Mechanisms maintaining reduced appetite and normoglycaemia after metabolic surgery. The role of bile acids.

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PhD Dissertation

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Abstract

Obesity is becoming the healthcare epidemic of this century. Weight loss surgery is the only effective treatment for morbid obesity. Furthermore glycaemic control in type 2 diabetic patients is improved after metabolic surgery.

Here I observed that with gastric bypass, type 2 diabetes can be improved and even rapidly put into a state of remission irrespective of weight loss. This is achieved via an improvement of both insulin resistance and insulin production. Reduced insulin resistance within the first week after surgery remains unexplained, but increased insulin production in the first week after surgery may be explained by the enhanced postprandial GLP-1 response.

In addition, I demonstrate that bile flow changes lead to increased gut hormone response in animal models. Roux-en-Y gastric bypass in humans causes changes in bile flow leading to increased plasma bile acid concentrations. This phenomenon may explain the improved glycaemic control following gastric bypass.

In conclusion I investigated the mechanism of diabetes remission after metabolic surgery and explored the role of gut hormones and bile acids in the changes in glucose homeostasis following metabolic surgery.

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List of Abbreviations

ANOVA   Analysis of Variance

AUC   Area Under the Curve

BMI   Body Mass Index

CCK   Cholecystokinin

CPAP   Conitnous Positive Airway Pressure

EDTA   Ethylenediaminetetraacetic acid

FGF19   Fibroblast Growth Factor 19

GIP   Glucose-dependent Insulinotropic Polypeptide

GLP-1   Glucagon-Like Peptide-1

HOMA-IR   Homeostatic Model Assessment-Insulin Resistance

NASH   Nonlcoholic Steatohepatitis

OXM   Oxyntomodulin

PCOS   Polycystic Ovary Syndrome

PP   Pancreatic Polypeptide

PYY   Peptide YY

SEM   Standard error of the mean

VAS   Visual Analogue Scales

1. Introduction

1.1 Metabolic surgery

Introduction

Weight loss surgery is now recognised as the most effective long term treatment for morbid obesity (Sjostrom et al 2007). The effect of these procedures is not restricted to sustained weight loss. Improvement in obesity related comorbidity may represent a more important effect than weight loss itself. The Diabetes Surgery Summit in Rome in 2007 suggested the use of the term "metabolic surgery" to reflect profound effect of weight loss procedures on the metabolic syndrome. This concept is gaining acceptance as evidenced by the number of scientific societies worldwide which include the word metabolic in their title. Of note is that the British Obesity and Metabolic Surgery Society, the American Society for Metabolic and Bariatric Surgery and the International Federation for the Surgery of Obesity and Metabolic Disorders have also changed its name to reflect this.

Obesity Staging, the Edmonton obesity staging system (EOSS)

Body Mass Index (BMI) is used as the main criterion for qualifying for weight loss surgery. The National Institute for Health and Clinical Excellence in the UK, as well as the National Institute of Health in the United Sates of America, recommend weight loss surgery in adults with BMI of 40 kg/m^2 and above or BMI between 35 kg/m^2 and 40 kg/m^2 with obesity related co-morbidity (The National Institute of Clinical Excellence, 2006; Gastrointestinal Surgery for Severe Obesity. NIH Consensus Statement Online 1991 Mar 25-27). However it is recognised that BMI is associated

with a number of limitations (Hu 2007). Sharma et al proposed a novel clinical staging system, the Edmonton obesity staging system which scores obese individuals on a 5-point ordinal scale and takes into account severity of co-morbidities and functional status (Sharma and Kushner 2009). The objective was to provide a simple framework to aid decision making in clinical practice (Sharma and Kushner 2009).

The ability of the Edmonton obesity staging system to predict mortality was examined in a nationally representative US sample by Padwal et al (Padwal, Pajewski, Allison and Sharma 2011). Data from the National Health and Human Nutrition Examination Surveys (NHANES) III 1988–1994 and the NHANES 1999–2004, with mortality data up to the end of 2006 were used (Padwal, Pajewski, Allison and Sharma 2011). Overweight or obese individuals aged 20 or older who had been randomized to the morning session at the mobile examination centre were scored according to the Edmonton obesity staging system (Padwal, Pajewski, Allison and Sharma 2011). The analysis was retrospective. Individuals with class III obesity following adjustment for metabolic syndrome or hypertriglyceridemic waist (this was defined as waist circumference ≥ 90 cm and a triglyceride levels ≥ 2 mmol/L for men; waist circumference ≥ 85 cm and triglyceride levels ≥ 1.5 mmol/L for women) had similar mortality risk compared to class II obese individuals (Padwal, Pajewski, Allison and Sharma 2011). In contrast individuals with Edmonton obesity staging system score of 2 or 3 had a 4 to 12-fold greater hazard ratio compared to individuals with Edmonton obesity staging system score of 0 or 1 (Padwal, Pajewski, Allison and Sharma 2011).

It has been suggested that the Edmonton obesity staging system may have a role in patient selection for weight loss surgery (Gill, Karmali and Sharma 2011). However it is important to note that this score has not been prospectively validated yet for use in this population.

Table 1. The Edmonton obesity staging system (Sharma and Kushner 2009).

Stage	Description	Management
0	No apparent obesity-related risk factors (e.g., blood pressure, serum lipids, fasting glucose, etc. within normal range), no physical symptoms, no psychopathology, no functional limitations and/or impairment of well being	Identification of factors contributing to increased body weight. Counselling to prevent further weight gain through lifestyle measures including healthy eating and increased physical activity.
1	Presence of obesity-related subclinical risk factors (e.g., borderline hypertension, impaired fasting glucose, elevated liver enzymes, etc.), mild physical symptoms (e.g., dyspnoea on moderate exertion, occasional aches and pains, fatigue, etc.), mild psychopathology, mild functional limitations and/or mild impairment of well being	Investigation for other (non-weight related) contributors to risk factors. More intense lifestyle interventions, including diet and exercise to prevent further weight gain. Monitoring of risk factors and health status.
2	Presence of established obesity-related chronic disease (e.g., hypertension, type 2 diabetes, sleep apnoea, osteoarthritis, reflux disease,	Initiation of obesity treatments including considerations of all behavioural, pharmacological and surgical treatment options. Close

	polycystic ovary syndrome, anxiety disorder, etc.), moderate limitations in activities of daily living and/or well being	monitoring and management of comorbidities as indicated.
3	Established end-organ damage such as myocardial infarction, heart failure, diabetic complications, incapacitating osteoarthritis, significant psychopathology, significant functional limitations and/or impairment of well being	More intensive obesity treatment including consideration of all behavioural, pharmacological and surgical treatment options. Aggressive management of comorbidities as indicated.
4	Severe (potentially end-stage) disabilities from obesity-related chronic diseases, severe disabling psychopathology, severe functional limitations and/or severe impairment of well being	Aggressive obesity management as deemed feasible. Palliative measures including pain management, occupational therapy and psychosocial support.

The King's College criteria

Aylwin et al have proposed a score with the objective to stratify obesity in a weight-independent manner but focusing on the disease burden, in other words moving away from morbid obesity and focusing on obese morbidity (Aylwin and Al-Zaman 2008). The criteria can be seen on table 2.

Table 2. The King's criteria (Aylwin and Al-Zaman 2008) are summarised below:

	Stage 0	Stage 1	Stage 2	Stage 3
Airway	Normal	snoring	Require CPAP	Cor pulmonale
BMI	<35	35-40	40-50	>50
Cardiovascular	<10% risk	10-20% risk	Heart disease	Heart failure
Diabetes	Normal	Impaired fasting glycaemia	Type 2 diabetes	Uncontrolled type 2 diabetes
Economic	Normal	Suffered discrimination	Unemployed due to obesity	Requires financial support
Functional	Can manage 3 flights of stairs	Manage 1 or 2 flights of stairs	Requires walking aids or wheel chair	House bound
Gonadal	Normal	PCOS	Infertility	Sexual dysfunction
Health status	Normal	Low mood or QoL	Depression or poor QoL	Severe depression
Image	Normal	Does not like	Body image	Eating disorder

		body	dysphoria	
Junction gastro-oesophagus	Normal	Heart burn	Oesophagitis	Barrett's Oesophagus
Kidney	Normal	proteinuria	GFR<60ml/min	GFR<30ml/min
Liver	Normal	Raised GGT	NASH	Liver failure

The criteria were modified and the score was used on patients undergoing weight loss surgery in a study by Aasheim et al (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011). Inter-observer reliability was assessed with eleven clinicians (six physicians, two surgeons, two medical students and one nurse specialist; of these, four physicians, one surgeon, one student and one nurse had previous experience with the modified King's Criteria) scored the same 12 individuals (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011). Different assessors assigned mostly similar scores for the same individual (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011). However there was high variability between assessors for some domains with Body Image being the one with the worst observer consistency (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011).

Aasheim et al demonstrated that according to the modified King's Criteria patients undergoing weight loss and metabolic surgery experienced improvement in their health postoperatively (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011). The authors suggested that in the clinical setting the modified King's Criteria added structure to a patient-centred clinical interaction. More importantly this work highlighted the need to focus on outcomes of metabolic surgery other than weight loss itself (Aasheim, Aylwin, Radhakrishnan, Sood, Jovanovic, Olbers and le Roux 2011).

The King's criteria are a useful tool for the risk stratification of patients preoperatively but also postoperatively with the comprehensive documentation of risk and the potential increase or decrease following surgery (le Roux and Pournaras 2010a). They will be used in this thesis as a roadmap presenting the benefit of surgery for patients, focusing on co-morbidity and functional improvement rather than weight loss (le Roux and Pournaras 2010).

Airway

Obstructive Sleep Apnoea is common in the obese population and in patient undergoing weight loss surgery in particular. In the Longitudinal Assessment of Bariatric Surgery study obstructive sleep apnoea was detected in 48.9% of the population consisting of 4776 consecutive individuals undergoing primary weight loss surgery (Flum et al 2009). Furthermore obstructive sleep apnoea was associated with a significantly higher incidence of adverse effects perioperatively (Flum et al 2009).

In a 2004 meta-analysis, 85.7% of patients achieved resolution of obstructive sleep apnoea (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004). A more recent meta-analysis confirmed that weight loss surgery significantly reduced the apnoea-hypopnoea index (Greenburg, Lettieri and Eliasson 2009). In the same study the levels of the index recorded suggested moderately severe obstructive sleep apnoea postoperatively (Greenburg, Lettieri and Eliasson 2009).

Therefore patients should be informed during the consent process that obstructive sleep apnoea may persist postoperatively (Greenburg, Lettieri and Eliasson 2009).

Body mass Index (BMI)

In Buchwald's landmark meta-analysis the mean percentage of excess weight loss at the time point the status of comorbidity was assessed was 47.5% for gastric banding, 61.6% for gastric bypass, 68.2% for gastroplasty, and 70.1% for biliopancreatic diversion or duodenal switch (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004). The Swedish Obese Subjects study provides the best available comparison of weight loss between weight loss surgery and non-surgical management of obesity in the long term (Sjostrom et al 2007). In the three surgical groups the total body weight loss was 25% ten years after gastric bypass, 16% after vertical-banded gastroplasty, and 14% after banding (Sjostrom et al 2007). In the non-surgical group weight remained within ±2% during the ten year period (Sjostrom et al 2007).

Cardiovascular

Weight loss is known to reduce cardiovascular risk. However, metabolic surgery is associated with improvement in cardiac function and the reversal of obesity related cardiomyopathy (Ashrafian, le Roux, Darzi and Athanasiou 2008). Improved cardiac function, ventricular remodelling and atherosclerotic load have also been demonstrated (Ashrafian, le Roux, Darzi and Athanasiou 2008).

Diabetes

The effect of metabolic surgery on glucose metabolism is the subject of this thesis and will be described in detail in subsequent chapters.

Economic

The cost-effectiveness of metabolic surgical procedures for the health care systems will be described further in a separate section. The benefit specifically to recipients of metabolic surgery has been shown in this study by Hawkins et al using data from Somerset, UK (Hawkins, Osborne, Finlay, Alagaratnam, Edmond and Welbourn 2007). They demonstrated that the number of individuals receiving income from work was increased postoperatively (Hawkins, Osborne, Finlay, Alagaratnam, Edmond and Welbourn 2007). The same group also showed that the number of hours worked was also increased and there was a reduction in state benefits claims postoperatively (Hawkins, Osborne, Finlay, Alagaratnam, Edmond and Welbourn 2007).

Functional

Obesity is associated with poor function, physical illness and disability (Weil, Wachterman, McCarthy, Davis, O'Day, Iezzoni and Wee 2002; Ferraro and Booth 1999). Bergkvist et al studied populations of patients attending an orthopaedic department in both the emergency and the elective/chronic setting (Bergkvist,

Hekmat, Svensson and Dahlberg 2009). Significant relationships between obesity and common orthopaedic conditions were demonstrated (Bergkvist, Hekmat, Svensson and Dahlberg 2009).

Miller et al conducted a longitudinal, observational study of 28 morbidly obese individuals followed for 12 months after laparoscopic gastric bypass using the Fitness Arthritis and Seniors Trial disability questionnaire for the estimation of physical function, the Short Physical Performance Battery and a lateral mobility task to assess performance tasks and maximal isometric knee torque for measurement of strength (Miller, Nicklas, You and Fernandez 2009). An increase in mobility and improvement in the performance of daily activities was demonstrated as early as three weeks postoperatively (Miller, Nicklas, You and Fernandez 2009).

De Souza et al used the 6-minute walk test in a group of 51 patients undergoing weight loss surgery (de Souza, Faintuch, Fabris, Nampo, Luz, Fabio, Sitta and de Batista Fonseca IC 2009). The test was performed preoperatively and seven to twelve months postoperatively and a significant improvement was reported. (de Souza, Faintuch, Fabris, Nampo, Luz, Fabio, Sitta and de Batista Fonseca IC 2009).

Gonadal

In a report from six centres participating in the Longitudinal Assessment of Bariatric Surgery-2 study a prevalence of polycystic ovary syndrome (PCOS) of 13.1% was

recorded (Gosman, King, Schrope, Steffen, Strain, Courcoulas, Flum, Pender and Simhan 2010). Furthermore 41.9% experienced infertility and 61.4% had a live birth with a previous history of infertility (Gosman, King, Schrope, Steffen, Strain, Courcoulas, Flum, Pender and Simhan 2010). In another study of 24 women with PCOS undergoing gastric bypass, a significant improvement of PCOS symptoms postoperatively was reported (Eid, Cottam, Velcu, Mattar, Korytkowski, Gosman, Hindi and Schauer 2005).

I have recently shown that 23 out 149 (15.4%) consecutive women of child bearing age (18-45 years old) undergoing weight loss surgery at Musgrove Park Hospital, Taunton were diagnosed with PCOS (Pournaras, Manning, Bidgood, Fender, Mahon and Welbourn 2010). Furthermore in the same population, for 11 of 149 (7.4%) women of childbearing age, subfertility was the main reason for undergoing weight loss surgery (Pournaras, Manning, Bidgood, Fender, Mahon and Welbourn 2010).

It is well documented that maternal obesity is associated with a higher risk for both mother and fetus (Gross, Sokol and King 1980; Morin 1998). A recent systematic review suggests that the risk of maternal complications, such as gestational diabetes and preeclampsia, as well as neonatal complications, such as premature delivery and low birth weight, are lower following weight loss surgery compared to obese individuals (Maggard 2008).

A study from George Fileding's unit demonstrated that gastric banding is safe and well-tolerated during pregnancy with a lower incidence of gestational diabetes and maternal hypertension (Skull, Slater, Duncombe and Fielding 2004).

Focusing on male fertility Shayeb et al studied 2035 men presenting to a fertility clinic and demonstrated that obese men were more likely to have lower semen volume and fewer morphologically normal spermatozoa than non-obese men (Shayeb, Harrild, Mathers and Bhattacharya 2011). These findings are consistent with another study showing obesity is associated with a higher risk of low sperm quality (Hammoud, Wilde, Gibson, Parks, Carrell and Meikle 2008). The effects of surgically induced weight loss on male fertility remain largely unknown.

Health status perceived

In the Swedish Obese Subjects study, health related quality of life improved significantly after weight loss surgery but not in the control group (Karlsson, Sjöström and Sullivan 1998). Using the Short Form-36 (SF-36) O'Brien et al demonstrated in a randomised controlled trial comparing of gastric banding versus conservative management in patients with a BMI of 30-35, that quality of life improved significantly after gastric banding in all domains of the SF-36 (O'Brien et al 2006). The above results are consistent with another prospective study comparing patients undergoing gastric bypass surgery with two control groups; patients who requested but did not undergo surgery and obese individuals (Kolotkin, Crosby, Gress, Hunt and Adams

2009). Health related quality of life was improved in the surgical group compared to the controls groups (Kolotkin, Crosby, Gress, Hunt SC and Adams 2009)

Image of body

The Melbourne group demonstrated that super-obese patients undergoing gastric banding surgery have reduced evaluation of appearance preoperatively which is improved with weight loss after surgery (Dixon, Dixon and O'Brien 2002). They also showed an associated psychological benefit with this improvement (Dixon, Dixon and O'Brien 2002). In the SOS study, a small effect of surgery on social interaction was reported as measured by health-related limitations in social interaction within the family, among friends and in the community (Karlsson, Taft, Rydén, Sjöström and Sullivan 2007). This was only present on patients who achieved a 10% weight loss or more (Karlsson, Taft, Rydén, Sjöström and Sullivan 2007). An improvement in the scores of patients on body image dissatisfaction subscale three years after biliopancreatic diversion has been also reported (Adami, Gandolfo, Campostano, Meneghelli, Ravera and Scopinaro 1998)

It has to be noted that individuals may face significant morbidity due to the severe change in body image and this needs to be accounted for, and aggressively treated appropriately. The cosmetic outcomes of weight loss surgery may be very poor. These interventions are not cosmetic surgery. A new field, post-bariatric body contouring surgery is emerging as a subspecialty of plastic surgery with the objective

to address specific cosmetic and occasionally functional issues amenable to plastic surgical interventions.

Junction gastro-oesophagus (gastro-oesophageal reflux disease)

A meta-analysis demonstrated that obesity is associated with a higher risk of gastro-oesophageal reflux symptoms, erosive oesophagitis, and oesophageal adenocarcinoma (Hampel, Abraham and El-Serag 2005). The same study showed a trend for progressive increase of the above with increasing weight (Hampel, Abraham and El-Serag 2005).

Gastric bypass surgery leads to the improvement of gastro-oesophageal reflux symptoms and oesophageal exposure to acid (Mejía-Rivas, Herrera-López, Hernández-Calleros, Herrera and Valdovinos 2008). This is to be expected considering that these procedures were initially designed for peptic ulcer disease in the era prior to pharmacological treatment.

In a study of 100 patients, 73% were reported to have gastro-oesophageal reflux symptoms (Merrouche, Sabaté, Jouet, Harnois, Scaringi, Coffin and Msika 2007). The different effects of gastric bypass and gastric banding on oesophageal function were demonstrated, with worsening of pH-metric data and occasional severe dyskinesia after gastric banding Merrouche, Sabaté, Jouet, Harnois, Scaringi, Coffin

and Msika 2007). Suter et al also demonstrated that postoperative oesophageal dysmotility and gastrooesophageal reflux are not uncommon after gastric banding suggesting routine preoperative evaluation (Suter, Dorta, Giusti and Calmes 2005). Low amplitude of contraction in the lower oesophagus and increased oesophageal acid exposure should be regarded as contraindications to gastric banding and patients with such findings should be offered an alternative weight loss surgical procedure (Suter, Dorta, Giusti and Calmes 2005). George Fielding's group propose the addition of routine repair of a hiatus hernia during gastric band placement when indicated as it significantly reduces the reoperation rate due to gastric prolapse and pouch dilatation (Gulkarov, Wetterau, Ren and Fielding 2008).

Kidney

Obesity is a risk factor for the progression of chronic kidney disease and a systematic review demonstrated that weight loss is associated with decreased proteinuria and microalbuminuria (Afshinnia, Wilt, Duval, Esmaeili and Ibrahim 2010). There were no available data regarding the durability of this decrease and the effect of weight loss on chronic kidney disease (Afshinnia, Wilt, Duval, Esmaeili and Ibrahim 2010).

A report of a patient with end-stage renal disease who experienced dramatic improvement of renal function after gastric bypass surgery, obviating the need for dialysis and transplantation has led to increased interest in this renal effect of weight loss surgery (Tafti, Haghdoost, Alvarez, Curet and Melcher 2009). Navarro-Dıaz et

al studied 61 obese patients undergoing weight loss surgery (Navarro-Díaz, Serra, Romero, Bonet, Bayés, Homs, Pérez and Bonal 2006). All renal parameters improved in the first 12 months postoperatively when the majority of weight loss occurred (Navarro-Díaz, Serra, Romero, Bonet, Bayés, Homs, Pérez and Bonal 2006). However, 24-h albuminuria still improved during the second year of follow-up (Navarro-Díaz, Serra, Romero, Bonet, Bayés, Homs, Pérez and Bonal 2006). The authors suggested that this decrease in 24-h albuminuria may not be related to glomerular filtration rate but possibly due to the decrease in BMI and the improvement of other metabolic factors (Navarro-Díaz, Serra, Romero, Bonet, Bayés, Homs, Pérez and Bonal 2006).

Liver

Non-alcoholic fatty liver disease (NAFLD) is a spectrum ranging from fatty liver, followed by non-alcoholic steatohepatitis (NASH) and the more severe form being cirrhosis and progress to hepatocellular carcinoma or liver failure (Kim and Younossi 2008). Non-alcoholic fatty liver disease has become the most common form of liver disease (Kim and Younossi 2008).

NAFLD and NASH appear to improve or completely resolve in the majority of patients after weight loss surgery (Mummadi, Kasturi, Chennareddygari and Sood 2008; Mathurin et al 2009).

Metabolic surgery and cancer

The effect of weight loss surgery on cancer incidence is not part of any scoring system of obesity (such as the Edmonton obesity staging system or the King's College criteria). However it is an effect of weight loss surgery which has been described and well documented. A recent systematic review and meta-analysis of prospective observational studies confirmed that increased BMI is associated with increased risk of common and less common cancers (Renehan, Tyson, Egger, Heller and Zwahlen 2008). In men, a 5 kg/m2 increase in BMI was associated with oesophageal adenocarcinoma, thyroid cancer, colonic cancer and renal cancer. In women, a 5 kg/m2 increase in BMI was associated with endometrial cancer, gallbladder cancer, oesophageal adenocarcinoma and renal cancer (Renehan, Tyson, Egger, Heller and Zwahlen 2008). Furthermore weaker positive associations were recorded between increased BMI and rectal cancer and malignant melanoma in men; postmenopausal breast, pancreatic, thyroid, and colon cancers in women; and leukaemia, multiple myeloma, and non-Hodgkin lymphoma in both sexes (Renehan, Tyson, Egger, Heller and Zwahlen 2008) .

