male					
Pre	78	20	25.6	1.00	<.001
1 yr	67	27	40.3	1.6 (1.3–2.1)	
2 yr	66	30	45.5	2.6 (1.6–4.3)	
5 yr	40	25	62.5	4.3 (2.0–9.0)	
10 yr	0	0	–	–	
Cohort 2					
No baseline cohort: female					
1 yr	177	29	16.4	1.00	<.001
2 yr	300	66	22.0	.7 (.6–.8)	
5 yr	545	172	31.6	.4 (.3–.6)	
10 yr	191	73	38.2	.3 (.2–.5)	
No baseline cohort: male					
1 yr	53	25	47.2	1.00	.278
2 yr	97	50	51.5	.9 (.7–1.1)	
5 yr	148	76	51.4	.8 (.5–1.2)	
10 yr	43	26	60.5	.7 (.3–1.4)	

CI = confidence interval; OR = odds ratio; PEth = phosphatidylethanol.

N is total number investigated at the particular occasion, and n is the number of patients with PEth values more than .05 at that occasion.

Several studies suggest a higher risk of alcohol overconsumption and AUD after bariatric surgery. Two studies, Walther et al. [35] and Siikaluoma et al. [37], showed increased drinking prevalence 2 years after surgery using PEth. Walther et al. found that bariatric surgery patients, although showing increased alcohol consumption per PEth values, had lower PEth values than blood donors, questioning the extent of the problem in the bariatric surgery group. Siikaluoma et al. reported 8.3% of patients with PEth >.30 μmol/L 2 years after surgery, close to our finding of 9.3%. Their study, however, did not use a cohort with baseline PEth data to a full extent. Our finding of an 8-fold higher risk in men is however in line with their findings of a much higher risk among men.

Our study categorizes the population into 2 distinct cohorts: individuals with baseline PEth levels and those without. Participants were consecutively enrolled into these cohorts based on the timing of their surgery, relative to the implementation of PEth as a standard clinical practice. In addition, our cohort benefits from an extended follow-up period and is considerably larger in longitudinal analysis using PEth. Although PEth has 100% specificity, its sensitivity varies due to several biological factors such as total body water, variability in alcohol uptake, and differences in formation and degradation rates. Although sensitivity does

not appear to be associated with sex or age, a high BMI (total body water) may reduce sensitivity [38]. If patients after surgery consume the same amount of alcohol, changes in distribution and absorption may lead to higher alcohol and PEth concentrations. This was a possible conclusion reached in a previous study [35]. Furthermore, after bariatric surgery, patients may not recognize relative increases in alcohol intake, potentially increasing their risk of AUD.

Women, who constitute the majority of patients undergoing bariatric surgery, demonstrate a different trend. Approximately 5% showed a PEth level indicative of excessive alcohol consumption 2 years after surgery, without a further rise by the 5-year mark. Further using PEth as a continuous variable showed no significant change over the first 5 years.

Furthermore, in the present study, we did not observe significant differences between RYGB and SG, despite their anatomical distinctions, although the study was not specifically powered to detect such differences. Although the effect of RYGB on alcohol metabolism is well established, the impact of SG remains more debated, which is relevant when interpreting potential mechanisms behind altered alcohol use [19,22,23]. Earlier reports have suggested that restrictive procedures confer a lower risk of AUD [15,16,39] although SG was often grouped together with older restrictive surgery such as vertical banded gastroplasty

Table 4
Results from simple logistic regression

	N	PetH > 0.3 $\mu\text{mol/L}$		Logistic regression	P value
		n	%	OR (–95% to 95% CI)	
Cohort 1					
Baseline preoperative group: female					
Pre	249	0	.0	1.00	<.001
1 yr	229	3	1.3	2.4 (1.5–3.8)	
2 yr	232	12	5.2	5.6 (2.2–14.4)	
5 yr	126	6	4.8	13 (3–55)	
10 yr	0	0	–	–	
Baseline preoperative group: male					
Pre	78	0	.0	1.00	<.001
1 yr	67	7	10.4	3.0 (2.0–4.5)	
2 yr	66	17	25.8	9 (4–21)	
5 yr	40	15	37.5	28 (8–94)	
10 yr	0	0	–	–	
Cohort 2					
No baseline group: female					
1 yr	177	13	7.3	1.00	<.001
2 yr	300	25	8.3	1.5 (1.2–1.8)	
5 yr	545	79	14.5	2.3 (1.5–3.4)	
10 yr	191	36	18.8	3.4 (1.9–6.2)	
No baseline group: male					
1 yr	53	12	22.6	1.00	.033
2 yr	97	27	27.8	1.3 (1.0–1.7)	
5 yr	148	52	35.1	1.8 (1.0–3.0)	
10 yr	43	17	39.5	2.3 (1.1–5.1)	