Adams et al demonstrated that all cause mortality after gastric bypass surgery was significantly reduced when compared with obese controls (Adams, Gress, Smith, Halverson, Simper, Rosamond, Lamonte, Stroup and Hunt 2007). This was a study comparing 7925 patients undergoing gastric bypass surgery and 7925 obese individuals applying for a driver's license in Utah matched for age, sex, and BMI (Adams, Gress, Smith, Halverson, Simper, Rosamond, Lamonte, Stroup and Hunt

2007). Cancer specific mortality was reduced by 60% (Adams, Gress, Smith, Halverson, Simper, Rosamond, Lamonte, Stroup and Hunt 2007).

Adams et al investigated this effect further in another study by using cancer incidence and mortality data through 2007 from the Utah Cancer Registry (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009). They compared 6,596 Utah patients who had gastric bypass in Utah and 9,442 severely obese individuals applying for a Utah Driver's License (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009). The follow-up was in excess of 24 years with a mean of 12.5 years (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009). Total cancer incidence was significantly lower in the surgical group with a hazard ratio of 0.76 (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009). Cancer specific mortality was decreased in patients undergoing gastric bypass surgery with a hazard ratio of 0.54 (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009). An intriguing finding of this study was that reduced mortality was observed for all cancers, not only the obesity related ones (Adams, Stroup, Gress, Adams, Calle, Smith, Halverson, Simper, Hopkins and Hunt 2009).

In the same issue of the New England Journal of Medicine in which Adams study was published, the Swedish Obese Subject study trial reported that weight loss surgery is associated with long-term weight loss and decreased overall mortality

(Sjöström et al 2007). Sjostrom et al intended to perform a randomised control trial comparing weight loss surgery with best medical treatment. However the study was not approved by the Ethics Committee due to the high risk associated with weight loss surgery. The fact that a similar trial is currently considered not ethical due to the high risk associated with non-surgical treatment following the reported outcomes of the SOS trial highlights the unique contribution of this study in the field. The study performed was a prospective, matched control trial including 4047 obese subjects; 2010 undergoing weight loss surgery and 2037 undergoing conventional treatment, recruited over a 13.4-year period and follow-up for a mean of 10.9±3.5 years (Sjöström et al 2007). The follow-up in terms of vital status was complete for participants with exception of three, achieving an impressive rate of 99.9% (Sjöström et al 2007). Two participants were deleted from the records upon their request and one withdrew from the study and subsequently obtained an unlisted social security number (Sjöström et al 2007). In the weight loss surgery group 101 deaths were recorded compared to 129 deaths in the control group with an unadjusted overall hazard ratio of 0.76 and with the most common cause of death myocardial infarction and cancer (Sjöström et al 2007).

A report on cancer incidence in the SOS trial was published in 2009 (Sjöström et al 2009). In the weight loss surgery group 117 cancers were detected compared 169 in the control group with a hazard ratio 0.67 (Sjöström et al 2009). In females incidence of cancer was lower in the surgical group compared to the control group, but there was no difference in males (Sjöström et al 2009). However the number of male participants was lower (Sjöström et al 2009).

Weight loss surgery is associated with a protective effect on cancer, although the mechanism of this interaction remains to be elucidated. Abdominal obesity is linked to alterations in insulin and the insulin-like growth factor-1 (IGF-1), sex steroids and adipokines (Sjöström et al 2009; Renehan, Frystyk and Flyvbjerg 2006; Key, Appleby, Reeves, Roddam et al 2003; Barb, Williams, Neuwirth and Mantzoros 2007). In the SOS study baseline sagittal trunk diameter came out as a strong multiple cancer predictor. In contrast weight and BMI did not. More importantly these findings highlight the fact the favourable effect of metabolic surgery is extended well beyond weight loss.

Cost effectiveness

Although the effect of metabolic surgery on comorbidity is slowly recognised by the scientific community there is still debate about the cost effectiveness of this type of surgery. In fact the surgical management of obesity and obesity related comorbidities is associated with polarised opinions among general surgeons not performing weight loss surgery, physicians and the public.

Nicholas Christou's group in Montreal performed a comparison in terms of health-related costs between 1035 patients undergoing weight loss surgery and to 5746 age- and sex-matched controls (Sampalis, Liberman, Auger and Christou 2004). The follow-up period was five years and the endpoint was all-cause hospitalisation with the cost of the surgical procedure included (Sampalis, Liberman, Auger and

Christou 2004). The total hospitalization cost was higher in the surgical group in the first year, however at 5 years the cost was higher for the control group allowing the authors to conclude that weight loss surgery decreases long-term direct health-care costs and the initial cost of this treatment can be recovered over a period of 3.5 years (Sampalis, Liberman, Auger and Christou 2004). Of note is the fact that the majority of the patients in the surgical group underwent open gastric bypass surgery (Sampalis, Liberman, Auger and Christou 2004). With the use of the laparoscopic approach the comparison may be more favourable for weight loss surgery (Sampalis, Liberman, Auger and Christou 2004).

Focusing on medication cost in another study from the USA 78 patients aged 55 to 75 undergoing laparoscopic gastric bypass surgery were assessed preoperatively and 6 months, 1 year, and yearly postoperatively thereafter (Snow, Weinstein, Hannon, Lane, Ringold, Hansen and Pointer 2004). The number of medications per patient fell by 66% (Snow, Weinstein, Hannon, Lane, Ringold, Hansen and Pointer 2004). With the cost of the surgical intervention as one-off, the crossover point for cost effectiveness was at 2.5 years (Snow, Weinstein, Hannon, Lane, Ringold, Hansen and Pointer 2004; Welbourn and Pournaras 2010).

The Health Technology Assessment report used for the 2002 National Institute of Clinical Excellence Guidelines for weight loss surgery estimated that the incremental cost effectiveness ratios per quality-adjusted life year were £8527 for gastric banding and £6289 for gastric bypass (Clegg, Colquitt, Sidhu, Royle, Loveman and Walker

2002; Welbourn and Pournaras 2010). Both of these were well below the conventional threshold of £30 000 often used by the National Institute of Clinical Excellence to determine cost effectiveness (Clegg, Colquitt, Sidhu, Royle, Loveman and Walker 2002; Welbourn and Pournaras 2010).

An updated Health Technology Assessment systematic review assessed the cost-effectiveness of weight loss surgery in 2009 (Picot, Jones, Colquitt, Gospodarevskaya, Loveman, Baxter and Clegg 2009). Picot el al reported that weight loss surgery was cost-effective compared to non-surgical treatment modalities in the published estimates of cost-effectiveness (Picot, Jones, Colquitt, Gospodarevskaya, Loveman, Baxter and Clegg 2009). However, these estimates were likely to be unreliable according to the authors (Picot, Jones, Colquitt, Gospodarevskaya, Loveman, Baxter and Clegg 2009). Hence they developed a novel economic model. According to this the cost of weight loss surgery was higher than non-surgical treatment the three patient populations used for analysis, but was associated with an improved effect (Picot, Jones, Colquitt, Gospodarevskaya, Loveman, Baxter and Clegg 2009). For morbid obesity incremental cost-effectiveness ratios ranged between £2000 and £4000 per quality-adjusted life year gained (Picot, Jones, Colquitt, Gospodarevskaya, Loveman, Baxter and Clegg 2009). These figures remain within the range regarded as cost-effective by National Institute of Clinical Excellence.

Cost effectiveness and type 2 diabetes

Focusing on type 2 diabetes related outcomes, O'Brien and his group in Melbourne reported cost-effectiveness results from a randomised control trial comparing gastric banding versus best medical treatment for type 2 diabetes (Keating, Dixon, Moodie, Peeters, Playfair and O'Brien 2009). The incremental cost effectiveness ratio for gastric banding was lower than the comparable figure for conventional therapy suggesting that within a 2-year period gastric banding was below the currently accepted cost-effectiveness threshold in Australia (Keating, Dixon, Moodie, Peeters, Playfair and O'Brien 2009).

Klein et al examined the administrative claims database of privately insured patients in the US covering 8.5 million lives 1999-2007, identified obese patients with diabetes, aged 18-65 years, who were treated with weight loss surgery and matched them with controls for demographic characteristics, comorbidities, and health-care costs (Klein, Ghosh, Cremieux, Eapen and McGavock 2011). The cost of laparoscopic surgery was fully recovered at 26 months (Klein, Ghosh, Cremieux, Eapen and McGavock 2011). Within one month medication costs were significantly lower for the surgical group (Klein, Ghosh, Cremieux, Eapen and McGavock 2011).

Conclusion

Weight loss surgery leads to an improvement in a number of obesity related comorbidites secondary to the weight loss itself but also due to weight loss independent effects.

Gut hormones and bile acids

Energy homeostasis

Energy intake and expenditure are regulated by the mechanisms of energy homeostasis and lead to a stable body mass over time (Morton, Cummings, Baskin, Barsh and Schwartz 2006; Flier 2004; Pournaras and le Roux 2009). From an evolutionary point of view one can hypothesise that survival in an environment with limited availability of food provided selection bias towards homeostatic systems which are more sensitive to reduced energy intake rather than energy excess (Schwartz, Woods, Seeley, Barsh, Baskin and Leibel 2003; Pournaras and le Roux 2009). This phenomenon may perhaps explain the current obesity epidemic (Pournaras and le Roux 2009). Non-surgical weight loss is associated with increased hunger and reduced metabolic rate which is a physiological response to reduced energy intake. Leptin and insulin are the key messengers of the status of energy stores from the periphery to the central nervous system (Morton, Cummings, Baskin, Barsh and Schwartz 2006; Flier 2004; Pournaras and le Roux 2009).

The central melanocortin system, which plays a crucial role in the regulation of energy homeostasis, is influenced by signals mediated through gut hormones (Ellacott, Halatchev and Cone 2006; Pournaras and le Roux 2009). These molecules cause hunger and postprandial satiety and hence regulate appetite control (Pournaras and le Roux 2009).

Weight loss procedures were developed with the objective to cause weight loss due to the reduction of gastric volume (laparoscopic adjustable gastric banding, laparoscopic sleeve gastrectomy), malabsorption of nutrients (biliopancreatic diversion, duodenal switch) or the effect of both (Roux-en-Y gastric bypass) (Pournaras and le Roux 2009). There is no evidence that calorie or protein malabsorption occurs after gastric bypass. It has been demonstrated that negating the effect of the satiety gut hormone change with octeotride is associated with increased food intake and reduced satiety (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom and Olbers 2007). A number of studies have suggested that gut hormone concentrations change after gastric bypass leading to the establishment of the concept of the gut-brain axis. The available data will be reviewed below.

Gut hormones

In this chapter anorexigenic and orexigenic gut hormones are reviewed in decreasing order in terms of level of evidence regarding their role in the mechanism of action of weight loss surgery namely peptide YY (PYY), glucagon-like peptide-1 (GLP-1), ghrelin, cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin (OXM) and pancreatic polypeptide (PP).

Peptide YY (PYY)

Peptide YY is 36-amino-acid peptide, member of the PP-fold peptide family. Y is the abbreviation for tyrosine. PYY was found throughout the small gut in very low

concentrations in the duodenum and jejunum increasing in the terminal ileum and even higher concentrations throughout the colon with the maximum in the rectum (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). Basal plasma concentrations of PYY were low but rose in response to food, remaining elevated for several hours postprandially (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). PYY levels are not altered by gastric distension (Oesch, Rüegg, Fischer, Degen and Beglinger 2006). PYY decreases appetite and reduces food intake by 33% over 24 hours in non obese individuals (Batterham, Cowley, Small, Herzog, Cohen, Dakin, Wren, Brynes, Low, Ghatei, Cone and Bloom 2002). Furthermore obese individuals are not resistant to the anorectic effects of PYY and have lower endogenous PYY suggesting that PYY deficiency may contribute to the pathogenesis of obesity (Batterham, Cohen, Ellis, Le Roux, Withers, Frost, Ghatei and Bloom 2003).

Glucagon-like peptide-1 (GLP-1)

GLP-1 is released postprandially by the same endocrine L-cells as PYY and OXM (Cummings and Overduin 2007). GLP-1 and PYY inhibit food intake additively (Neary, Small, Druce, Park, Ellis, Semjonous, Dakin, Filipsson, Wang, Kent, Frost, Ghatei and Bloom 2005). GLP-1 has an additional role as an incretin, a hormone which is secreted from gastrointestinal tract cells into the systemic circulation in response to the presence of nutrients intraluminally (Baggio and Drucker 2007). GLP-1 leads to glucose-dependent insulin secretion, induction of ß-cell proliferation and enhanced resistance to apoptosis (Baggio and Drucker 2007). GLP-1 has

further effects on glucose regulation with slowing of gastric emptying and glucose-dependent inhibition of glucagon secretion (Baggio and Drucker 2007).

Ghrelin

The only known orexigenic gut hormone is ghrelin, is a 28-amino acid peptide produced from the fundus of the stomach and the proximal intestine (Kojima, Hosoda, Date, Nakazato, Matsuo and Kangawa 1999; Frühbeck, Diez Caballero and Gil 2004). Central and peripheral administration leads to increased food intake (Wren, Small, Abbott, Dhillo, Seal, Cohen, Batterham, Taheri, Stanley, Ghatei and Bloom 2001; Wren, Seal, Cohen, Brynes, Frost, Murphy, Dhillo, Ghatei and Bloom 2001). The preprandial elevation and the postprandial fall in ghrelin levels suggest that ghrelin may play a physiological role in meal initiation (Cummings, Purnell, Frayo, Schmidova, Wisse and Weigle 2001). Diet induced weight loss of 17% of initial body weight was associated with a 24% increase in the 24 hour ghrelin profile suggesting that ghrelin may also have a role in the long-term regulation of body weight (Cummings, Weigle, Frayo, Breen, Ma, Dellinger and Purnell 2002). Data from Imperial demonstrate that obese individuals have lower fasting ghrelin levels and reduced postprandial ghrelin suppression compared to normal weight individuals (le Roux, Patterson, Vincent, Hunt, Ghatei and Bloom 2005).

Cholecystokinin (CCK)

CCK is secreted by I cells located in the duodenum, jejunum, and proximal ileum in response to a meal (Chandra and Liddle 2007). CCK has been implicated in gastric

emptying and distension, gallbladder contraction, pancreatic secretion, and intestinal motility as well as postprandial satiety (Chandra and Liddle 2007; Kellum, Kuemmerle, O'Dorisio, Rayford, Martin, Engle, Wolf and Sugerman 1990). Intraperitoneal administration of CCK in mice diminished food intake by 90% (Kopin, Mathes, McBride, Nguyen, Al-Haider, Schmitz, Bonner-Weir, Kanarek and Beinborn 1999). In contrast intraperitoneal administration of CCK in mice, lacking CCK-A receptors did not reduce food intake (Kopin, Mathes, McBride, Nguyen, Al-Haider, Schmitz, Bonner-Weir, Kanarek and Beinborn 1999). The latter group of mice had the same weight as wild-type mice and Kopin et al suggested that CCK plays a role in appetite but is not essential for homeostasis (Kopin, Mathes, McBride, Nguyen, Al-Haider, Schmitz, Bonner-Weir, Kanarek and Beinborn 1999).

Glucose-dependent insulinotropic peptide or Gastric Inhibitory Peptide

GIP is also an incretin with similar to GLP-1 effect on islet β-cells acting through structurally distinct yet related receptors (Yip and Wolfe 2000). It is produced in the proximal gut and is released postprandially (Yip and Wolfe 2000). It was initially named so due to the gastric acid inhibitory properties, but the effect on insulin seems to be more important from a physiological point of view (Yip and Wolfe 2000).

Oxyntomodulin (OXM)

Infusion of OXM reduced ad libitum energy intake at a buffet meal and also reduced hunger scores without causing nausea or affecting food palatability (Cohen, Ellis, Le Roux, Batterham, Park, Patterson, Frost, Ghatei and Bloom 2003). In a randomised,

double-blind human trial the effect of OXM treatment was tested with self administered injections of OXM three times daily 30 minutes before each meal. In the treatment group body weight was reduced by 2.3 kg compared to 0.5 kg in the control group (Wynne, Park, Small, Patterson, Ellis, Murphy, Wren, Frost, Meeran, Ghatei and Bloom 2005).

Pancreatic polypeptide (PP)

PP is a gut hormone released from the pancreas in response to nutrients. Plasma levels of PP increased rapidly after a meal in healthy volunteers and remained elevated after six hours (Adrian, Bloom, Bryant, Polak, Heitz and Barnes 1976). Plasma PP has been shown to be reduced in conditions associated with increased food intake such as Prader-Willi syndrome (Zipf, O'Dorisio, Cataland and Sotos 1981). Plasma PP has been shown to be increased in anorexia nervosa (Uhe, Szmukler, Collier, Hansky, O'Dea and Young 1992). Infusion of PP leads to decreased appetite and food intake (Batterham, le Roux, Cohen, Park, Ellis, Patterson, Frost, Ghatei and Bloom 2003).

Bile acids

Bile consists of bile acids, cholesterol, phosphatidylcholine and bilirubin. The primary bile acids in humans are cholic acid and chenodeoxycholic acid (Vlahcevic, Pandak and Stravitz 1999). They are synthesized with the aid of enzymes found in the endoplasmic reticulum, cytosol, mitochondria, and peroxisomes and they are conjugated to glycine or taurine before they are secreted into bile canaliculi

(Vlahcevic, Pandak and Stravitz 1999). Conjugated bile acids are the major solutes in bile; they are less toxic and are more efficient promoters of intestinal absorption of dietary lipid than unconjugated bile acids (Vessey, Crissey and Zakim 1977; Pircher, Kitto, Petrowski, Tangirala, Bischoff, Schulman and Westin 2003).

Following a meal, bile flows into the duodenum and proximal gut (Houten, Watanabe and Auwerx 2006). Bile acids are absorbed by both passive diffusion and active transport in the terminal ileum, and then transported to the liver via the portal vein in the so called enterohepatic recirculation (Houten, Watanabe and Auwerx 2006). They are subsequently taken up at the basolateral sinusoidal membrane and exported again at the apical canalicular membrane of the hepatocytes into the bile canaliculus (Houten, Watanabe and Auwerx 2006). Each BA molecule may complete 4–12 cycles between the liver and intestine per day (Houten, Watanabe and Auwerx 2006).

Bile acids have been recently recognised as potential targets for drug treatment in obesity and diabetes. However this concept is not entirely novel. The use of bile acids as appetite suppressants was first reported in 1968 (Bray and Gallagher 1968).

In a randomized, double-blind, crossover study of cholestyramine compared with placebo for a period of 6 weeks each 21 patients with type 2 diabetes mellitus were included (Garg and Grundy 1994). The aim was to assess efficacy and tolerability of the bile acid sequestrant cholestyramine (Garg and Grundy 1994). Unexpectedly in

the post hoc analysis improved glycaemic control with mean plasma glucose values lowered by 13% and a median reduction in urinary glucose excretion were reported as well as a trend for lower glycated haemoglobin (Garg and Grundy 1994).

Suzuki et al compared a different bile acid sequestrant, colestimide, to acarbose in a randomised open label study and showed a significant decrease in glucose levels (Suzuki, Oba, Futami, Suzuki, Ouchi, Igari, Matsumura, Watanabe, Kigawa and Nakano 2006). Zieve et al showed in a double blind, placebo controlled study that a 12-week treatment with colesevelam, another bile acid sequestrant, in addition to oral antihyperglycaemic medications, was associated with a significant reduction in HbA1c compared to placebo (Zieve, Kalin, Schwartz, Jones and Bailey 2007). In another study comparing colestimide to pravastin, using a randomised open label design, colestimide therapy for three months led to reduced HbA1c and fasting glucose levels (Yamakawa, Takano, Utsunomiya, Kadonosono and Okamura 2007).

Three similar multicenter studies assessed the efficacy of colevesham in patients not adequately controlled receiving insulin therapy alone or in combination with oral antidiabetic agents in one, receiving sulfonylurea monotherapy or sulfonylurea in combination with additional oral antidiabetic agents in the second and receiving metformin monotherapy or metformin in combination with additional oral antidiabetic agents in the third (Goldberg, Fonseca, Truitt and Jones 2008; Fonseca, Rosenstock, Wang, Truitt and Jones 2008; Bays, Goldberg, Truitt and Jones 2008). A prospective, randomized, double-blind, placebo-controlled, parallel-group design

was used in all studies and all three confirmed that colesevelam improved glycaemic control (Goldberg, Fonseca, Truitt and Jones 2008; Fonseca, Rosenstock, Wang, Truitt and Jones 2008; Bays, Goldberg, Truitt and Jones 2008).

Different possible mechanisms have been suggested in attempt to dissect the effects of bile acids on glucose metabolism. Disruption of enterohepatic circulation of bile acids may have an effect on the Farnesoid X Receptor (FXR) pathway, the intracellular signalling pathway for bile acids (Guzelian and Boyer 1974). Bile acids promote GLP-1 secretion through TGR5 in STC-1 cells (Katsuma, Hirasawa and Tsujimoto 2005). The effect of GLP-1 on glucose metabolism has already been described in the GLP-1 section. Bile acids inhibit gluconeogenesis in both FXR dependent and independent manner (Thomas, Pellicciari, Pruzanski, Auwerx and Schoonjans 2008; De Fabiani, Mitro, Gilardi, Caruso, Galli and Crestani 2003; Yamagata, Daitoku, Shimamoto, Matsuzaki, Hirota, Ishida and Fukamizu 2004; Ma, Saha, Chan and Moore 2006). Bile acids increase energy expenditure and therefore reduce insulin resistance. This effect is dependent on induction of the cyclic-AMP-dependent thyroid hormone activating the enzyme type 2 iodothyronine deiodinase (D2) (Watanabe, Houten, Mataki, Christoffolete, Kim, Sato, Messaddeq, Harney, Ezaki, Kodama, Schoonjans, Bianco and Auwerx 2006). In addition bile acids act via the phosphatidylinositol 3 (PI3) kinase/AKT/glycogen synthase (kinase) 3 (GSK3)/glycogen synthase (GS) pathway (Han, Studer, Gupta, Fang, Qiao, Li, Grant, Hylemon and Dent 2004). Through this pathway bile acids cooperate with insulin in the regulation of glucose storage in hepatocytes (Han, Studer, Gupta, Fang, Qiao, Li, Grant, Hylemon and Dent 2004). Bile acids through fibroblast growth

factor 19 (FGF19) may cause increased metabolic rate and decreased adiposity as well as increased energy expenditure (Tomlinson, Fu, John, Hultgren, Huang, Renz, Stephan, Tsai, Powell-Braxton, French and Stewart 2002). All these are beneficial for insulin sensitivity. FGF19 also regulates hepatic protein and glycogen metabolism in an insulin-independent manner (Kir, Beddow, Samuel, Miller, Previs, Suino-Powell, Xu, Shulman, Kliewer and Mangelsdorf DJ 2011).

The effect of metabolic surgery on gut hormones and bile acids

The changes in gut hormones and bile acids after RYGB, the commonest weight loss operation are summarised in Table 1.

Metabolic Surgery and PYY

In both a human model of gastric bypass and a rodent model of jejuno-intestinal bypass increased postprandial PYY responses were shown (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel and Bloom 2006). In an a follow-up study it was confirmed that gastric bypass leads to enhanced postprandial PYY and GLP-1 responses in the first week after surgery (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). This was associated with enhanced postprandial satiety as measured with visual analogue scores (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). In a comparison of good versus poor responders to gastric bypass, the poor responders had attenuated PYY and GLP-1 postprandial responses compared to patients with good weight loss

(le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). These findings demonstrated an association between the enhanced postprandial satiety gut hormone response and appetite control after gastric bypass surgery.

In the same study causation was shown with another experiment (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). Using a randomised double-blind saline controlled design and including patients who had undergone gastric bypass and were weight stable, inhibition of the gut hormone response with the somatostatin analogue octeotride led to increased food intake and reduced satiety (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). Octeotride had no effect on patients who had previously undergone gastric banding and acted as a control group (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). Hence gastric bypass can be considered a surgically induced a behavioural modification (appetite control) which is due to the modulation of the satiety gut hormone response.

Korner et al performed a prospective study of patients undergoing gastric bypass and gastric banding (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). PYY levels were measured preoperatively, 26 and 52 weeks postoperatively using a liquid mixed standard meal (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). Although there

was no difference in fasting PYY levels at any time point, a progressive increase in fasting PYY in the gastric bypass was reported, which reached significance compared to preoperatively at the 52 week time point (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). Postprandial levels of PYY (30 minutes) increased 3.5 times at both postoperative time points after gastric bypass and were significantly higher compared to the gastric banding group (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). When postprandial PYY was assessed with the area under the curve (AUC), this was increased at 26 weeks and increased even further with a significant difference at 52 weeks (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). Postprandial PYY AUC increased 26 weeks after gastric banding but not after 52 weeks (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009). Of note is that the two groups were not matched for BMI preoperatively as the patients in the gastric bypass group were heavier, but both groups had similar BMI at 26 and 52 weeks making the findings of this study an important contribution in the evidence base of PYY increase after gastric bypass surgery (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009).

Laferrère's group performed a similar comparative study in which participants underwent gastric bypass or gastric banding (Bose, Machineni, Oliván, Teixeira, McGinty, Bawa, Koshy, Colarusso and Laferrère 2010). The two groups were matched for preoperative weight and age. They were studied preoperatively, postoperatively following weight loss of 12 kg and at one year postoperatively with a

50 g (in 200 mL) oral glucose test being used (Bose, Machineni, Oliván, Teixeira, McGinty, Bawa, Koshy, Colarusso and Laferrère 2010). Postprandial PYY levels increased at the fist postoperative time point following gastric bypass but not gastric banding (Bose, Machineni, Oliván, Teixeira, McGinty, Bawa, Koshy, Colarusso and Laferrère 2010). No further changes were noted at 12 months (Bose, Machineni, Oliván, Teixeira, McGinty, Bawa, Koshy, Colarusso and Laferrère 2010). This study demonstrated that gastric bypass leads to an increased postprandial PYY response controlling for the effect of surgery and weight loss by using a matched control group and matching for similar weight loss at the first postoperative time point (Bose, Machineni, Oliván, Teixeira, McGinty, Bawa, Koshy, Colarusso and Laferrère 2010).

The same group performed a comparative study of the effect of equivalent weight loss (10kg) after gastric bypass and diet on PYY (Oliván, Teixeira, Bose, Bawa, Chang, Summe, Lee and Laferrère 2009). Again the PYY response to an oral glucose tolerance test was enhanced following bypass but not after diet again controlling for the effect of weight loss (Oliván, Teixeira, Bose, Bawa, Chang, Summe, Lee and Laferrère 2009).

Exploring the effects of other procedures on PYY, Garcia-Fuentes et al showed increased PYY fasting levels 7 months after biliopancreatic diversion compared to preoperatively and there was a significant difference compared to patients undergoing gastric bypass (Garcia-Fuentes, Garrido-Sanchez, Garcia-Almeida, Garcia-Arnes, Gallego-Perales, Rivas-Marin, Morcillo, Cardona and Soriguer 2008).

Sleeve gastrectomy has also been suggested to have an effect on PYY. Karamanakos et al performed a prospective double blind study comparing gastric bypass and sleeve gastrectomy (Karamanakos, Vagenas, Kalfarentzos and Alexandrides 2008). Time points were preoperatively and 1, 3, 6, and 12 months postoperatively (Karamanakos, Vagenas, Kalfarentzos and Alexandrides 2008). Fasting PYY levels increased significantly after sleeve gastrectomy as well as gastric bypass (Karamanakos, Vagenas, Kalfarentzos and Alexandrides 2008). In a subgroup of patients, samples were collected 2 hours after a standard 420 kcal mixed meal and PYY postprandial levels increased significantly after both sleeve gastrectomy and gastric bypass compared to fasting levels (Karamanakos, Vagenas, Kalfarentzos and Alexandrides 2008).

Peterli et al performed a similar randomized, prospective study comparing gastric bypass and sleeve gastrectomy (Peterli, Wölnerhanssen, Peters, Devaux, Kern, Christoffel-Courtin, Drewe, von Flüe and Beglinger 2009). In this study preoperative fasting PYY levels were higher in the bypass group compared to gastric banding with no significant difference (Peterli, Wölnerhanssen, Peters, Devaux, Kern, Christoffel-Courtin, Drewe, von Flüe and Beglinger 2009). Fasting PYY levels decreased postoperatively in both groups (Peterli, Wölnerhanssen, Peters, Devaux, Kern, Christoffel-Courtin, Drewe, von Flüe and Beglinger 2009). However an increased postprandial PYY response was reported one week after both sleeve gastrectomy and gastric bypass (Peterli, Wölnerhanssen, Peters, Devaux, Kern, Christoffel-Courtin, Drewe, von Flüe and Beglinger 2009).

Metabolic Surgery and GLP-1

As expected, in accordance to the PYY response, the postprandial GLP-1 response is also enhanced after gastric bypass. Rubino et al showed no difference in fasting GLP-1 levels in a group of patients, both diabetic and non diabetic undergoing gastric bypass (Rubino, Gagner, Gentileschi, Kini, Fukuyama, Feng and Diamond 2004). Morinigo et al performed two prospective studies of patients undergoing gastric bypass (Morínigo, Moizé, Musri, Lacy, Navarro, Marín, Delgado, Casamitjana and Vidal 2006; Morínigo, Lacy, Casamitjana, Delgado, Gomis and Vidal 2006). Fasting GLP-1 did not increase at the 6 week or the 12 month time point.

De Carvalho et al investigated prospectively eleven normal glucose tolerant and eight abnormal glucose metabolism obese patients undergoing initially diet-restriction followed by gastric bypass with a silastic ring around the gastric pouch, the FOBI procedure (de Carvalho, Marin, de Souza, Pareja, Chaim, de Barros Mazon, da Silva, Geloneze, Muscelli and Alegre 2009). GLP-1 levels at 30 and 60 min after a glucose tolerance test increased nine months postoperatively.

Laferrère et al investigated a group of nine obese, type 2 diabetic women who underwent gastric bypass (Laferrère, Teixeira, McGinty, Tran, Egger, Colarusso, Kovack, Bawa, Koshy, Lee, Yapp and Olivan 2008). They used a group of matched individuals who were on low calorie diet as controls. A 50-gr oral glucose test was performed prior to the intervention and one month afterwards. In addition to GLP-1

levels, the incretin effect was also calculated. Fasting GLP-1 levels remained unchanged in both groups. Both the postprandial GLP-1 and the incretin effect were increased following gastric bypass but not after diet-induced weight loss.

The same group studied 11 participants with type 2 diabetes preoperatively and 1, 6, and 12 months after gastric bypass (Bose, Teixeira, Olivan, Bawa, Arias, Machineni, Pi-Sunyer, Scherer and Laferrère 2010). The blunted incretin effect improved at 1 month and remained unchanged at 6 and 12 months after gastric bypass. The blunted GLP-1 levels followed a similar response after gastric bypass with the improvement at 1 month remaining unchanged at 12 months.

Morinigo et al showed on their study of 9 patients undergoing gastric bypass that a significant increase in the GLP-1 postprandial response using a standard mixed liquid meal (Morínigo, Moizé, Musri, Lacy, Navarro, Marín, Delgado, Casamitjana and Vidal 2006). In a subsequent study from the same group, a 12-month prospective study using the same standard test meal, participants had normal glucose tolerance, impaired glucose tolerance or type 2 diabetes (Morínigo, Lacy, Casamitjana, Delgado, Gomis and Vidal 2006). Postprandial GLP-1 was increased at the 6-week time point for participants with normal or impaired glucose tolerance but not in participants with type 2 diabetes. The postprandial GLP-1 response was similar among the three groups at 12 months.

Lugari et al studied 22 non-diabetic patients undergoing biliopancreatic diversion and 9 age-matched healthy volunteers using a mixed meal preoperatively and postoperatively when patients reached 50% excess weight loss (Lugari, Dei Cas, Ugolotti, Barilli, Camellini, Ganzerla, Luciani, Salerni, Mittenperger, Nodari, Gnudi and Zandomeneghi 2004). An overall increase in circulating GLP-1 levels was reported whilst plasma DPP-IV activity remained abnormally increased postoperatively.

Further work on the effect of biliopancreatic diversion on GLP-1 was performed by Valverde et al (Valverde, Puente, Martín-Duce, Molina, Lozano, Sancho, Malaisse and Villanueva-Peñacarrillo 2005). An oral glucose tolerance test was used in patients at 1, 3 and 6 months after biliopancreatic diversion. As a control group participants were also tested 6 months after vertical banded gastroplasty. One month after biliopancreatic diversion the postprandial GLP-1 response was increased. Both the basal plasma GLP-1 concentration and its incremental area during the oral glucose tolerance test continued to increase. However a more pronounced increase in basal and incremental plasma GLP-1 was observed after biliopancreatic diversion compared to vertical banded gastroplasty.

Mingrone's group studied 10 patients with type 2 diabetes undergoing biliopancreatic diversion (Guidone, Manco, Valera-Mora, Iaconelli, Gniuli, Mari, Nanni, Castagneto, Calvani and Mingrone 2006). Following an oral glucose tolerance test postprandial

GLP-1 response increased one week postoperatively and did not increase further at 4 weeks.

The effect of sleeve gastrectomy on GLP-1 was studied in the randomised control trial of gastric bypass vs. sleeve gastrectomy by Peterli et al (Peterli, Wölnerhanssen, Peters, Devaux, Kern, Christoffel-Courtin, Drewe, von Flüe and Beglinger 2009). The postprandial GLP-1 response was more enhanced one week after gastric bypass compared to sleeve gastrectomy. This exaggerated GLP-1 response remained unchanged 3 months after gastric bypass but increased further after sleeve gastrectomy.

Varderas et al performed a prospective study of non-diabetic undergoing sleeve gastrectomy using a standard liquid meal preoperatively and two months postoperatively (Valderas, Irribarra, Rubio, Boza, Escalona, Liberona, Matamala and Maiz 2011). The incremental area under the curve of GLP-1 increased after sleeve gastrectomy.

A study from the USA showed no change in fasting GLP-1 levels 6 and 12 months after gastric banding (Shak, Roper, Perez-Perez, Tseng, Francois, Gamagaris, Patterson, Weinshel, Fielding, Ren and Blaser 2008).

Usinger et al investigated the GLP-1 response in 10 patients before and 6 weeks after gastric banding also using 75 g-oral glucose tolerance test (Usinger, Hansen, Kristiansen, Larsen, Holst and Knop 2011). This study did not show any change in fasting or postprandial GLP-1.

Finally in Korner's comparative study fasting levels and 30minute postprandial levels of GLP-1 did not change 26 and 52 weeks after gastric banding (Korner, Inabnet, Febres, Conwell, McMahon, Salas, Taveras, Schrope and Bessler 2009).

Metabolic Surgery and Ghrelin

Cummings et al showed in a landmark study, which led to great interest in the interaction of weight loss surgery and gut hormones, a profound suppression of ghrelin levels post gastric bypass (Cummings, Weigle, Frayo, Breen, Ma, Dellinger and Purnell 2002). Data from other centres have been heterogeneous (Pournaras, le Roux 2009). Studies showed decreased fasting and postprandial (Geloneze, Tambascia, Pilla, Geloneze, Repetto and Pareja 2003; Morínigo, Casamitjana, Moizé, Lacy, Delgado, Gomis and Vidal 2004), unchanged fasting and postprandial (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007; Faraj, Havel, Phélis, Blank, Sniderman and Cianflone 2003; Stoeckli, Chanda, Langer and Keller 2004) and increased fasting ghrelin levels after gastric bypass (Vendrell, Broch, Vilarrasa, Molina, Gómez, Gutiérrez, Simón, Soler and Richart 2004).

Lin et al showed that the divided gastroplasty creating a small proximal gastric pouch, as part of gastric bypass, results in an early decline in circulating ghrelin levels that are not observed with other gastric procedures (Lin, Gletsu, Fugate, McClusky, Gu, Zhu, Ramshaw, Papanicolaou, Ziegler and Smith 2004). An intact vagus nerve is required for exogenous ghrelin to increase appetite and food intake (le Roux, Neary, Halsey, Small, Martinez-Isla, Ghatei, Theodorou and Bloom 2005). Perioperative factors may play a role. Sundbom et al showed that ghrelin levels fall transiently on postoperative day 1 after gastric bypass, increased after 1 month to preoperative levels, and rose further at 6 and 12 months (Sundbom, Holdstock, Engström and Karlsson 2007). The authors suggest that this is due to vagal dysfunction (Sundbom, Holdstock, Engström and Karlsson 2007). Differences in the surgical technique may also contribute to the variation in the results as with a vertical pouch, ghrelin producing cells are more likely to be excluded, compared to a horizontal pouch (Pories 2008). In obese individuals, insulin resistance and hyperinsulinaemia are inversely associated with ghrelin concentrations (McLaughlin, Abbasi, Lamendola, Frayo and Cummings 2004). A potential hypothesis is that preoperative differences as well as differences in the postoperative improvement in of insulin and insulin resistance may affect ghrelin levels and hence contribute to the variation in results (Pournaras and le Roux 2009).

Finally, the different assays used for ghrelin may be responsible for the diversity in the results published in the literature (Chandarana, Drew, Emmanuel, Karra, Gelegen, Chan, Cron and Batterham 2009). Serine-3 of ghrelin is acylated with an eight carbon fatty acid, octanoate (Yang, Brown, Liang, Grishin and Goldstein 2008).

This is essential for ghrelin's effects. De Vriese et al demonstrated in a human study that ghrelin is degraded by several esterases (De Vriese, Gregoire, Lema-Kisoka, Waelbroeck, Robberecht and Delporte 2004). This process renders ghrelin inactive (De Vriese, Gregoire, Lema-Kisoka, Waelbroeck, Robberecht and Delporte 2004).

Chandarana et al showed that fasting plasma acyl-ghrelin concentrations were markedly higher in samples processed with 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride in addition to hydrochloric acid and aprotinin compared to samples processed with hydrochloric acid and aprotinin or aprotinin alone (Chandarana, Drew, Emmanuel, Karra, Gelegen, Chan, Cron and Batterham 2009). Furthermore they detected higher acyl-ghrelin levels in hydrochloric acid and aprotinin samples compared to aprotinin only samples. These subtle differences in the processing of the samples may explain the different results amongst different centres.

Despite the fact that ghrelin has led to considerable interest in the gut brain axis, the inconsistent response among studies and centres have made a challenging candidate for elucidating the mechanism of metabolic surgery.

Metabolic Surgery and CCK

Rubino et al showed no change in fasting CCK three weeks after gastric bypass (Rubino, Gagner, Gentileschi, Kini, Fukuyama, Feng and Diamond 2004). Kellum et

al studied patients before and six months after gastric bypass and vertical banded gastroplasty showing no change in the CCK response to a glucose meal (Kellum, Kuemmerle, O'Dorisio, Rayford, Martin, Engle, Wolf and Sugerman 1990).

Foschi et al studied eight patients before and after weight loss of 20% of the initial BMI following vertical banded gastroplasty (Foschi, Corsi, Pisoni, Vago, Bevilacqua, Asti, Righi and Trabucchi 2004). A control group of healthy volunteers was used. There was no difference in basal CCK levels between the two groups. Following an acidified liquid meal peak CCK levels were increased after vertical banded gastroplasty.

Naslund et al showed increased basal CCK levels 20 years after jejuno-ileal bypass (Naslund, Gryback, Hellstrom, Jacobsson, Holst, Theodorsson and Backman 1997).

The same group studied eight patients before and 9 months after jejuno-ileal bypass using a standard mixed meal (Naslund, Melin, Gryback, Hagg, Hellstrom, Jacobsson, Theodorsson, Rössner and Backman 1997). Postprandial CCK levels were lower than non-obese controls both pre and postoperatively.

Metabolic Surgery and GIP

Rubino et al studied the GIP response in diabetic and non-diabetic patients before and three weeks after gastric bypass (Rubino, Gagner, Gentileschi, Kini, Fukuyama, Feng and Diamond 2004). Gastric bypass led to reduced fasting GIP levels in diabetic patients whereas no changes in GIP levels were found in non-diabetics. Bose et al also showed no difference in fasting GIP levels after gastric bypass (Bose, Teixeira, Olivan, Bawa, Arias, Machineni, Pi-Sunyer, Scherer and Laferrère 2010). Work from Phil Schauer's unit in accordance with the findings of the previous studies showed no change in fasting GIP one and four weeks following gastric bypass (Kashyap, Daud, Kelly, Gastaldelli, Win, Brethauer, Kirwan and Schauer 2010). Breitman et al showed no change in fasting GIP two and eight weeks after gastric bypass (Breitman, Saraf, Kakade, Yellumahanthi, White, Hackett and Clements 2011).

In the work by Blandine Laferrere GIP levels in response to an oral glucose tolerance test increased at one month and remained at this levels 6 and 12 months after gastric bypass (Laferrère, Heshka, Wang, Khan, McGinty, Teixeira, Hart and Olivan 2007; Bose, Teixeira, Olivan, Bawa, Arias, Machineni, Pi-Sunyer, Scherer and Laferrère 2010).

Guidone et al studied 10 patients with type 2 diabetes before and one and four weeks after biliopancreatic diversion using an oral glucose tolerance test (Guidone, Manco, Valera-Mora, Iaconelli, Gniuli, Mari, Nanni, Castagneto, Calvani and

Mingrone 2006). Fasting GIP levels as well as the postprandial GIP response decreased one week postoperatively and remained unchanged at four weeks.

Fasting GIP levels do not change six weeks after gastric banding according to study by Usinger et al (Usinger, Hansen, Kristiansen, Larsen, Holst and Knop 2011). Shak et al showed no change in fasting GIP 6 and 12 months after gastric banding (Shak, Roper, Perez-Perez, Tseng, Francois, Gamagaris, Patterson, Weinshel, Fielding, Ren and Blaser 2008).

Metabolic Surgery and Enteroglucagon and OXM

Kellum et al showed that gastric bypass was associated with an exaggerated enteroglucagon response to glucose (Kellum, Kuemmerle, O'Dorisio, Rayford, Martin, Engle, Wolf and Sugerman 1990). Furthermore enteroglucagon appears to be a marker of the dumping syndrome after gastric bypass. Laferrère et al demonstrated a 2-fold peak of OXM levels rose in response to oral glucose and the peak of OXM was significantly correlated with GLP-1 and PYY (Laferrère, Swerdlow, Bawa, Arias, Bose, Oliván, Teixeira, McGinty and Rother 2010).

Metabolic Surgery and PP

No changes in PP were detected after gastric bypass in one study (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel and Bloom 2006). Sundbom

et al showed that PP concentrations decreased on day 1 after gastric bypass and subsequently returned to preoperative levels (Sundbom, Holdstock, Engström and Karlsson 2007). A prospective study of patients undergoing gastric banding showed that a low PP meal response preoperatively was associated with greater weight loss postoperatively (Dixon, le Roux, Ghatei, Bloom, McGee and Dixon 2011). The findings allowed the authors to hypothesise that low PP postprandial response may be a predictor of greater weight loss after gastric banding.

Metabolic Surgery and bile acids

Patti et al has shown in a cross-sectional study of three groups; patients after gastric bypass, individuals matched to preoperative BMI and individuals matched to current BMI (Patti, Houten, Bianco, Bernier, Larsen, Holst, Badman, Maratos-Flier, Mun, Pihlajamaki, Auwerx and Goldfine 2009). Total serum bile acid concentrations were higher after gastric bypass and were inversely correlated with 2-h post-meal glucose and fasting triglycerides as well as positively correlated with adiponectin and peak GLP-1. Total bile acids strongly correlated inversely with thyrotrophic hormone.

The increase in plasma bile acids was reflected in both primary and secondary bile acids. The authors found no change in FGF19, a marker of bile acid absorption from the ileum and regulator of bile acids synthesis. The main limitation of this study was the cross-sectional design. Although control groups matched for preoperative and postoperative weight loss, the effect of rapid weight loss itself cannot be excluded.

Hence prospective studies need to be conducted to establish the role of bile acids in the effect of metabolic surgery.

Table 3: Summary of gut hormone changes after RYGB (Pournaras and le Roux 2010).