CI = confidence interval; OR = odds ratio; PEth = phosphatidylethanol.
N is total number investigated at the particular occasion, and n is the number of patients with PEth values more than .3 at that occasion.

and gastric banding. SG differs substantially from these procedures and may influence alcohol metabolism as well as neurohormonal responses. Notably, regions producing gastric alcohol dehydrogenase are partially removed during SG and bypassed in RYGB. Consistent with these

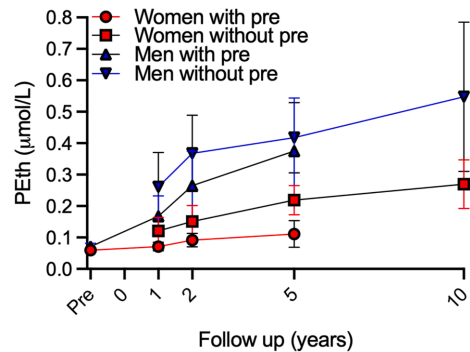


Fig. 1. Mean phosphatidylethanol (PEth) ranging from presurgical up to 10 years, cohorts 1 and 2, male and female.

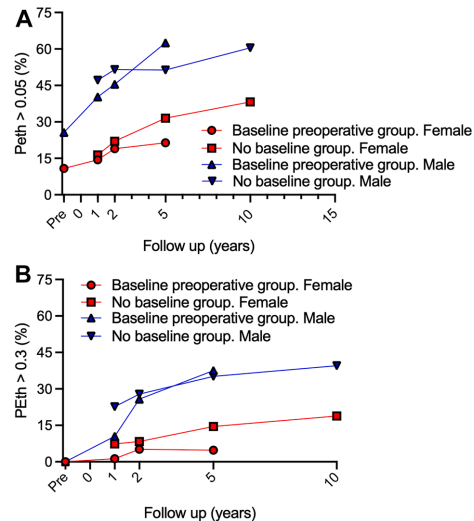


Fig. 2. Fraction of patients reaching phosphatidylethanol (PEth) > .05 $\mu\text{mol/L}$ (A) and fraction > .3 $\mu\text{mol/L}$ (B). Logistic regression: $P < .001$ over time span.

mechanistic considerations, more recent studies indicate that SG patients are also at risk of increased alcohol consumption after surgery [40,41].

Several studies have used the AUDIT to examine the prevalence of AUDs after bariatric surgery, consistently showing an increased risk of AUD within the first 2 years after surgery. Questionnaires regarding alcohol or drug use often face the challenge of underreporting, particularly in the preoperative setting where patients know such information is affecting their acceptance to surgery. A specific marker could be highly advantageous compared with the limitations of AUDIT.

This study has some limitations. Despite, to our knowledge, the largest cohort analyzed with PETH as an endpoint, the sample size remains relatively small, particularly in the later years of follow-up (within the baseline group). In addition, the second cohort lacks baseline values and thus does not hold strict statistical value when compared with the baseline group. This second cohort seems however to be drawn from the same population as the baseline and follow-up data are similar in absolute values (Fig. 1, Table 1) and thus indicative of potential future trends.

Further studies are needed to validate our findings, particularly through comparisons with general populations. Investigating the reasons for the increased incidence of excessive alcohol consumption among males after bariatric surgery is important. Our results highlight the importance of addressing alcohol use in follow-up. Although PETH is highly specific, it does not differentiate between high alcohol consumption and AUD; it merely reflects consumption levels.

Conclusions

Most patients undergoing bariatric surgery do not develop excessive alcohol consumption. Women, who represent the majority of this population, typically remain low-level alcohol consumers over time. However, a substantial subgroup increases their alcohol intake postoperatively. This study provides objective, biomarker-based evidence of a sustained rise in heavy drinking among a minority of patients, particularly men, who demonstrated an 8-fold higher risk than women. By 5 years, over one-third of male patients exceeded the clinical PETH threshold for excessive drinking. This trend persisted up to 10 years, with no clear difference between RYGB and SG. These findings support routine monitoring of alcohol use—especially in men—and endorse the integration of PETH testing into long-term postoperative care protocols.

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Disclosures

The authors report no conflicts of interest related to this work.

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