Gut hormone	Basal level	Postprandial
GLP-1	Unchanged	Increased
PYY	Unchanged	Increased
Ghrelin	Inconclusive	Decreased
CCK	Unchanged	Unchanged
GIP	Decreased	Unknown
PP	Unchanged	Unchanged
Bile acids	Increased	Unknown

Hypothesis

This thesis deals with the following hypotheses. Firstly I hypothesise that gastric bypass leads to improved glycaemic control in a weight loss independent manner. Secondly, that one of the mechanisms for this observation involves gut hormones and particularly the incretin GLP-1. Finally, I hypothesise that another mechanism is the change in bile flow which contributes to improved glucose metabolism.

2. Glycaemic control after metabolic surgery

2.1 Introduction

The purpose of this chapter is to provide the evidence that the effect of gastric bypass on glycaemic control is not only related to weight loss. During the period of the research undertaken, the concept of diabetes remission was introduced and subsequently new criteria were proposed. Initially the discontinuation of medication with acceptable measurements of fasting glucose was reported (Pories, Swanson, MacDonald, Long, Morris, Brown, Barakat, deRamon, Israel and Dolezal 1995). Anecdotally this could lead to patients stopping their medication as they considered themselves "cured". There were also conflicts within the multidisciplinary team with diabetologists being sceptical regarding the effects of metabolic surgery and metabolic surgeons being over enthusiastic regarding the effects of surgery on diabetes. The lack of a strict definition did not help this. The common example of patients remaining postoperatively on Metformin, an effective and proven cost-effective treatment, and hence not considered on remission was associated with disappointment of patients and surgeons. More important was the issue of follow-up from a diabetes point of view. A patient who is regularly told that they are cured of their diabetes are likely to forego life-saving follow up such as regular BP recordings, eye tests, renal function test.

A consensus group comprising experts in endocrinology, diabetes education, transplantation, metabolism, metabolic surgery and haematology–oncology proposed new definitions of partial and complete remission of type 2 diabetes (Buse, Caprio, Cefalu, Ceriello, Del Prato, Inzucchi, McLaughlin, Phillips, Robertson, Rubino, Kahn and Kirkman 2009). The input of haematologists and oncologists was deemed valuable as they do deal with the concept of remission of cancer in their routine practice. The implication of these recommendations was that a standard metric was proposed for reporting rates of diabetes remission allowing comparison between different procedures from different units in future. Secondly, the more strict criteria for glycaemic control remission may affect treatment options suggesting that patients may benefit from hypoglycaemic treatment after metabolic surgery. Finally opening the debate of optimal glycaemic control after metabolic surgery can now be facilitated.

The remission rate after metabolic surgery using the new criteria was established for the first time in the second study described in this chapter.

2.2 Methods

Study 1: Glycaemic control after gastric bypass and gastric banding

This study was performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC Protocol

Number: 05/Q2202/96). Exclusion criteria were pregnancy, substance abuse, more than two alcoholic drinks per day. Written informed consent was obtained from all participants.

Selection criteria for study 1 included patients with type 2 diabetes undergoing either gastric bypass or gastric banding operations. This was not a randomised study. Data were collected prospectively on 34 consecutive patients with type 2 diabetes. All operations were performed by the same surgeon in one centre within a period of three years. It was patient's choice which determined the type of operation performed. Participants were informed about the different operations by the physicians, the surgeons and the dieticians as part of the preoperative consultation. They were also encouraged to attend patient support groups organised by the British Obesity Surgery Patients Association.

Fasting glucose, HbA1c, and dosage of anti-diabetic medication were recorded pre-operatively and during follow-up at 3, 6, 12, 18, 24 and 36 months. Remission of type 2 diabetes was previously defined as being off diabetes medications with normal fasting blood glucose (5.6 mmol/l) or HbA1c of less than 6% (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004; Buchwald, Estok, Fahrbach, Banel, Jensen, Pories, Bantle and Sledge 2009). This definition was used in meta-analyses estimating the effect of weight loss surgery on type 2 diabetes (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004; Buchwald, Estok, Fahrbach, Banel, Jensen, Pories, Bantle and Sledge 2009).

In this study (study 1) remission of type 2 diabetes was defined using the WHO definition of type 2 diabetes. Therefore remission was achieved when all the below criteria were met:

1. Fasting plasma glucose below 7 mmol/L in the absence of medical treatment for at least 3 days.

2. A two hour plasma glucose below 11.1 mmol/L following an oral glucose tolerance test as specified by the World Health Organisation (Report of a WHO Consultation, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1 Diagnosis and classification of diabetes mellitus. World Health Organization, Department of Noncommunicable Disease Surveillance, Geneva, 1999).

3. HbA1c below 6% after 3 months of last hypoglycaemic agent usage. This criterion was added as it was often used in the surgical literature at the time of designing the experiment (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004; Buchwald, Estok, Fahrbach, Banel, Jensen, Pories, Bantle and Sledge 2009; O'Brien, Dixon, Laurie, Skinner, Proietto, McNeil, Strauss, Marks, Schachter, Chapman and Anderson 2006; Schauer, Burguera, Ikramuddin, Cottam, Gourash, Hamad, Eid, Mattar, Ramanathan, Barinas-Mitchel, Rao, Kuller and Kelley 2003).

The technique used for the bypass was a combination of linear stapler and hand sewn enterotomy closure for both the gastrojejunostomy and jejuno-jejunostomy, in an antegastric-retrocolic configuration (Higa, Boone, Ho and Davies 2000). This was

a modification of the technique first described by Higa et al (Higa, Boone, Ho and Davies 2000).

A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Reverse Trendelenburg position was used with the operating on the right of the patient and assistant positioned between the legs of the patient. Pneumoperitoneum was obtained with a Verres needle inserted below the left costal margin at the mid-clavicular line. Thromboprophylaxis included the routine use of TED stockings and 40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively and lower limb pneumatic compression devices intraoperatively. Five ports were used. An isolated lesser curve-based, 15–20-ml gastric pouch was created and a retrocolic antegastric Roux limb was made 100 cm long for patients with a BMI equal to or less than 50 kg/m2 and 150 cm long for patients with BMI of more than 50 kg/m2. The bilio-pancreatic limb was 25 cm. The gastrojejunostomy was closed over a 34 Fr bougie. A blue dye leak test via a nasogastric tube was routinely performed. All hernia defects (jejunojejunostomy, Petersen's and mesocolon) were routinely closed with a purse-string suture.

An enhanced recovery protocol was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. The first routine postoperative review in the outpatient department was 6 weeks after discharge with a minimum regular follow-up program for uncomplicated patients afterwards of 6 months, 12 months postoperatively, and yearly thereafter, and more intensive follow-up for complex patients.

The surgical technique used for gastric banding was the pars flaccida described by the Melbourne group (O'Brien, Dixon, Laurie and Anderson 2005). A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure as with the gastric bypass. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010). Thromboprophylaxis included the routine use of TED stockings and 40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively. Lower limb pneumatic compression devices intraoperatively. Five ports were used. The Swedish Adjustable Gastric band® (Ethicon Endo-Surgery) and the LAP-BAND® (Allergan) bands were used for all gastric banding procedures. The pars flaccida dissection technique with gastro-gastric tunnelling sutures was the operative method utilised (O'Brien, Dixon, Laurie and Anderson 2005). The same enhanced recovery protocol as the one sued for gastric bypass was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. Follow-up was also similar with the first postoperative review in

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the outpatient department 6 weeks after discharge. Patients were then seen monthly and adjustments were performed until optimal reduction in hunger or restriction was achieved.   The adjustments were performed using the indicators described by Favretti et al (Favretti, O'Brien and Dixon 2002) as seen in table 4. Patients were seen at least six times in the first year and then once a year minimum (Pournaras, Osborne, Hawkins, Vincent, Mahon, Ewings, Ghatei, Bloom, Welbourn and le Roux 2010).

Table 4. Protocol used for band adjustments from Favretti, O'Brien, Dixon 2002).

Consider adding fluid	Adjustment not required	Consider removing fluid
Inadequate weight loss	Adequate rate of weight loss	Vomiting, heartburn, reflux into the mouth
Rapid loss of satiety after meals	Eating reasonable range of food	Coughing spells, wheezing and choking, especially at night
Increased volume of meals	No negative symptoms	Difficulty coping with broad range of foods
Hunger between meals		Maladaptive eating behaviour

Latest follow-up (24-36 months) data were collected. No patient was lost to follow up.

Study 2: Diabetes remission with the new criteria.

For this study data collection was prospective in three different centres. These were Imperial Weight Centre, Imperial College, London, UK; Musgrove Park Hospital, Taunton, UK; Department of Gastrointestinal Surgery, Oslo University Hospital, Aker, Oslo, Norway. Permission was obtained from the Imperial College Healthcare NHS Trust Clinical Governance & Patient Safety Committee (Ref:09/808).

All participants were operated between August 2004 and July 2009. Inclusion criteria were preoperative diagnosis of type 2 diabetes and weight loss surgery. Patients were reviewed 6 weeks, 6 months and 12 months postoperatively. Median follow-up was 23 months (range 12-75 months).

According to the 2009 consensus of the American Diabetes Association partial remission of diabetes was defined as hyperglycaemia (HbA1c<6.5% and fasting glucose 5.6–6.9 mmol/l) at least 1 year after surgery in the absence of active hypoglycaemic pharmacologic therapy or ongoing procedures. Complete remission was defined as a return to normal measures of glucose metabolism (HbA1c < 6%, fasting glucose < 5.6 mmol/l) at least 1 year after surgery without hypoglycaemic pharmacologic therapy or ongoing procedures (Buse, Caprio, Cefalu, Ceriello, Del Prato, Inzucchi, McLaughlin, Phillips, Robertson, Rubino, Kahn and Kirkman 2009).

Statistical analysis

Data were analyzed using SPSS version 14 (SPSS Inc., Chicago, IL). Results were expressed as number (%), mean ± SD or median (range). Time to glucose below 7 mmol/L (below the World Health Organization definition of diabetes which includes fasting plasma glucose ≥ 7.0 mmol/l) off all medication was compared between operative groups by log-rank test (study 1). The Mann-Whitney test was used for non-parametric demographic data and the unpaired t-test was used for parametric demographic data in study 1. Fisher's exact test and the Freeman-Halton extension of the Fisher's exact test were used to compare categorical data and one-way ANOVA was used to compare continuous data for study 2. A p value of ≤ 0.05 was considered significant.

2.3 Results

Study 1: Glycaemic control after gastric bypass and gastric banding

The number of diabetic and non diabetic patients undergoing gastric bypass (n = 109) and gastric banding (n = 107) during the study period (between January 2004 and January 2007) was similar suggesting that the clinicians involved in the patients care and particularly the surgeon did not have a preference for one procedure versus

the other, hence reducing although not eliminating the risk of bias. Thirty four consecutive patients with type 2 diabetes on hypoglycaemic treatment presenting for weight loss surgery were identified (16%). Twenty two of these chose to undergo Roux-en-Y gastric bypass and 12 laparoscopic adjustable gastric banding. The two groups were well matched for demographic characteristics, duration of diabetes or pre- and post-operative BMI (Table 5).

Table 5. **Study 1: Patient characteristics presented as Mean (standard deviation) except where the median (range) is indicated. *p<0.05 (t-test, Mann-Whitney, Fisher's exact test).**

|	Bypass	Banding	*p value
Age (years)	46.0 ± 9.6	47.4 ± 10.9	0.71
Pre-op Weight (kg)	137.4 ± 22.9	137.7 ± 31.8	0.97
Pre-op BMI	47.4 ± 7.2	47.1 ± 7.1	0.90
Pre-op HbA1c	9.1 ± 1.9	8.4 ± 1.7	0.29
Duration of diabetes (years)	7.0 (1-18)	5.5 (1-14)	0.61
Proportion on insulin pre-op (%)	12/22 (54%)	4/12 (33%)	0.04
Follow-up (months)	29.5 ± 8.0	33.0 ± 7.5	0.21
% Total Weight Loss	29.5 ± 8.4	28.5 ± 10.0	0.76
% Excess BMI Loss	63.3 (30.8-103.2)	62.3(45.1-105.9)	0.92
BMI at follow-up	33.0 ± 5.2	32.6 ± 5.1	0.80
HbA1c at follow-up	6.2 ± 1.2	6.5 ± 1.2	0.59

Although patients lost weight postoperatively they remained in the obese state with a mean BMI of 33 ± 5.2 kg/m^2 after gastric bypass and 32.6 ± 5.1 kg/m^2 after gastric banding. There was no statistically significant difference between the two groups in weight at 3, 6, 12, 18, 24 and 36 months (Figure 1).

HbA1c improved significantly after both procedures ($p < 0.001$ compared to preoperatively for both groups). Fasting plasma glucose levels were 6.6 ± 2.7 mmol/L and 7.3 ± 2.4 mmol/L for the gastric bypass and gastric banding groups respectively ($p = 0.43$) at latest follow-up. The time to a fasting plasma glucose below 7 mmol/L off all medication was significantly shorter for gastric bypass than gastric banding, with a hazard ratio of 8.2, ($p = 0.001$, 95% confidence interval 1.8 to 36.7).

Fifteen out of 22 patients undergoing gastric bypass patients (68%) were in remission as defined for study 1 at 12 months postoperatively (Figure 2). In contrast no gastric banding patients achieved remission and this was a statistically significant difference at the level of $p<0.001$. Weight loss at the same time point was similar ($p=0.14$) between the groups with 25.2 ± 8.2 % for gastric bypass and 20.4 ± 9.1 % for gastric banding. Sixteen out of 22 patients undergoing gastric bypass (72%) were in remission compared to two gastric band patients (17%) at maximum follow up and again this was significant ($p = 0.01$). However weight loss remained similar ($p = 0.76$) between the groups with 29.5 ± 8.4 % for gastric bypass and 28.5 ± 10 % for gastric banding. There was no correlation between weight loss and HbA1c
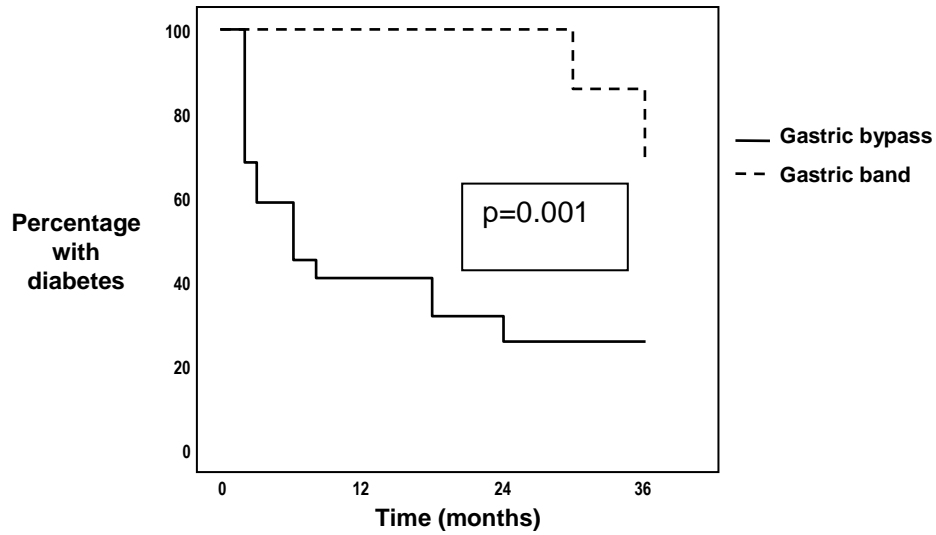
improvement after gastric bypass with Pearson r of 0.3196, 95% confidence interval

-0.1180   to   0.6532   and   p   of   0.1471   (Figure   3).

Figure 1. Weight loss over time for gastric bypass (n = 22) and gastric banding (n = 12) patients over a 3 year period.
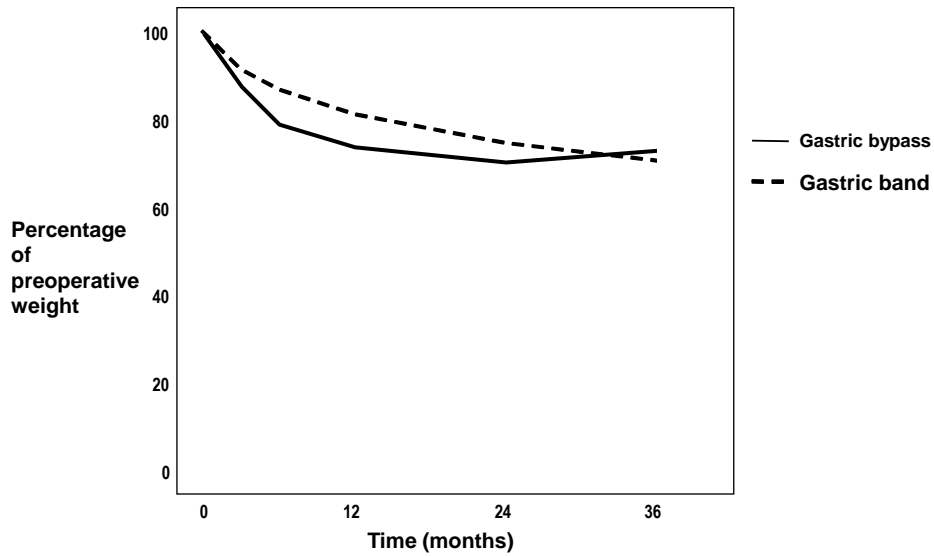

Months	0	3	6	12	24	36
Band n=22	12	12	12	12	12	8
Bypass n=12	22	22	22	22	22	11

Figure 2. Kaplan-Meier curve for time to remission of type 2 diabetes in patients who had gastric bypass (solid line) or gastric banding (broken line) over a 3 year period.

Months	0	3	6	12	24	36
Band n=22	12	12	12	12	12	8
Bypass n=12	22	22	22	22	22	11

Figure 3. Correlation between % weight loss and HbA1c improvement after gastric bypass. Pearson r=0.3196, 95% confidence interval -0.1180 to 0.6532, p=0.1471.
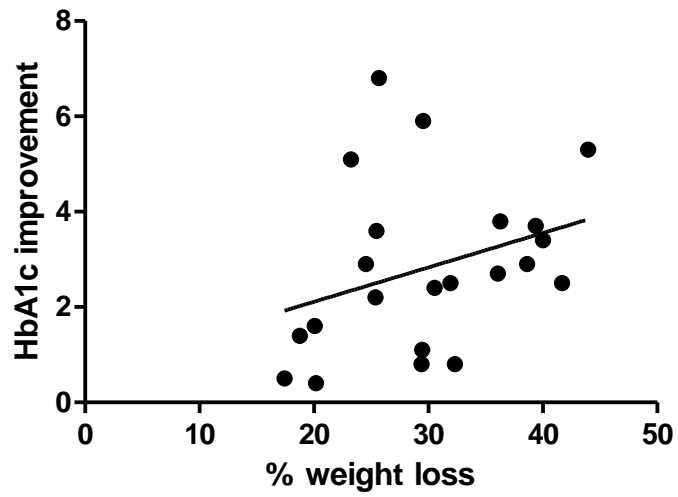
The severity of diabetes as demonstrated by the number of patients on insulin treatment preoperatively was worse in the bypass group (12 of 22 gastric, 54% compared to the gastric banding group (4 of the 12, 33%) (p = 0.04).

In terms of length of stay there was no difference in median length of stay for gastric bypass between patients with diabetes (n = 22, 4 days range 1-114) or without diabetes (n = 87, 3 days range 1- 44) (p=0.47). For gastric banding there was no difference in median length of stay between patients with diabetes (n = 12, 1 day range 1-2) or without diabetes (n = 95, 1 day range 1-3) (p=0.68). Diabetic and non diabetic patients after bypass stayed longer in hospital than patients after banding (p <0.001).

There was one early complication in a diabetic patient who was re-operated on day 1 post gastric bypass for a small bowel enterotomy. The patient made a full recovery and was discharged following a 114-day stay (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010). The non diabetic patient that required hospital admission for 44 days had an anastomotic leak and also made a full recovery (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Study 2: Diabetes remission with the new American Diabetes Association criteria.

The demographic characteristics of the 209 patients included in this study can be seen on Table 6. The prevalence of type 2 diabetes before surgery was 36 out of 136 patients (26%) at Oslo University Hospital Aker, 93 out of 551 (17%) at Musgrove Park Hospital and 80 out of 319 (25%) at Imperial College Healthcare NHS Trust.

Table 6. Patient characteristics and type 2 diabetes remission rates 23 months (range 12-75) postoperatively.

	Total n=209	Gastric bypass n=160	Sleeve gastrectomy n=19	Gastric banding n=30	p values
Age (years)	48 ± 10	47 ± 9	53 ± 14	46 ± 10	0.041
Women, N (%)	137 (66%)	105 (66%)	11 (58%)	21 (70%)	0.695
Insulin usage before surgery, N (%)	63 (30%)	51 (32%)	6 (32%)	6 (19%)	0.457
BMI (kg/m^2) before surgery	48 ± 7	48 ± 7	50 ± 8	47 ± 9	0.390
after surgery	35 ± 7	34 ± 6	42 ± 6	36 ± 8	0.002
Fasting glucose (mmol/L) before surgery	9.6 ± 3.6	9.8 ± 3.6	8.9 ± 4.2	7.4 ± 0.7	0.144
after surgery	6.3 ± 2.6	6.0 ± 2.1	8.0 ± 5.3	6.5 ± 2.1	0.004
HbA1C (%) before surgery	8.0 ± 1.9	8.1 ± 1.9	7.5 ± 1.5	7.7 ± 1.5	0.388
after surgery	6.2 ± 1.2	6.2 ± 1.2	6.8 ± 1.7	6.3 ± 0.7	0.081
Complete remission with 2009 criteria, N (%)	72 (34%)	65 (41%)	5 (26%)	2 (7%)	0.0004
Partial remission with 2009 criteria, N (%)	28 (13%)	25 (16%)	1 (5%)	2 (7%)	0.301
Remission with previous definition, N (%)	103 (49%)	92 (58%)	6 (32%)	5 (17%)	<0.0001

The remission rate of type 2 diabetes following after weight loss surgery was significantly lower with the new criteria (72 of 209 patients, 34%) than with the previous definition (103 of 209, 49%) (p=0.003). As seen on figure 4, the newly defined remission rate was 41% after gastric bypass (65 of 160 patients), 26% for sleeve gastrectomy (five of 19 patients) and 7% for gastric banding (two of 30 patients). The remission rate after gastric bypass was significantly lower compared to the previous definition used (p=0.003).

There was a significant improvement in HbA1c levels for all groups postoperatively (p<0.001) and HbA1c levels were 6.2 ± 1.2% after gastric bypass, 6.8 ± 1.7% after sleeve gastrectomy and 6.3 ± 0.7% after gastric banding (p=0.081 between groups).

Figure 4. Diabetes remission for gastric bypass, sleeve gastrectomy and gastric banding with the new American Diabetes Association definition and the previous definition. * p<0.001 between groups # p<0.01 compared to previous definition
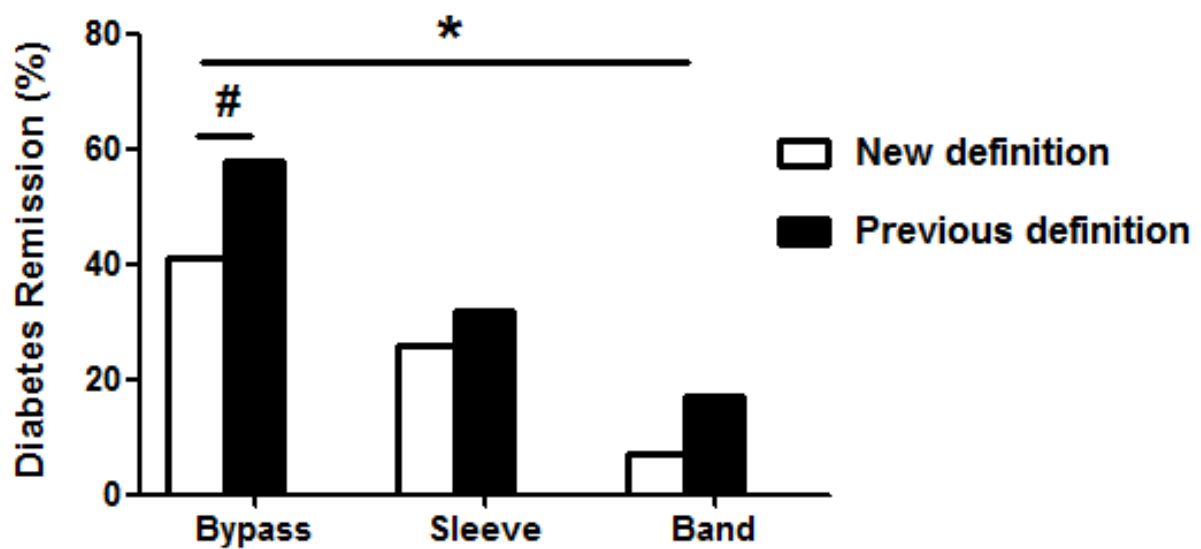
2.4 Discussion

In study 1 we performed a comparison in glycaemic control and diabetes remission in patients undergoing gastric bypass and gastric banding in a single centre. Although this was not a randomised trial, both groups were matched for age, sex and BMI before and after surgery. At latest follow up significantly more patients had fasting plasma glucose concentration below 7 mmol/L without hypoglycaemic treatment after gastric bypass than after gastric banding (72% vs 17%). Furthermore the diabetes remission rate over time was more rapid after gastric bypass. The findings of this study suggest that the improved glycaemic control may be independent of weight loss.

Different studies comparing glycaemic control after gastric bypass and gastric banding also suggested that glycaemic and diabetes related outcomes were more favourable after gastric bypass (Bowne, Julliard, Castro, Shah, Morgenthal and Ferzli 2006; Parikh, Ayoung-Chee, Romanos, Lewis, Pachter, Fielding G and Ren 2007). The remission rate for the gastric bypass group is comparable to other studies; however the remission rate for the gastric banding group was lower than previously published O'Brien, Dixon, Laurie, Skinner, Proietto, McNeil, Strauss, Marks, Schachter, Chapman and Anderson 2006; Schauer, Burguera, Ikramuddin, Cottam, Gourash, Hamad, Eid, Mattar, Ramanathan, Barinas-Mitchel, Rao, Kuller and Kelley 2003; Bowne, Julliard, Castro, Shah, Morgenthal and Ferzli 2006; Parikh, Ayoung-Chee, Romanos, Lewis, Pachter, Fielding and Ren 2007; Kim, Daud, Ude, DiGiorgi, Olivero-Rivera, Schrope, Davis, Inabnet and Bessler 2006; Weber, Mueller, Bucher, Wildi, Dindo, Horber, Hauser and Clavien 2004; Dixon and O'Brien 2002).

This can be explained by the stricter definition used for the remission of type 2 diabetes in comparison to other studies (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007; Parikh, Ayoung-Chee, Romanos, Lewis, Pachter, Fielding G and Ren 2007; Kim, Daud, Ude, DiGiorgi, Olivero-Rivera, Schrope, Davis, Inabnet and Bessler 2006; Weber, Mueller, Bucher, Wildi, Dindo, Horber, Hauser and Clavien 2004; Dixon and O'Brien 2002; Levy, Fried and Santini 2007). Remission for this study was defined as fasting plasma glucose below 7 mmol/L and 2 hour post oral glucose tolerance test plasma glucose below 11.1 mmol/L with a HbA1c below 6% being off all hypoglycaemic treatment. Also all participants required antihyperglycaemic agents and a significant proportion of them needed insulin reflecting severe disease with reduced beta cell reserve. The duration of the disease prior to surgery was prolonged in our population with a median of 7 years in the bypass group and 5.5 years in the banding group. All these factors are associated with inferior outcomes after weight loss surgery (Schauer, Burguera, Ikramuddin, Cottam, Gourash, Hamad, Eid, Mattar, Ramanathan, Barinas-Mitchel, Rao, Kuller and Kelley 2003).

Schauer et al reported inferior weight loss after gastric bypass in diabetic patients compared to non diabetic patients, although the reason for this has not been elucidated (Schauer, Burguera, Ikramuddin, Cottam, Gourash, Hamad, Eid, Mattar, Ramanathan, Barinas-Mitchel, Rao, Kuller and Kelley 2003). This may explain the similar weight loss between the two groups in this study. It is recognised that frequent follow-up for band adjustments is associated with better weight loss and this may also be the cause of the similar weight loss between the groups (Shen, Dugay,

Rajaram, Cabrera, Siegel and Ren 2004). Finally Kim et al reported similar weight loss between banding and bypass although their population was not diabetic (Kim, Daud, Ude, DiGiorgi, Olivero-Rivera, Schrope, Davis, Inabnet and Bessler 2006).

Limitations of study 1 are the lack of randomisation, and the relatively small size of the groups. However the groups were well matched.

Study 2 is the first report evaluating the effect of the 2009 criteria on diabetes remission rates after weight loss surgery. The complete diabetes remission rate was significantly lower than the previously defined remission rate on the same population. Glycaemic control as measure with HbA1c was similar between different procedures although there was a significant difference in the usage of hypoglycaemic medication.

The concept of remission changed throughout the period of this research project. Although for study 1 we used a very strict definition, the 2009 criteria are robust and are rapidly becoming accepted as the gold standard (Buse, Caprio, Cefalu, Ceriello, Del Prato, Inzucchi, McLaughlin, Phillips, Robertson, Rubino, Kahn and Kirkman 2009). The impressive outcomes of weight loss surgery, the reported remission of type 2 diabetes and the introduction of "metabolic surgery" have led to increased interest in the field and study 1 has contributed to the evidence base supporting the weight loss independent effect of gastric bypass on glucose metabolism. It is study 2, however, that can lead to a more rational approach in the field of metabolic

surgery with realistic expectations from surgeons, diabetologists and patients. Furthermore the findings of study 2 may lead to a focus on achieving good glycaemic control rather than remission from type 2 diabetes. And more importantly a shift in the metabolic surgical literature away from glycaemic control and aiming towards the reduction of microvascular and macrovascular complications associated with diabetes (UKPDS 33 1998; Ray, Seshasai, Wijesuriya, Sivakumaran, Nethercott, Preiss, Erqou and Sattar 2009).

Remission may be low in this population due to the reduced beta cell reserve. Patients treated in the NHS tend to have a higher comorbidity burden, with long standing type 2 diabetes, often on insulin. Data regarding these characteristics were not available for study 2, but from study 1 the above statement can be supported. Patients on the early stages of type 2 diabetes are more likely to achieve remission Schauer, Burguera, Ikramuddin, Cottam, Gourash, Hamad, Eid, Mattar, Ramanathan, Barinas-Mitchel, Rao, Kuller and Kelley 2003). On the other hand, patients with long standing, difficult to control type 2 diabetes may benefit more from surgery as other treatment options are limited and less likely to succeed. Operating on this population may be associated with a higher perioperative risk. Identifying the patients who are likely to benefit more from metabolic surgery, calculate the risk and optimise outcomes remain burning issues in the field.

Limitations of the study include the relatively low numbers of type 2 diabetes patients in the gastric banding and sleeve gastrectomy groups and the lack of randomisation.

Also there was no data available on the duration of diabetes. The 20.8% prevalence of patients with diabetes is similar to the one reported in other studies (Buchwald, Avidor, Braunwald, Jensen, Pories, Fahrbach and Schoelles 2004; Buchwald, Estok, Fahrbach, Banel, Jensen, Pories, Bantle and Sledge 2009). The proportion of patients with insulin treated type 2 diabetes was similar to the one reported in the UK & Ireland National Bariatric Surgery Registry (Welbourn, Fiennes, Kinsman and Walton 2011).

In conclusion, gastric bypass leads to the remission of type 2 diabetes in a weight loss independent manner. The 2009 criteria are associated with a lower remission rates after metabolic surgery than previously thought. However metabolic surgery leads to improved glycaemic control.

3. The mechanism of action of metabolic surgery is partly due to gut hormone modulation.

3.1 Introduction

The question raised from chapter 2 is that if it is not the weight loss which leads to the remission of type 2 diabetes then what the mechanism is. In a group of patients for whom access to the gastric remnant was available, I was able to test two different routes of a meal test controlling for weight loss. I hypothesised that the first postoperative days, before any substantial weight loss has occurred, is the period when most of these changes occur. Therefore I investigated the postprandial changes in glucose, insulin and gut hormones in the first weeks after gastric bypass. Finally establishing the long term effect of gastric bypass was possible in the third study of this chapter.

3.2 Methods

Study 1: The effect of the altered nutrient route in glucose metabolism and the gut hormone response

This study was approved by the Clinical Governance and Patient Safety committee of Imperial College Healthcare NHS Trust (reference number: 10/807) and was performed according to the declaration of Helsinki. Informed consent was obtained

from five patients who had undergone gastric bypass in the Imperial Weight Centre. All patients were type 2 diabetic prior to surgery and were in remission, achieving normoglycaemia with no hypoglycaemic medication at the time of participation in the study. The weight loss achieved was 29.9 ± 4.6 % and the mean BMI was reduced from 43.2 ± 1.9 preoperatively to 29.9 ± 2.4 kg/m2 (p=0.001).

Secondary to surgical complications participants in this study were unable to feed using the enteral route and hence all had a functioning gastrostomy tube providing access to the gastric remnant, duodenum and proximal jejunum. The causes of the surgical complications are listed in table 7. All participants had fully recovered (tolerating an oral diet and sustaining a stable weight) by the time they entered the study (14 ± 4 months postoperatively, range 9-24 months) following conservative or surgical treatment.

Table 7: List of complications leading to inability to feed with the enteral route.

Patient 1	Münchausen syndrome
Patient 2	Twist at the gastro-eosophageal junction causing severe dysphagia.
Patient 3	Twist at the gastro-eosophageal junction causing severe dysphagia.
Patient 4	Twist at the gastro-eosophageal junction causing severe dysphagia.
Patient 5	Twist at the gastro-eosophageal junction causing severe dysphagia.

Participants were examined at 08.00 following an overnight fast on two different occasions with a 3 to 6 day-interval. A 410ml solution containing 75 g of glucose, 287 kcal (Lucozade Energy Original, GlaxoSmithKline) was given orally on day one (to ensure that participants could tolerate the volume orally), and via gastrostomy on the second occasion. Figure 5 is a schematic illustration of the gastrointestinal glucose route after oral and gastrostomy loading. A venous catheter was placed in a large vein and blood samples were collected prior to and 15, 30, 60, 90, 120, 150 and 180 minutes following the glucose test. All samples were collected in tubes containing EDTA and aprotinin and were immediately centrifuged and stored in a -80°C freezer until further analysis.

Figure 5: Glucose route after oral (black arrows) and after gastrostomy load (empty arrows).
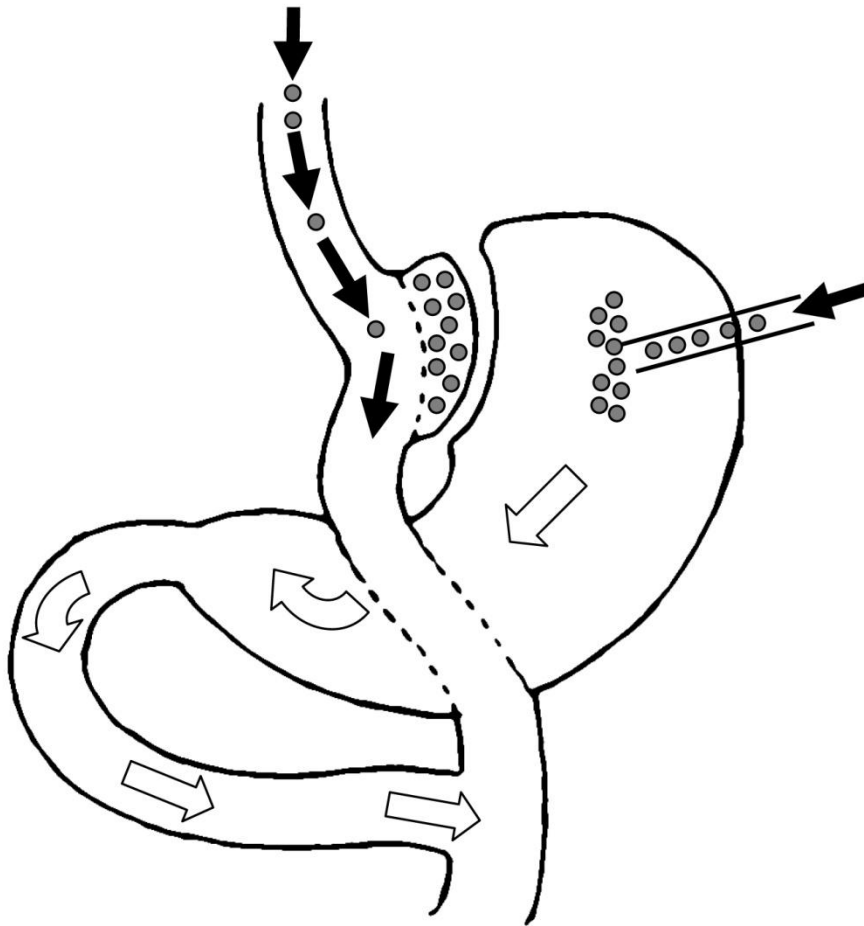
Glucose levels were measured with an automated glucose analyser (Abbott laboratories, Chicago USA) and insulin levels with an automated chemiluminescent immunoassay (Abbott laboratories, Chicago, USA).

For the measurement of gut hormones all samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay (Savage, Adrian, Carolan, Chatterjee and Bloom 1987). The assay measured the biologically active components, the full length (PYY_{1-36}) and the fragment (PYY_{3-36}). Antiserum (Y21) was produced in a rabbit against synthetic porcine PYY (Bachem, UK) coupled to bovine serum albumin with glutaraldehyde and used at a final dilution of 1:50000. This antibody cross-reacts fully with the biologically active circulating forms of human PYY, but not with pancreatic polypeptide, neuropeptide Y, or any other known gastrointestinal hormone (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). ^{125}I-labeled PYY was prepared by the Iodogen method and purified by high pressure liquid chromatography. The specific activity of the ^{125}I-labelled PYY was 54 Bq per femtomole. The assay was performed in a total volume of 700 µL of 0.06 M phosphate buffer, pH 7.26, containing 0.3 % bovine serum albumin. The samples were incubated for 3 days at 4 ºC before separation of free and antibody-bound label by sheep anti-rabbit antibody. Two hundred µL of plasma was assayed, while 200 µL of PYY-free, Haemacel colloid fluid was added to standards and other reference tubes to neutralise any non-specific assay interference. The assay has been reported to detect changes of 2 pmol/L, with an intra-assay coefficient of variation (CV) of 5.8 % and an interassay CV of 9.8 %.

Plasma GLP-1 was measured in duplicate by an established in-house radioimmunoassay. The GLP-1 assay detected changes of 7.5 pmol/L, with an intra-assay CV of 6.1 % (Kreymann, Williams, Ghatei and Bloom 1987).

Study 2: Changes in glucose metabolism and the gut hormone response in the initial postoperative period after gastric bypass.

This study was performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC Protocol Number: 05/Q2202/96). Exclusion criteria included pregnancy, substance abuse, more than 2 alcoholic drinks per day. Written informed consent was obtained from all participants.

A group of type 2 diabetic, morbidly obese patients undergoing gastric bypass in the Department of Bariatric and Metabolic Surgery, Musgrove Park Hospital, Taunton (n=17), were compared with three different control groups including patients a) with type 2 diabetes and obesity undergoing gastric banding (n=9) to control for the effect of general anaesthetic, laparoscopy and surgical trauma b) patients with type 2 diabetes and obesity undergoing very low calorie diet for one week (n=15) to control for the effect of reduced food intake in the immediate postoperative period and c) non insulin resistant obese subjects undergoing gastric bypass (n=5). All participants underwent a two week diet of 1000 kcal prior to the intervention. Diabetic patients were optimised in terms of glycaemic control with the aid of pharmacotherapy and lifestyle intervention under the guidance of physicians with a

special interest in obesity and type 2 diabetes for a minimum six month period preoperatively. None of the participants was on insulin therapy during the period of the study. Furthermore there was no difference in the usage of oral hypoglycaemic treatment between the groups including patients with diabetes. Time points were pre-operatively and day 2, 4, 7 and 42 postoperatively. For the very low calorie diet group there was no 42 day time point as this intervention only lasted for one week.

The technique used for the bypass was a combination of linear stapler and hand sewn enterotomy closure for both the gastrojejunostomy and jejuno-jejunostomy, in an antegastric-retrocolic configuration (Higa, Boone, Ho and Davies 2000). This was a modification of the technique first described by Higa et al (Higa, Boone, Ho and Davies 2000).

A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Reverse Trendelenburg position was used with the operating on the right of the patient and assistant positioned between the legs of the patient. Pneumoperitoneum was obtained with a Verres needle inserted below the left costal margin at the mid-clavicular line. Thromboprophylaxis included the routine use of TED stockings and

40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively and lower limb pneumatic compression devices intraoperatively. Five ports were used. An isolated lesser curve-based, 15–20-ml gastric pouch was created and a retrocolic antegastric Roux limb was made 100 cm long for patients with a BMI equal to or less than 50 kg/m2 and 150 cm long for patients with BMI of more than 50 kg/m2. The bilio-pancreatic limb was 25 cm. The gastrojejunostomy was closed over a 34 Fr bougie. A blue dye leak test via a nasogastric tube was routinely performed. All hernia defects (jejunojejunostomy, Petersen's and mesocolon) were routinely closed with a purse-string suture.

An enhanced recovery protocol was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. The first routine postoperative review in the outpatient department was 6 weeks after discharge with a minimum regular follow-up program for uncomplicated patients afterwards of 6 months, 12 months postoperatively, and yearly thereafter, and more intensive follow-up for complex patients.

The surgical technique used for gastric banding was the pars flaccida described by the Melbourne group (O'Brien, Dixon, Laurie and Anderson 2005). A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure as with the gastric bypass. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy,

Titcomb, Humadi, Edmond, Mahon and Welbourn 2010). Thromboprophylaxis included the routine use of TED stockings and 40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively. Lower limb pneumatic compression devices intraoperatively. Five ports were used. The Swedish Adjustable Gastric band® (Ethicon Endo-Surgery) and the LAP-BAND® (Allergan) bands were used for all gastric banding procedures. The pars flaccida dissection technique with gastro-gastric tunnelling sutures was the operative method utilised (O'Brien, Dixon, Laurie and Anderson 2005). The same enhanced recovery protocol as the one sued for gastric bypass was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. Follow-up was also similar with the first postoperative review in the outpatient department 6 weeks after discharge. Patients were then seen monthly and adjustments were performed until optimal reduction in hunger or restriction was achieved. The adjustments were performed using the indicators described by Favretti et al (Favretti, O'Brien and Dixon 2002) as seen in table 4. Patients were seen at least six times in the first year and then once a year minimum (Pournaras, Osborne, Hawkins, Vincent, Mahon, Ewings, Ghatei, Bloom, Welbourn and le Roux 2010).

Following a 12 hour fast, a venous catheter was placed in a large vein and blood samples were collected. For the bypass surgery group further samples were obtained at 15, 30, 60, 90, 120, 150 and 180 minutes following a standard semi-liquid meal containing 400 kcal. The meal macronutrient content was 48.8% carbohydrate, 10.2% protein and 41% fat. All samples were collected in tubes

containing EDTA and aprotinin and were immediately centrifuged and stored in a -80°C freezer until further analysis. Glucose levels were measured with an automated glucose analyser (Abbott laboratories, Chicago USA) and insulin levels with an automated chemiluminescent immunoassay (Abbott laboratories, Chicago, USA). Delta insulin was defined as the difference between a 0 minute and 15 minute insulin measurement (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lönroth, Fändriks, Ghatei, Bloom and Olbers 2007). Insulin resistance was measured with the Homeostatic Model Assessment (HOMA-IR) Matthews, Hosker, Rudenski, Naylor, Treacher and Turner 1985).

Plasma GLP-1 was measured in duplicate by an established in-house radioimmunoassay. The GLP-1 assay detected changes of 7.5 pmol/L, with an intra-assay CV of 6.1 % (Kreymann, Williams, Ghatei and Bloom 1987).

Study 3: The long term gut hormone response after gastric bypass.

This study was also performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC Protocol Number: 05/Q2202/96). Exclusion criteria included pregnancy, substance abuse, more than two alcoholic drinks per day and aerobic exercise for more than 30 min three times per week. Written informed consent was obtained from all participants.

Thirty-four participants were studied cross-sectionally at four different time points, preoperatively (n=17), and 12 (n=6), 18 (n=5) and 24 (n=6) months after gastric bypass. Another group of patients (n=6) were studied prospectively.

The technique used for the bypass was a combination of linear stapler and hand sewn enterotomy closure for both the gastrojejunostomy and jejuno-jejunostomy, in an antegastric-retrocolic configuration (Higa, Boone, Ho and Davies 2000). This was a modification of the technique first described by Higa et al (Higa, Boone, Ho and Davies 2000).

A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Reverse Trendelenburg position was used with the operating on the right of the patient and assistant positioned between the legs of the patient (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010). Pneumoperitoneum was obtained with a Verres needle inserted below the left costal margin at the mid-clavicular line. Thromboprophylaxis included the routine use of

TED stockings and 40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively and lower limb pneumatic compression devices intraoperatively. Five ports were used. An isolated lesser curve-based, 15–20-ml gastric pouch was created and a retrocolic antegastric Roux limb was made 100 cm long for patients with a BMI equal to or less than 50 kg/m2 and 150 cm long for patients with BMI of more than 50 kg/m2 (. The bilio-pancreatic limb was 25 cm . The gastrojejunostomy was closed over a 34 Fr bougie. A blue dye leak test via a nasogastric tube was routinely performed. All hernia defects (jejunojejunostomy, Petersen's and mesocolon) were routinely closed with a purse-string suture (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

An enhanced recovery protocol was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Following a 12 hour fast, a venous catheter was placed in a large vein and blood samples were collected. For the bypass surgery group further samples were obtained at 15, 30, 60, 90, 120, 150 and 180 minutes following a standard semi-liquid meal containing 400 kcal. The meal macronutrient content was 48.8% carbohydrate, 10.2% protein and 41% fat. All samples were collected in tubes containing EDTA and aprotinin and were immediately centrifuged and stored in a -80°C freezer until further analysis. GLP-1 and PYY levels were measure as

described in study 2. Visual analogue scales (VAS) were used to measure hunger and satiety immediately before consumption of the meal and at 60, 120, and 180 minutes later. The VAS can be seen in Figure 6.

Figure 6. Visual Analogue Scales for hunger and satiety.

Please put a mark crossing the line at the point that best describes your responses to these two questions. A mark at 0 would indicate that you have no hunger at all or that you feel completely empty. A mark at 100 would indicate that you feel extremely hungry or that you feel completely full.

How hungry do you feel?

0   100

(not hungry at all)   (extremely hungry)

How full do you feel?

0   100

(completely empty)   (completely full)

For the measurement of gut hormones all samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay (Savage, Adrian, Carolan, Chatterjee and Bloom 1987). The assay measured the biologically active components, the full length (PYY_{1-36}) and the fragment (PYY_{3-36}). Antiserum (Y21) was produced in a rabbit against synthetic porcine PYY (Bachem, UK) coupled to bovine serum albumin with glutaraldehyde and used at a final dilution of 1:50000. This antibody cross-reacts fully with the biologically active circulating forms of human PYY, but not with pancreatic polypeptide, neuropeptide Y, or any other known gastrointestinal hormone (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). ^{125}I-labeled PYY was prepared by the Iodogen method and purified by high pressure liquid chromatography. The specific activity of the ^{125}I-labelled PYY was 54 Bq per femtomole. The assay was performed in a total volume of 700 µL of 0.06 M phosphate buffer, pH 7.26, containing 0.3 % bovine serum albumin. The samples were incubated for 3 days at 4 ⁰C before separation of free and antibody-bound label by sheep anti-rabbit antibody. Two hundred µL of plasma was assayed, while 200 µL of PYY-free, Haemacel colloid fluid was added to standards and other reference tubes to neutrilise any non-specific assay interference. The assay has been reported to detect changes of 2 pmol/L, with an intra-assay coefficient of variation (CV) of 5.8 % and an interassay CV of 9.8 %.

Plasma GLP-1 was measured in duplicate by an established in-house radioimmunoassay. The GLP-1 assay detected changes of 7.5 pmol/L, with an intra-assay CV of 6.1 % (Kreymann, Williams, Ghatei and Bloom 1987).

Statistical analysis

For studies 1 and 2 results were analysed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). Data were expressed as mean ± SEM. Values for the area under the curve were calculated with the use of the trapezoidal rule. For study 1 analysis of variance (ANOVA) with post hoc Dunnett test were used for HOMA-IR, delta insulin and GLP-1 responses. For study 2 plasma levels of insulin, GLP-1, PYY and glucose following the two glucose loadings were analyzed with a two-way group (between subjects) x time (within subjects) ANOVA. Post-hoc Bonferroni tests for each concentration were applied when there was a significant group x time interaction. For study 3 results were analysed using SPSS statistical software (SPSS Inc., Chicago, IL). End points were compared with the use of 2-tailed, paired Student t tests or ANOVA. A $p \leq 0.05$ was considered significant for all three studies.

3.3 Results

*Study 1: The effect of the altered nutrient route in glucose metabolism and the gut hormone response*

There was a significant difference in plasma insulin, GLP-1 and PYY (all $p<0.01$) between the oral and gastrostomy glucose loading (Figures 7a, b, c and d and table 8). There was a significant main effect of time and a significant group x time

interaction for insulin, GLP-1 and PYY (all p<0.001). Glucose levels recovered to

baseline earlier after the oral route compared to gastrostomy (p<0.001). There was

a significant group x time interaction (p<0.001), but no significant main group effect

for glucose levels (p=0.84).

Table 8: Two-way ANOVA values comparing oral and gastrostomy tube glucose load as a function of group and time for glucose, insulin, insulin, GLP-1 and PYY plasma levels following oral and gastrostomy tube glucose load as a function of group and time.

	Time	**Group**	**Time** **X Group**
Glucose	F(7,57)=5.81; p<0.001	F(1,57=0.04; p=0.84	F(7;57)=4.26; p<0.001
Insulin	F(7,57)=7.64; p<0.001	F(1,57)=8.88; p=0.004	F(7,57)=5.87; p<0.001
GLP-1	F(7,58)=10.51; p<0.001	F(1,58)=32.08; p<0.001	F(7,58)=9.10; p<0.001
PYY	F(7,58)=8,79; p<0.001	F(1,58)=96.77; p<0.001	F(7,58)=7.61; p<0.001

Figure 7: Plasma levels of (a) glucose, (b) insulin, (c) GLP-1 and (d) PYY following oral (black circles) and gastrostomy (open circles) glucose load. Data are expressed as mean ± SEM. When two-way ANOVA revealed a significant group x time interaction, post-hoc Bonferroni test was used for time point to time point analysis between the two groups (*p<0.05, ***p<0.001).
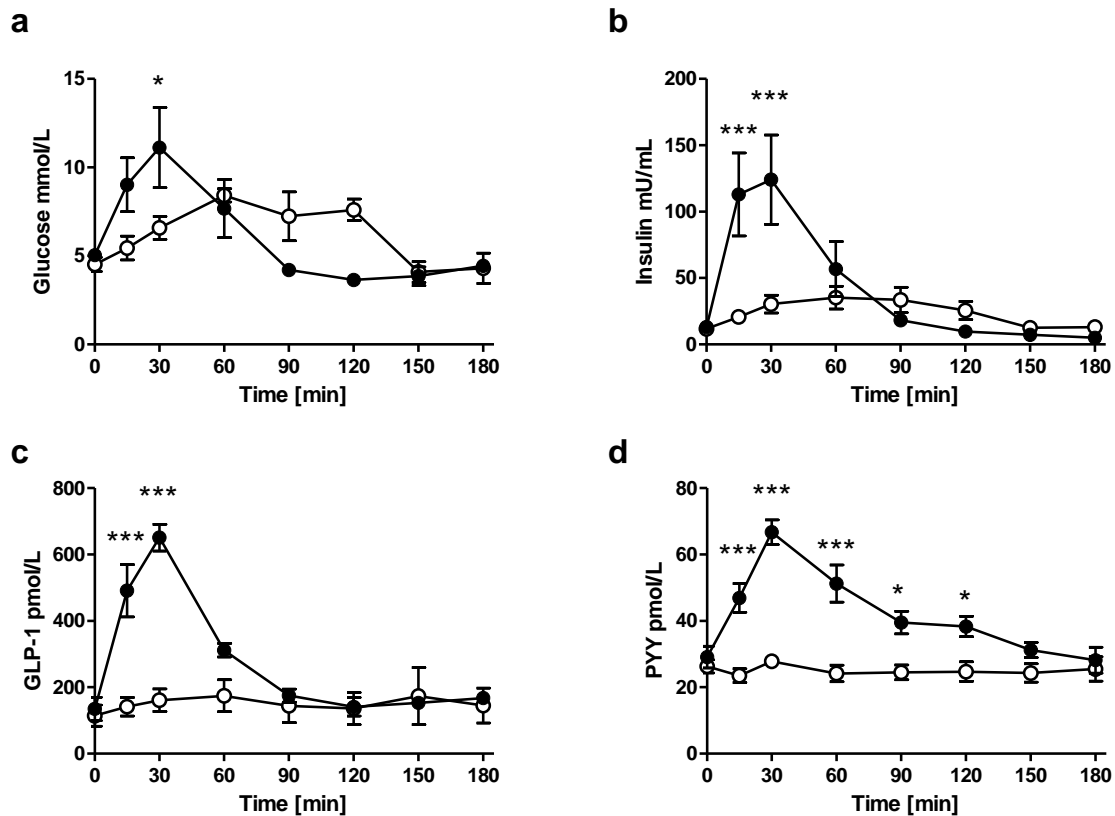
Study 2: Changes in glucose metabolism and the gut hormone response in the initial postoperative period after gastric bypass.

The demographic characteristics of the participants of the different groups are summarised in Table 9. Insulin resistance was reduced by 44% at 7 days after gastric bypass in the diabetic patients (Figure 8). As early as day 4, there was a significant difference in the HOMA-IR between the bypass and the banding group ($p<0.05$). Insulin resistance did not change after gastric banding or a 1000 kcal diet. (Fig. 2). HOMA-IR remained unchanged within the normal range in the non-diabetic gastric bypass group (Figure 8). The insulin response as measured with delta insulin increased at 2 days after gastric bypass in both diabetic and non-diabetic groups as seen in Figure 9. In accordance with the enhanced insulin production, the postprandial GLP-1 response also increased in both groups (Figure 10).

Table 9. Demographic characteristics of patients undergoing standard meal tests pre-operatively and at day 2, 4, 7 and 42. Comparisons were made between patients with diabetes (DM) who had very low calorie diet, gastric banding, gastric bypass or patients who did not have diabetes but underwent gastric bypass.

	Diet + DM	Band + DM	Bypass + DM	Bypass non DM	p-value
Number	15	9	17	5	
Age (years)	43.7 ± 10	48.1 ± 8.7	48.3 ± 8.6	42.4 ± 6.9	NS
Pre-op Weight (kg)	139.0 ± 37.5	129.5 ± 28.2	138.9 ± 35.5	143.8 ± 21.6	NS
Pre-op BMI	46.9 ± 8.1	43.5 ± 11.7	48.0 ± 5.7	52.2 ± 3.3	NS
Pre-op glucose	5.9 ± 1.1	6.1 ± 1.2	7.1 ± 2	5.9 ± 1.1	NS
Pre-op HOMA-IR	7.1 ± 3	8.8 ± 9.6	9.2 ± 7.8	2.1 ± 0.4	<0.05

Figure 8. Insulin resistance measure with HOMA in patients with type 2 diabetes following a 1000 kcal diet with no surgery over 7 days (n=15) and gastric band (n=9), gastric bypass (n=17) and patients without diabetes following gastric bypass (n=5) over a period of 42 days. The solid line indicates the level at which HOMA-IR is considered to indicate insulin resistance.

* p<0.05 compared to pre-operative state

 # p<0.05 compared with gastric banding at same time point

† p<0.05 compared to very low calorie diet at same time point
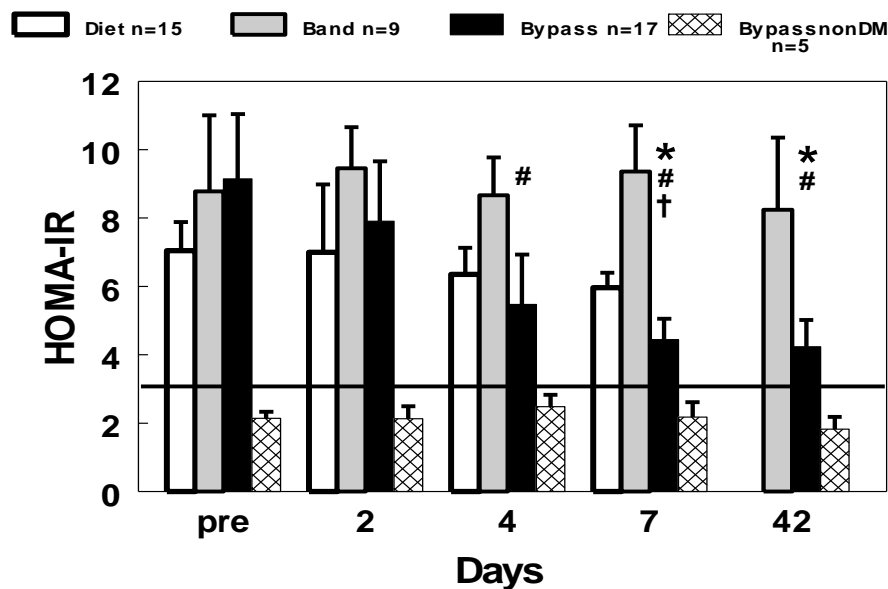
Figure 9. Delta insulin defined as the difference between fasting and 15 min postprandial insulin after gastric bypass in patients with type 2 diabetes (n=17) and without type 2 diabetes (n=5). * p<0.05 compared to pre-operative state for both groups.
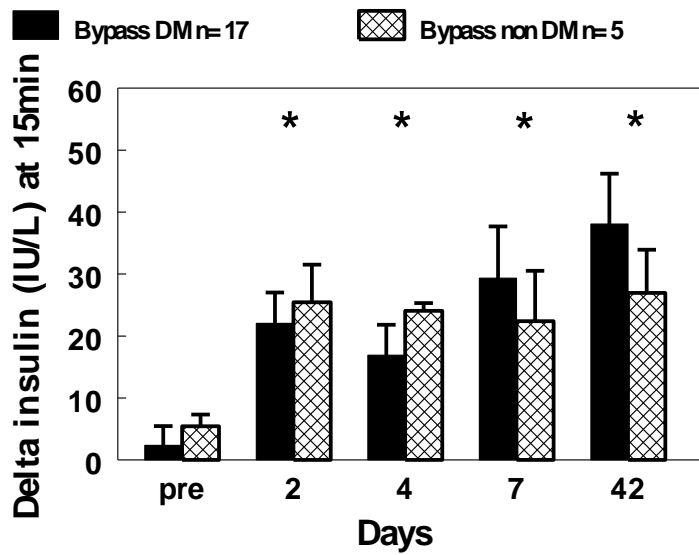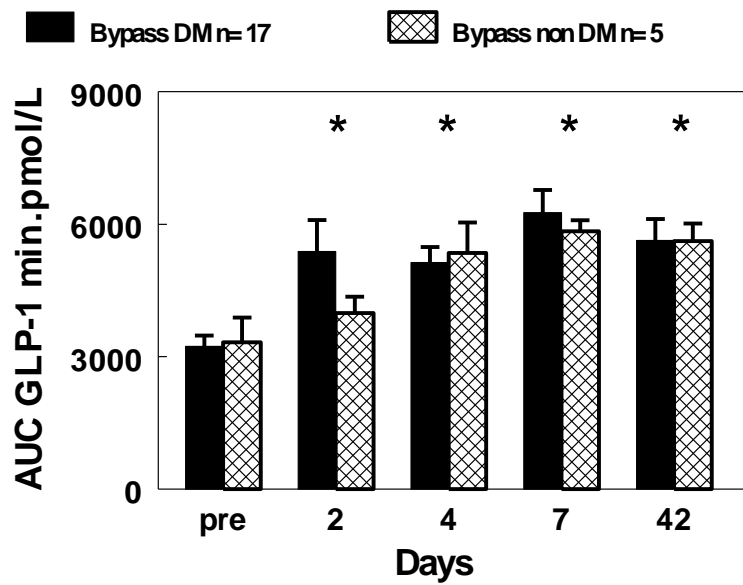
Figure 10. Postprandial GLP-1 response after a 400 kcal meal following gastric bypass in patients with type 2 diabetes (n=17) and without type 2 diabetes (n=5). Measured with the area under the curve over a 3-hour period. * p<0.05 compared to pre-operative state for both groups.

Study 3: The long term gut hormone response after gastric bypass.

The demographic characteristics of the groups of this study are demonstrated in Table 10. The BMI in the postoperative groups was significantly lower than the preoperative group, but there was no significant difference between the three postoperative groups (Figure 11). The PYY postprandial response curve was increased postoperatively in both the cross-sectional and the prospective comparisons, ($p<0.05$) (Figure 12 and Figure 13). This was not the case for the GLP-1 response ($p=0.189$ for the prospective comparison) (Figure 14 and Figure 15). Satiety was increased postoperatively ($p<0.05$) (Figure 16).

Table 10: Demographic characteristics of patients in cross-sectional study and prospective study.

Group	Number (n)	Age	Sex (No of women)
preoperative	17	47.8 ± 2.0	11
12 month postoperatively	6	45.2 ± 4.0	5
18 months postoperative	5	49.6 ± 3.3	3
24 months postoperative	6	43.3 ± 4.1	6
prospective study	6	47.8 ± 2.0	5

Figure 11. BMI before and 12, 18 and 24 months after gastric bypass.

* p<0.05 compared to the pre-operative group

BMI (kg/m2)
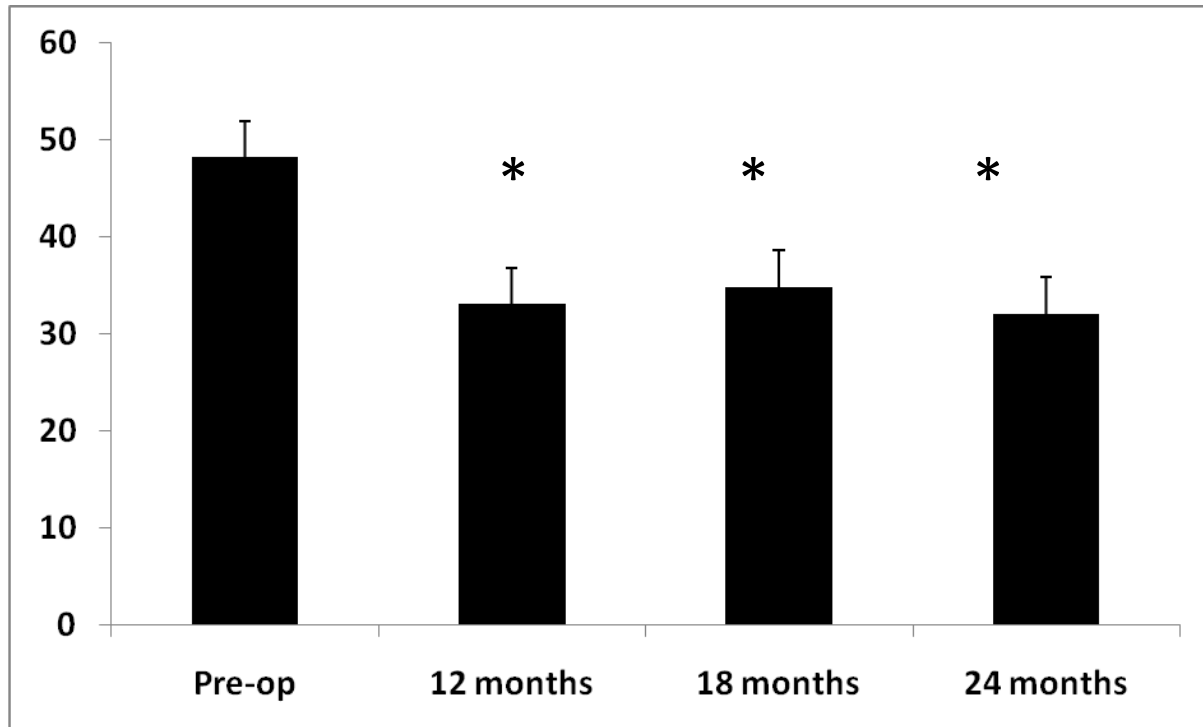
Figure 12. The PYY postprandial response in the cross-sectional study (12, 18 and 24 months).

* p<0.05 compared to the pre-operative group
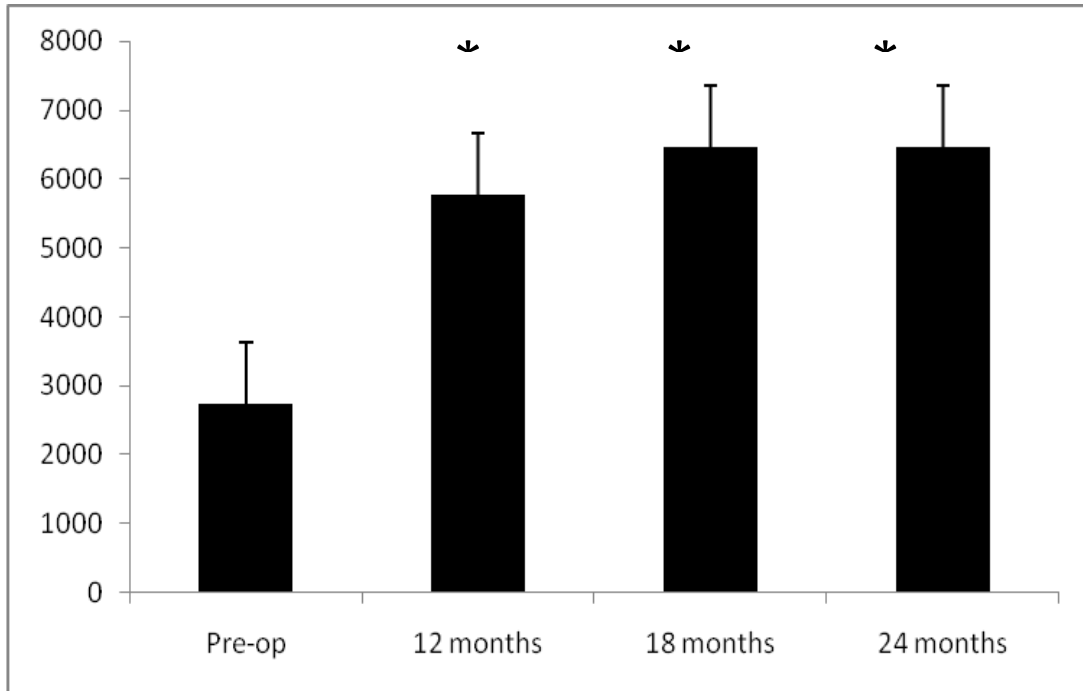
PYY (pmol/L/min)

Figure 13. The PYY postprandial response in the prospective study (18-24 months).
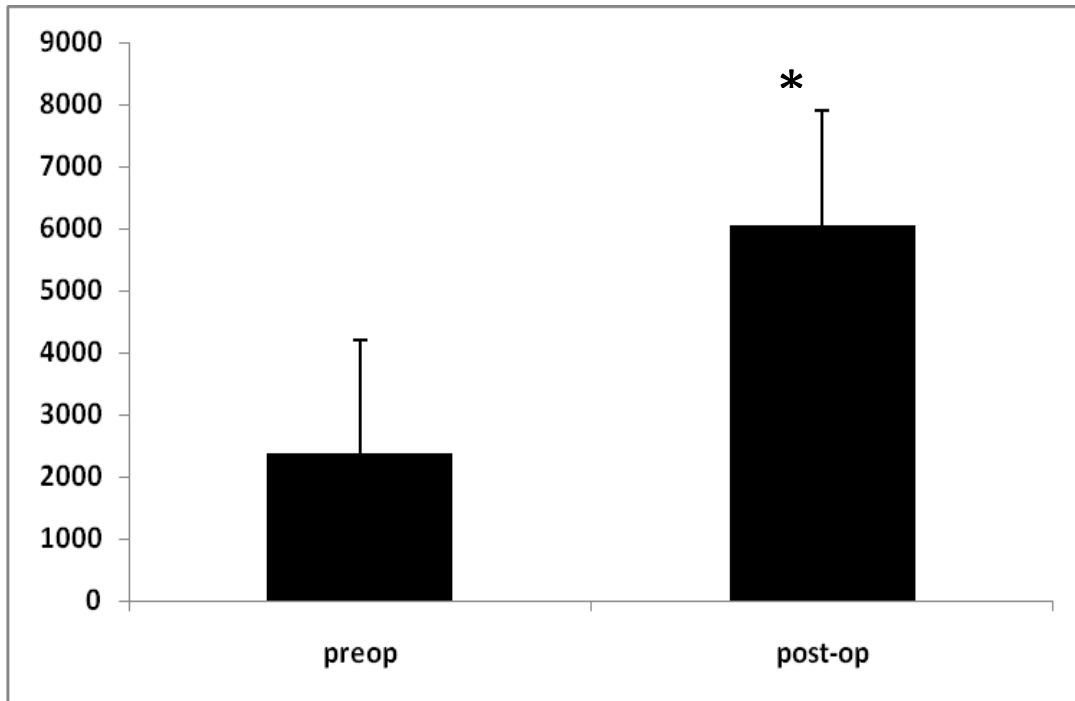
PYY (pmol/L/min)

Figure 14. The GLP-1 response in the cross-sectional study (12, 18 and 24 months).
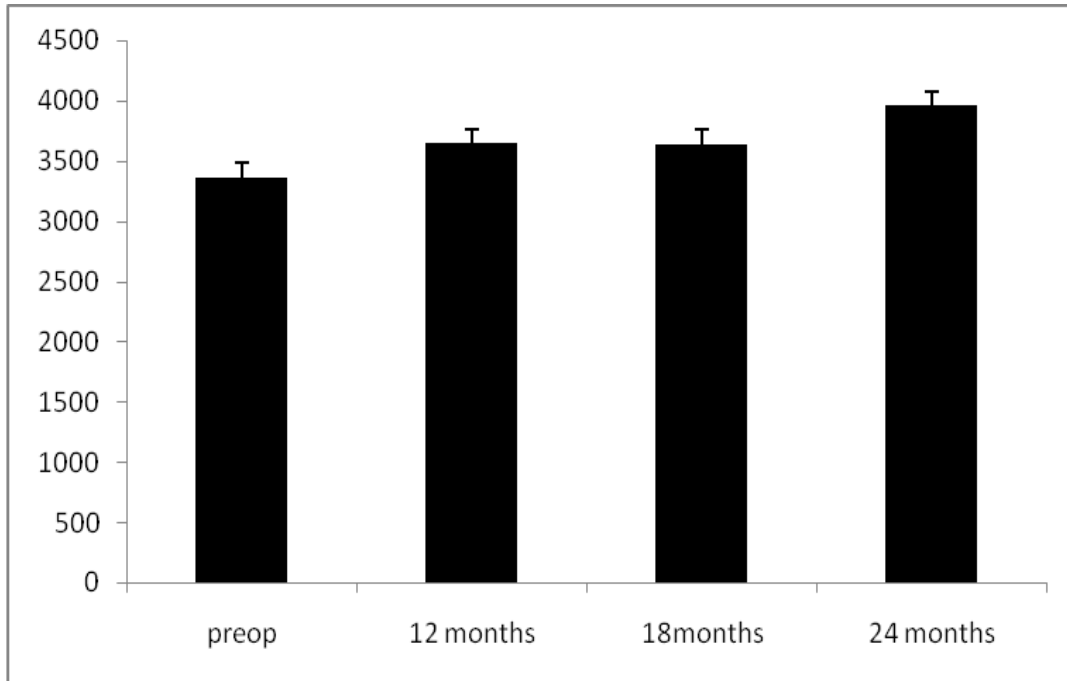
GLP-1 (pmol/L/min)

Figure 15. The GLP-1 postprandial response in the prospective study (18-24 months).
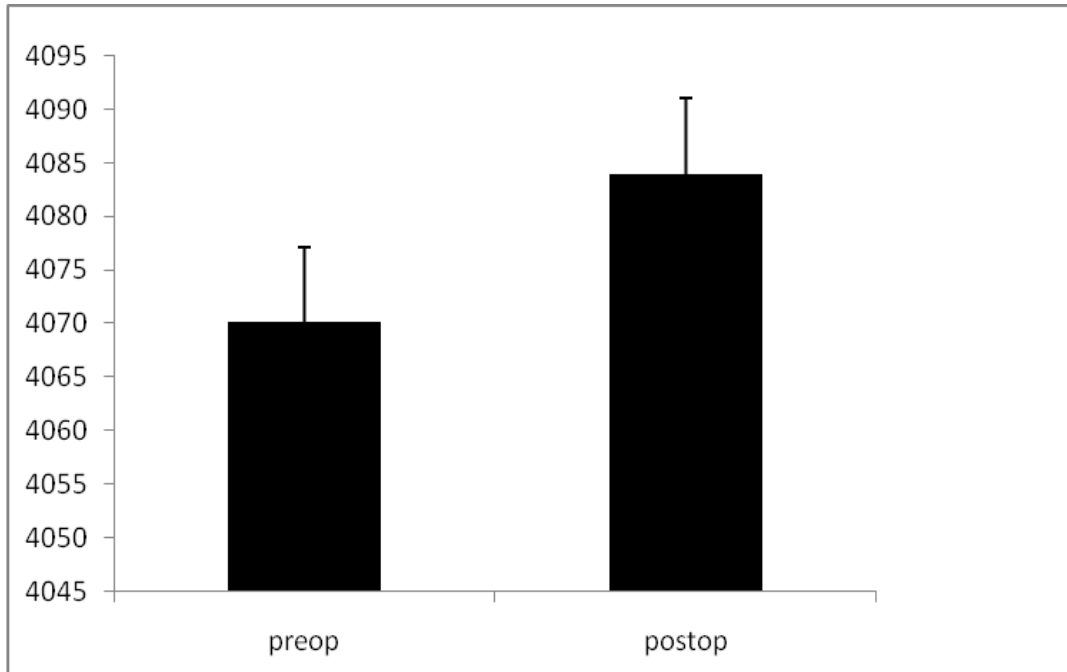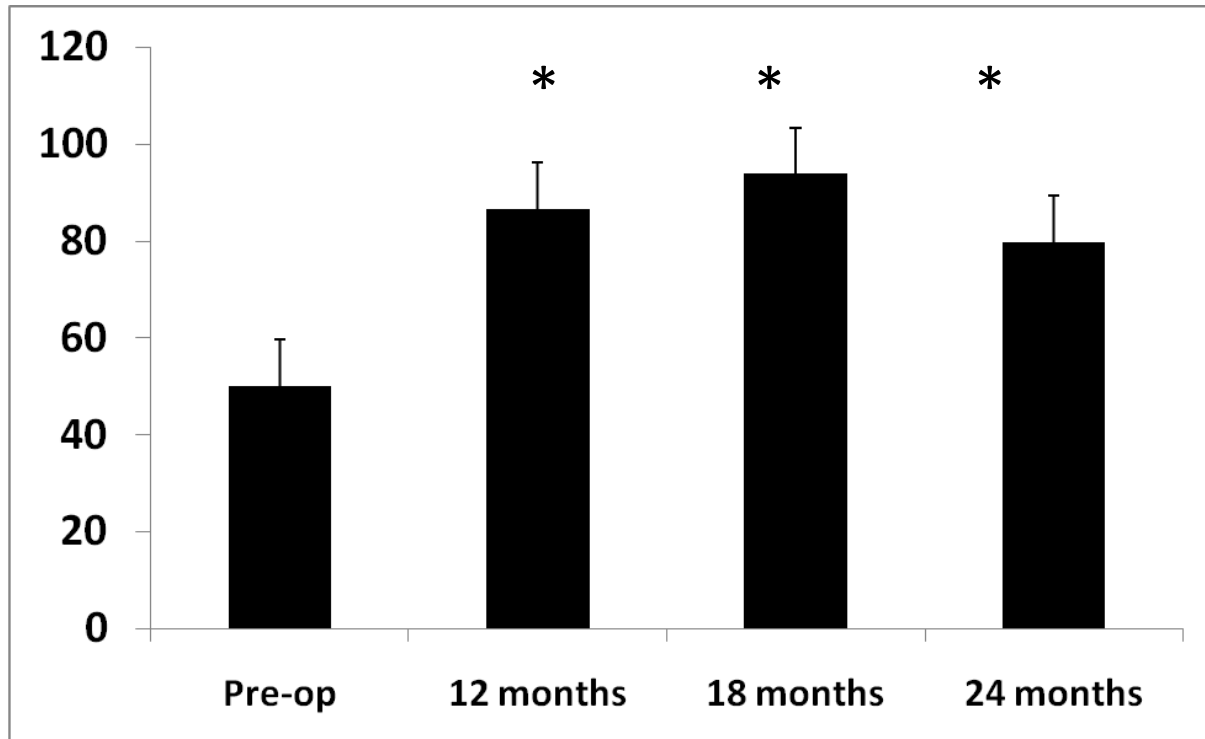
GLP-1 (pmol/L/min)

Figure 16. Satiety as measured with the VAS in the prospective study.

* p<0.05 compared to the pre-operative group

VAS (mm)

3.4 Discussion

Gastric bypass has an effect on glucose handling which is independent of weight loss and study 1 offers confirmation for this. Gastric bypass leads to the improvement of glycaemic control via a dual mechanism of increased insulin production and at the same time reduced insulin resistance in the first week after surgery as demonstrated in study 2. The increased insulin production is associated with an enhanced GLP-1 response. This improved glucose homeostasis can be explained in part by the altered nutrient delivery as seen in study 1. Finally the enhanced gut hormone response seen in the above studies is sustained in the long term, accompanied by increased postprandial satiety demonstrated in both the cross-sectional and prospective designs of study 3.

The paradoxical reduction in insulin resistance is independent of weight loss, the effect of the surgical trauma, which usually leads to increased insulin resistance and reduced food intake (Robertson, Bickerton, Dennis, Vidal, Jewell and Frayn 2005). This weight loss independent effect suggested in the chapter two of this thesis and further evidenced by the data from studies 1 and 2 in this chapter has been attributed to alterations in the hormonal milieu (Rubino, Gagner, Gentileschi, Kini, Fukuyama, Feng and Diamond 2004; Rubino, Forgione, Cummings, Vix, Gnuli, Mingrone, Castagneto and Marescaux 2006). However the findings of this study are in contrast to a study by WJ Lee et al who showed reduced insulin resistance after banding and diet comparable to gastric bypass (Lee, Lee, Ser, Chen, and Chen

2008). Of note is the fact that the time point used by Lee et al was four weeks when the effect of weight loss may have caused bias (Lee, Lee, Ser, Chen, and Chen 2008).

The enhanced insulin response as measured with delta insulin is associated with enhanced GLP-1 response supporting the hypothesis that the increased GLP-1 response is responsible in part for the effect of bypass on insulin production. On the other hand, the increase in insulin production is marked and perhaps other mechanisms may contribute. In addition the reduced insulin resistance measured at fasting is associated with unchanged fasting GLP-1 levels and therefore cannot be explained by the GLP-1 response.

The concept of proximal gut exclusion was first suggested in an animal model, the Goto-Kakizaki, a type of non-obese rats who spontaneously develop type 2 diabetes (Rubino, Forgione, Cummings, Vix, Gnuli, Mingrone, Castagneto and Marescaux 2006). Rubino demonstrated in a landmark experiment that glucose homeostasis improved after duodenal-jejunal bypass, compared to gastrojejunostomy (Rubino, Forgione, Cummings, Vix, Gnuli, Mingrone, Castagneto and Marescaux 2006). When the gastrojejunostomy group underwent a procedure which excluded the duodenum, these rats experienced improved glucose tolerance (Rubino, Forgione, Cummings, Vix, Gnuli, Mingrone, Castagneto and Marescaux 2006). And when the duodenal passage of nutrients was restored in the former group, impaired glucose tolerance recurred supporting the proximal gut hypothesis further.

Previously a report of a single case of a patient similar to our cohort has been described in the literature (Dirksen, Hansen, Madsbad, Hvolris, Naver, Holst and Worm 2010). The authors presented results similar to ours comparing the oral and the gastrostomy tube route of nutrients (Dirksen, Hansen, Madsbad, Hvolris, Naver, Holst and Worm 2010).

Cross-sectional studies have previously suggested that gastric bypass is associated with an enhanced PYY and GLP-1 postprandial response (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia and Wardlaw 2005; Rodieux, Giusti, D'Alessio, Suter and Tappy 2008; Vidal, Nicolau, Romero, Casamitjana, Momblan, Conget, Morínigo and Lacy 2009). Fasting levels of PYY have been showed to increase post gastric bypass as demonstrated in prospective studies (Reinehr, Roth, Schernthaner, Kopp, Kriwanek and Schernthaner 2007; Garcia-Fuentes, Garrido-Sanchez, Garcia-Almeida, Garcia-Arnes, Gallego-Perales, Rivas-Marin, Morcillo, Cardona and Soriguer 2008) whilst there are also data showing increased postprandial PYY (Borg, le Roux, Ghatei, Bloom, Patel and Aylwin 2006; Stratis, Alexandrides, Vagenas and Kalfarentzos 2006; Karamanakos, Vagenas, Kalfarentzos and Alexandrides 2008; Morínigo, Vidal, Lacy, Delgado, Casamitjana and Gomis 2008) and GLP-1 (Borg, le Roux, Ghatei, Bloom, Patel and Aylwin 2006; Laferrère, Teixeira, McGinty, Tran, Egger, Colarusso, Kovack, Bawa, Koshy, Lee, Yapp and Olivan 2008; de Carvalho, Marin, de Souza, Pareja, Chaim, de Barros Mazon, da Silva, Geloneze, Muscelli and Alegre 2009; Morínigo, Lacy, Casamitjana, Delgado, Gomis and Vidal 2006) levels up to 12 months postoperatively.

For the first study the non random allocation of the oral and gastrostomy route of the glucose load is a limitation. However, it was important to ensure that patients can tolerate the glucose load orally and taking into consideration any possible intolerance or hypoglycaemic episodes. Therefore instead of the standard dose, the same glucose load could be given to the participants. This was not necessary in the study as all participants tolerated the standard load. All tests were performed within 6 days for each participant minimising the effect of bias or changes in glucose homeostasis. Limitations of study 2 include the use of HOMA-IR as a marker of insulin resistance instead of the gold standard, the Hyperinsulinaemic Euglycaemic Glucose Clamp. Although this approach would have been more accurate, particularly in elucidating the effect of the bypass on insulin resistance postprandially, it was deemed more invasive and associated with considerably higher risk in the initial postoperative period. Furthermore electing a time point and therefore reducing repeated measurement would not have been possible as there was no available information regarding the time scale of the change of insulin resistance after gastric bypass. Finally for the long term investigation of the gut hormone response the limitations are the small number of participants and the cross-sectional design. This may be responsible for the lack of statistical difference in the GLP-1 response. The prospective part of the study confirmed the findings of the cross-sectional study.

In conclusion gastric bypass leads increased insulin production and reduced insulin resistance. The increased insulin production is associated with an increased postprandial incretin response. Furthermore gastric bypass leads to increased

postprandial gut hormone response, associated with increased satiety in the long term. However the mechanism of this increased gut hormone response as well as the weight loss independent reduction in insulin resistance has not been investigated in the above experiments and this is the topic of the next chapter.

4. The role of bile acids in improving glycaemic control after metabolic surgery.

4.1 Introduction

Following the results presented in the previous chapters I hypothesised that the altered anatomy after gastric bypass affects bile delivery to the terminal ileum and leads to elevated plasma bile acids after gastric bypass surgery. Changes in bile flow result in increased satiety gut hormone responses, reduced food intake and weight loss. In this chapter this hypothesis was tested in humans after gastric bypass surgery and two animal models of altered bile flow were used in order to explore the potential mechanisms involved further.

4.2 Methods

The canine studies were approved by the ethics committee of Onderstepoort Vetinary School, University of Pretoria, South Africa and the rat studies were approved by the Home Office UK (PL 70-6669).

Study 1: Human model

The human study was approved by the Somerset Research and Ethics committee approved the study (LREC Protocol Number: 05/Q2202/96) and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Exclusion criteria included pregnancy, substance

abuse, more than 2 alcoholic drinks per day. Twelve patients undergoing gastric bypass (seven females and five males) with a mean age of 45.2 ± 2.7 years and a mean BMI of 49.8 ± 1.5 were recruited. Six patients undergoing gastric banding (four females and 2 males), with a mean age of 45.4 ± 2.6 years and a mean BMI of 44 ± 2.0 kg/m^2 were used as a control group. All procedures were performed laparoscopically by one surgeon.

The technique used for the bypass was a combination of linear stapler and hand sewn enterotomy closure for both the gastrojejunostomy and jejuno-jejunostomy, in an antegastric-retrocolic configuration (Higa, Boone, Ho and Davies 2000). This was a modification of the technique first described by Higa et al (Higa, Boone, Ho and Davies 2000).

A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy, Titcomb, Humadi, Edmond, Mahon and Welbourn 2010).

Reverse Trendelenburg position was used with the operating on the right of the patient and assistant positioned between the legs of the patient. Pneumoperitoneum was obtained with a Verres needle inserted below the left costal margin at the mid-clavicular line. Thromboprophylaxis included the routine use of TED stockings and

40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively and lower limb pneumatic compression devices intraoperatively. Five ports were used. An isolated lesser curve-based, 15–20-ml gastric pouch was created and a retrocolic antegastric Roux limb was made 100 cm long for patients with a BMI equal to or less than 50 kg/m2 and 150 cm long for patients with BMI of more than 50 kg/m2. The bilio-pancreatic limb was 25 cm. The gastrojejunostomy was closed over a 34 Fr bougie. A blue dye leak test via a nasogastric tube was routinely performed. All hernia defects (jejunojejunostomy, Petersen's and mesocolon) were routinely closed with a purse-string suture.

An enhanced recovery protocol was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. The first routine postoperative review in the outpatient department was 6 weeks after discharge with a minimum regular follow-up program for uncomplicated patients afterwards of 6 months, 12 months postoperatively, and yearly thereafter, and more intensive follow-up for complex patients.

The surgical technique used for gastric banding was the pars flaccida described by the Melbourne group (O'Brien, Dixon, Laurie and Anderson 2005). A preoperative low-calorie, low-carbohydrate diet was used for 2 weeks aiming to reduce perioperative risk by reducing liver size and improving intraoperative exposure as with the gastric bypass. Intravenous antibiotic prophylaxis with 1.5g cefuroxime and 500mg metronidazole at induction was routinely used (Pournaras, Jafferbhoy,

Titcomb, Humadi, Edmond, Mahon and Welbourn 2010). Thromboprophylaxis included the routine use of TED stockings and 40 mg of low molecular weight heparin preoperatively and then for 7 days postoperatively. Lower limb pneumatic compression devices intraoperatively. Five ports were used. The Swedish Adjustable Gastric band® (Ethicon Endo-Surgery) and the LAP-BAND® (Allergan) bands were used for all gastric banding procedures. The pars flaccida dissection technique with gastro-gastric tunnelling sutures was the operative method utilised (O'Brien, Dixon, Laurie and Anderson 2005). The same enhanced recovery protocol as the one sued for gastric bypass was used with patients being offered a glass of water in the recovery room progressing to free fluids on postoperative day 1 and a soft diet thereafter. Follow-up was also similar with the first postoperative review in the outpatient department 6 weeks after discharge. Patients were then seen monthly and adjustments were performed until optimal reduction in hunger or restriction was achieved. The adjustments were performed using the indicators described by Favretti et al (Favretti, O'Brien and Dixon 2002) as seen in table 4. Patients were seen at least six times in the first year and then once a year minimum (Pournaras, Osborne, Hawkins, Vincent, Mahon, Ewings, Ghatei, Bloom, Welbourn and le Roux 2010).

Following a 12 hour fast, blood was obtained in tubes containing EDTA and aprotinin. Samples were immediately centrifuged and stored in a -80°C freezer until analysis.

Plasma FGF19 concentration was measured using a quantitative sandwich enzyme linked immunosorbent assay (ELISA) technique (FGF19 Quantikine ELISA kit, Catalogue Number DF1900; R&D Systems, Minneapolis, MN 55413, USA).

The FGF19 Quantikine ELISA method utilises a 96 well polystyrene microplate pre-coated with a mouse monoclonal antibody specific for FGF19. FGF19 standards were provided by the manufacturer (10ng of recombinant human FGF-19 in a buffered protein solution, with preservatives, lyophilized). Serum samples (10ng) were pipetted into the microplate wells. FGF-19 molecules were bound to the immobilised antibody during a 2 hour incubation period. Buffer concentrate (21mL of a concentrated solution of buffered surfactant with preservatives) was utilised to wash unbound molecules. An enzyme-linked polyclonal antibody specific for FGF-19 (21mL of polyclonal antibody against FGF19 conjugated to horseradish peroxidise with preservatives) was added to the wells for an incubation period of 2 hours. Following further wash with the objective to remove unbound antibody-enzyme reagent, a substrate solution formed of colour reagents (12.5mL of stabilised hydrogen peroxide and 12.5mL of stabilised chromogen) was added to the wells for a 30 minute period.

Colour development in proportion to the amount of FGF19 bound in the initial step was observed at this stage. The colour development was terminated with the use of an acid based stop solution. The optical density of each well was then measured with a microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 540nm or 570nm. The assay was conducted according to the manufacturer's guidelines. Prior to the assay commencement, the unopened

kit was stored between 2-8⁰C as recommended. All serum samples were measured in duplicate. The assay results lead to generation of a standard curve, plotting optical density for the standards versus the concentrations of the standards. These data were transformed linearised using the log/log paper and regression analysis applied to the log transformation. The FGF19 concentration in each sample was then calculated using the absorbance values obtained by the assays on the standard curve and reading the corresponding concentration.

In order to avoid cross contamination during the procedure, pipette tips were changed between additions of each standard level, between sample additions, and between reagent additions. Separate reservoirs were used for each reagent. Washing of the plates was completed using an automatic plate washer, and to ensure accuracy plate sealers were suitably employed during incubation steps.

The EDTA plasma samples were analysed for fractionated bile acids using high-performance liquid chromatography (HPLC) tandem mass spectrometry. The method was based on that of Tagliocozzi et al and developed using an Ascentis Express fused core C18 analytical column (Sigma-Aldrich Co., Poole, UK) on a JascoTM LC 2000 HPLC system (Tokyo, Japan) coupled to a triple quadruple mass spectrometer API 3200TM (Applied Biosystems, Cheshire, UK) (Tagliacozzi, Mozzi, Casetta, Bertucci, Bernardini, Di Ilio, Urbani and Federici 2003). Bile acids were quantified using peak area analysis corrected by comparison to the respective internal standard, glycine, taurine or unconjugated deuterium-labelled DCA. Bile acids analysed were chenodeoxycholic acid, DCA, cholic acid and their respective glycine and taurine conjugates. The method was linear between 0.1 and 10 mmol/L

for all bile acids and their conjugates with coefficients of variation ranging from 3.6%
to 8.0% at the lower limit of quantitation (0.1 mmol/L).

Multiple batches were used; interassay coefficient of variations ranging from 1.5% to
6.8% for these bile acids and their conjugates. The method allowed 12 different bile
acids to be measured within the range of 0.1-10µM. The two main plasma bile acids
in humans, glycochenodeoxycholic acid (GCDC) and Glycocholic acid (GCA) were
compared as well as total bile acids, total unconjugated bile acids, total glycine-
conjugated bile acids and total taurine-conjugated bile acids.

Study 2: Canine model

Four male and four female Beagles were fasted overnight and then given a standard
400 g test meal of dog chow (Husky, Purina, South Africa). The composition of the
test meal was 7.5% protein, 2% fat, 1% fibre, 7.5% crude ash and 82% moisture.
Five mL of blood were collected every 30 minutes from 30 minutes before the meal
up to 150 minutes postprandially.

The following day a fentanyl patch was placed and food was withheld overnight.
Following laparotomy the common bile duct was transacted and an 8 French Foley
catheter was placed into the gall bladder. An 8 French feeding tube was advanced
through the pylorus to the duodenum with the most distal point being 5-8 cm distal to
the pylorus. The altered anatomy can be seen in Figure 17. For the first 12 hours
postoperatively the dogs were given ad libitum access to water but not to food.

Figure 17. Illustration of the anatomy of the canine experiment showing the canulation. A Gastrostomy tube was placed into the duodenum close to the Ampulla of Vater. The common bile duct was ligated and the gallbladder was canulated to allow drainage of bile.
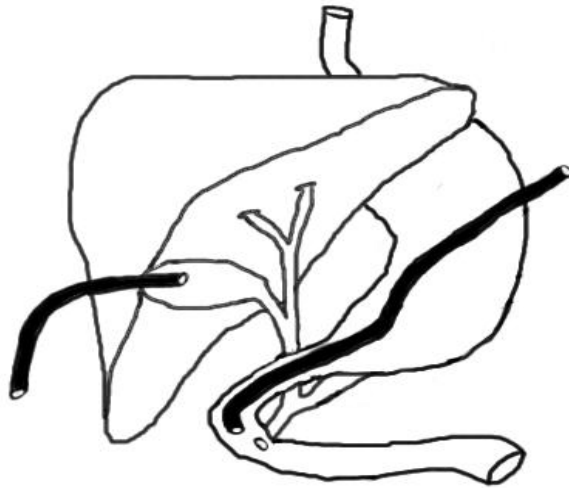
One dog was terminated due to signs of jaundice and infection. The other dogs received the standard meal of 400 g normal chow at the start of the light phase. At this time point, as much bile as possible was aspirated from the Foley catheter and injected through the gastrostomy tube, followed by a 5 mL flush of saline. The objective was to prevent jaundice and enable as close as normal digestion.

On day 4, 5 and 6 the dogs were randomised to a 180-minute cross over designed protocol of venous blood collection every 30 minutes, following a standard meal of 400 g dog food only without bile, bile only, without food or 400 g of dog food and bile in combination.

For the measurement of gut hormones all samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay (Savage, Adrian, Carolan, Chatterjee and Bloom 1987). The assay measured the biologically active components, the full length (PYY_{1-36}) and the fragment (PYY_{3-36}). Antiserum (Y21) was produced in a rabbit against synthetic porcine PYY (Bachem, UK) coupled to bovine serum albumin with glutaraldehyde and used at a final dilution of 1:50000. This antibody cross-reacts fully with the biologically active circulating forms of human PYY, but not with pancreatic polypeptide, neuropeptide Y, or any other known gastrointestinal hormone (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). ^{125}I-labeled PYY was prepared by the Iodogen method and purified by high pressure liquid chromatography. The specific activity of the ^{125}I-labelled PYY was 54 Bq per femtomole. The assay was performed in a total volume of 700 μL of 0.06 M phosphate buffer, pH 7.26, containing 0.3 % bovine serum

albumin. The samples were incubated for 3 days at 4 ºC before separation of free and antibody-bound label by sheep anti-rabbit antibody. Two hundred µL of plasma was assayed, while 200 µL of PYY-free, Haemacel colloid fluid was added to standards and other reference tubes to neutrilise any non-specific assay interference. The assay has been reported to detect changes of 2 pmol/L, with an intra-assay coefficient of variation (CV) of 5.8 % and an interassay CV of 9.8 %.

Plasma GLP-1 was measured in duplicate by an established in-house radioimmunoassay. The GLP-1 assay detected changes of 7.5 pmol/L, with an intra-assay CV of 6.1 % (Kreymann, Williams, Ghatei and Bloom 1987).

Study 3: Rodent model

Sixteen male Wistar obese rats were randomised to a sham operation which maintained the normal bile delivery to the duodenum or to an operation that would deliver bile to the ileum.   The bile in duodenum group underwent transections 1 cm proximal and distal to the drainage point of the common bile duct and reanastomosis to maintain the normal anatomy but allow for a similar surgical insult. The bile in ileum group had the same transection of the duodenum, but the proximal and distal ends of the transected duodenum were anastomosed end to end and continuity restored. The segment of the duodenum containing the common bile duct was anastomosed side to side to the distal jejunum, 10 cm proximally to the terminal ileum. This allowed bile and pancreatic juices to bypass the duodenum and most of the jejunum. The altered anatomy can be seen in Figure 18.

Figure 18. Illustration of the functional anatomy of the bile in ileum group. Transections 1 cm proximal and distal to the drainage point of the common bile duct were performed. The proximal and distal ends of the transected duodenum were anastomosed end to end and continuity of the gastrointestinal tract was restored. The segment of the duodenum containing the common bile duct was anastomosed side to side to the distal jejunum, 10 cm proximally to the terminal ileum.
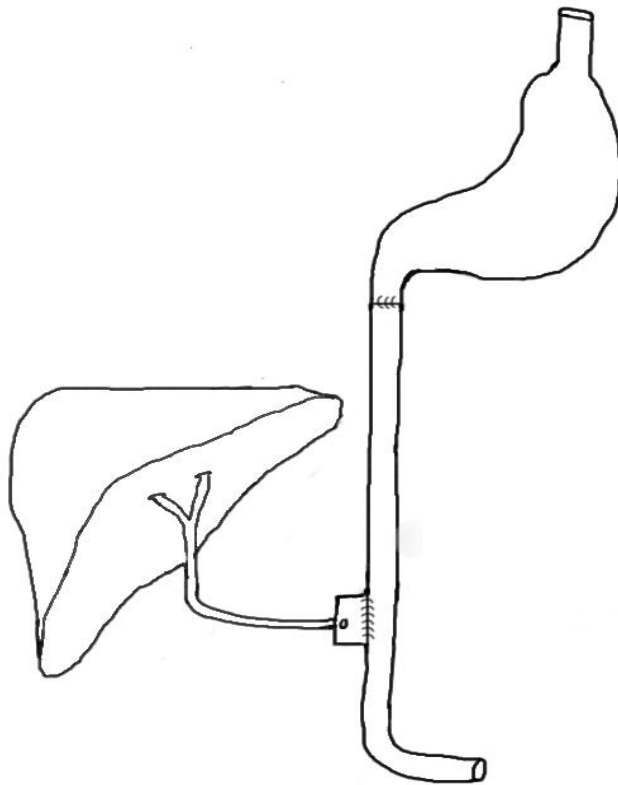
Body weight and food consumption was measured daily at the beginning of the light phase for 28 days. Faeces were collected over a 24 hour period on day 25. The rats were then fasted for 12 h before they were terminated and blood samples were collected.

To evaluate nutrient absorption, faeces were collected over 24 hours on postoperative days 25 from all rats. The ballistic bomb calorimeter used was designed by Miller and Payne in the 1950s (Miller and Payne 1959). The process consisted of three steps. The sample was initially prepared, then a defined amount of sample was burnt in excess of oxygen and finally the peak temperature detected was compared with the temperature when a standard material was burnt.

The preparation of the sample, rodent faeces for this experiment, was done over a 48-hour period. Rats were kept separate in single cages and on a predetermined day all saw dust was removed for a 24 hour period. The faeces were collected at the end of this period in sterile containers and kept at 4 °C until further analysis. The samples were dried overnight in an oven. The next day the specimen from each rat was ground producing a fine powder with the objective to enable satisfactory mixing. A representative sample weighing 400 mg was placed in a clean crucible.

Due to the low bulk density samples typically were compacted to ensure uniform and complete combustion. Five hundred µL of water was added and the crucible was placed on a hot plate to allow evaporation of the water and a formation of a cake like substrate.

Significant malabsorption can cause high fat content in the samples, thus causing a release that may exceed the measurement capability of the instruments rendering the measurement invalid. With the recommended oxygen pressure of 25 atmospheres the sample should burn completely without causing a high pressure in the bomb. With the above considerations a sample weighing 400 mg was selected.

The crucible was then placed on the pillar of the bomb and a standard 5 cm length sewing cotton was fitted to the firing wire with its free end in contact with the sample. The bomb body was fitted and the thermocouple inserted. Oxygen was admitted to a pressure of 25 atmospheres. The galvanometer zeroed and the firing button was pushed. The peak reading from the galvanometer was recorded during a 30 second period. The gas pressure was released and the bomb body was removed and allowed to cool in cold water awaiting assessment of the following sample (Jackson, Davis and Macdonald 1977).

For the assessment of inflammation serum CRP levels were measured (Rat Serum CRP ELISA kit Catalogue Number 1010; Alpha Diagnostics International, 6203 Wooldake Center Drive, San Antonio, Texas 78244, USA).

For the measurement of gut hormones all samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay (Savage, Adrian, Carolan, Chatterjee and Bloom 1987). The assay measured the biologically active components, the full length (PYY_{1-36}) and the fragment (PYY_{3-36}). Antiserum (Y21) was produced in a rabbit against synthetic porcine PYY (Bachem, UK) coupled to bovine serum albumin with glutaraldehyde and used at a final dilution

of 1:50000. This antibody cross-reacts fully with the biologically active circulating forms of human PYY, but not with pancreatic polypeptide, neuropeptide Y, or any other known gastrointestinal hormone (Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak and Bloom 1985). ^{125}I-labeled PYY was prepared by the Iodogen method and purified by high pressure liquid chromatography. The specific activity of the ^{125}I-labelled PYY was 54 Bq per femtomole. The assay was performed in a total volume of 700 μL of 0.06 M phosphate buffer, pH 7.26, containing 0.3 % bovine serum albumin. The samples were incubated for 3 days at 4 °C before separation of free and antibody-bound label by sheep anti-rabbit antibody. Two hundred μL of plasma was assayed, while 200 μL of PYY-free, Haemacel colloid fluid was added to standards and other reference tubes to neutrilise any non-specific assay interference. The assay has been reported to detect changes of 2 pmol/L, with an intra-assay coefficient of variation (CV) of 5.8 % and an interassay CV of 9.8 %.

Plasma GLP-1 was measured in duplicate by an established in-house radioimmunoassay. The GLP-1 assay detected changes of 7.5 pmol/L, with an intra-assay CV of 6.1 % (Kreymann, Williams, Ghatei and Bloom 1987).

Statistical Analysis

Results were analysed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. Data were expressed as means ± standard error of the mean (SEM) when the data follow a Gaussian distribution. When the data did not follow a Gaussian distribution the median (range) was used. Values for

the area under the curve were calculated with the use of the trapezoidal rule. End points were compared with the use of 2-tailed, paired Student t tests for parametric data and Mann-Whitney U test for non parametric data. Results were considered significant if $p < 0.05$.

4.3 Results

Study 1: Human model

There was no statistically significant difference in fasting plasma FGF19 levels between patients undergoing gastric banding [140ng/L (26-466)] and gastric bypass [123ng/L (41-406)] preoperatively (p=0.37). FGF19 levels were lower than those recorded in non-obese individuals (Walters, Tasleem, Omer, Brydon, Dew and le Roux CW 2009). In the gastric banding group there was no significant change between preoperative levels of fasting FGF19 and day 4 or 42 postoperatively. After gastric bypass, fasting levels of plasma FGF19 were significantly increased as early as day 4 postoperatively compared to preoperatively (p<0.01) as seen in Figure 19. The enhanced FGF19 levels remained high 42 days postoperatively (p<0.05).

Figure 19. Fasting plasma FGF19 concentrations (median and interquartile ranges) at day 0, 4 and 42 in six gastric banding patients (white bars) and twelve gastric bypass patients (black bars). * p<0.05 Mann Whitney U test.
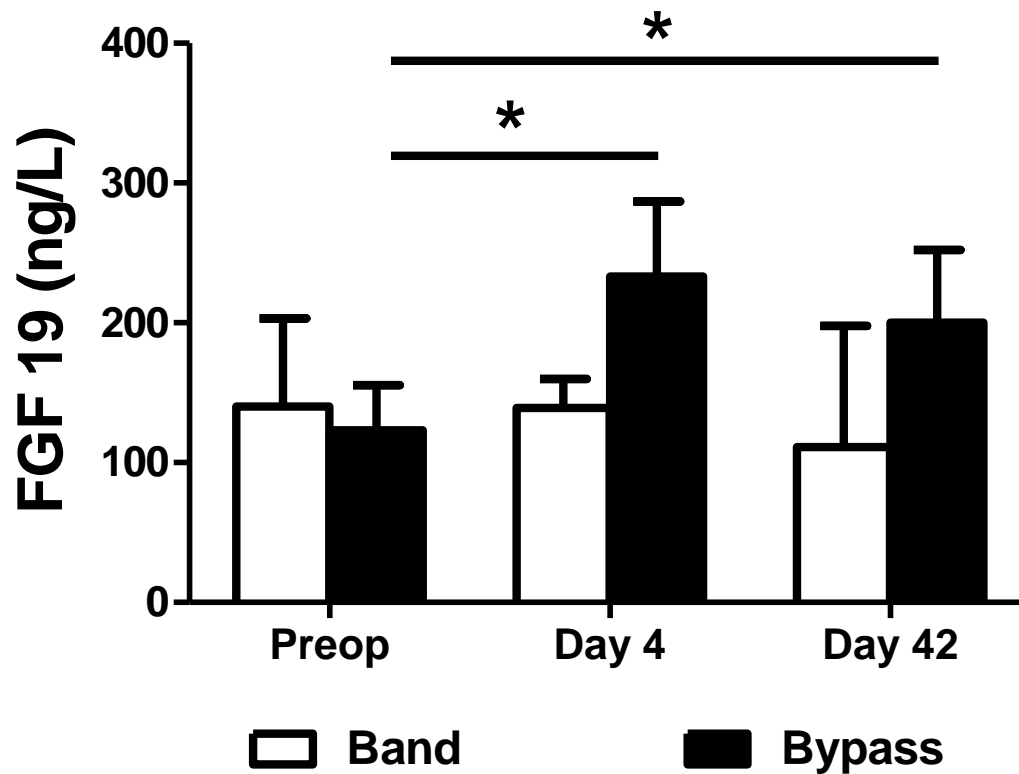
Levels of fasting total plasma bile acids measured in patients undergoing gastric banding and gastric bypass preoperatively were similar. On day 4 fasting plasma bile acids were increased after gastric bypass and there was a significant difference compared to the gastric banding group. On day 42 after gastric bypass fasting plasma total bile acid levels were higher compared to preoperative levels as seen in Figure 20. There was no difference in fasting plasma total bile acid levels before and 42 days after gastric banding.

Similar results with and an increase in the gastric bypass group but not in the gastric banding group were observed for GLP-1 in the same subjects as seen in Chapter 3, study 2.

Figure 20. Fasting total plasma bile acid concentrations at day 0, 4 and 42 in seven gastric banding patients (white bars) and twelve gastric bypass patients (black bars). * $p < 0.05$ Mann Whitney U test.
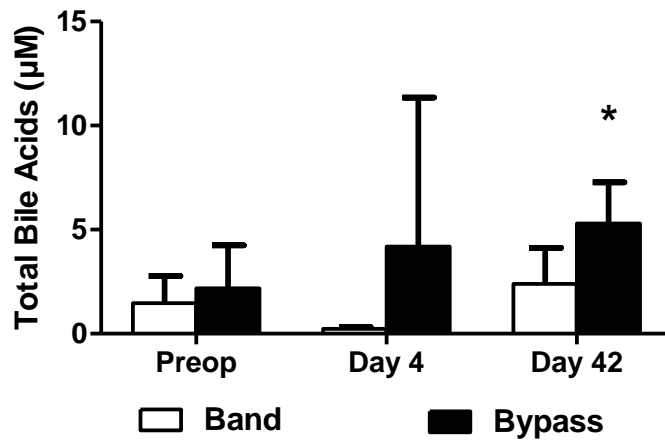
Study 2: Canine model

The responses of GLP-1 and PYY were investigated in this canine model after stimulation with food alone, bile alone, or a combination of both. At the time of these experiments, no assay for canine FGF19 level measurement was available. Baseline GLP-1 and PYY levels were the same preoperatively and postoperatively validating this model (6936 vs. 7290 for GLP-1 and 6386 vs. 5856 for PYY). Figure 21 shows the area under the curve (AUC) over 180 minutes for the postprandial GLP-1 response after the standard meal of 400 g dog and Figure 22 for PYY. In the operated dogs both food alone and bile alone lead to a significant GLP-1 and PYY response from baseline, although the responses were attenuated. The response to bile and or food alone was inferior to the combination of food and bile either pre- or post-operatively.

Figure 21. The shows the area under the curve (AUC) for the postprandial GLP-1 response after 400 g of food in dogs pre-operatively or postoperatively either receiving food alone without bile (Food), bile alone without food (Bile) or food and bile in combination (Food+Bile).* $p < 0.05$
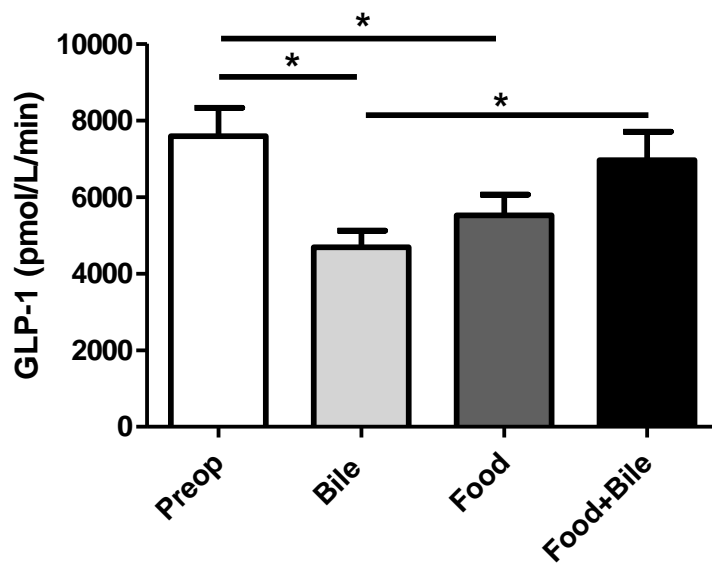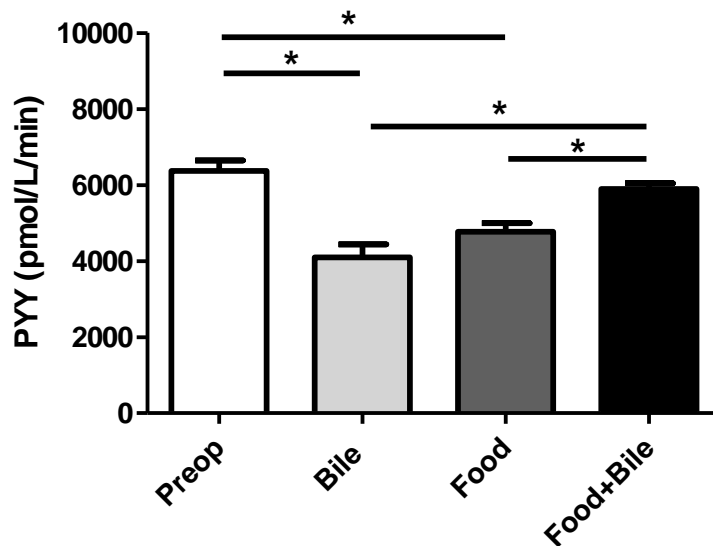
Figure 22. The shows the area under the curve (AUC) for the postprandial PYY response after 400 g of food in dogs pre-operatively or postoperatively either receiving food alone without bile (Food), bile alone without food (Bile) or food and bile in combination (Food+Bile).* p < 0.05

Study 3: Rodent model

In this rodent model, rats had bile draining into their ileum or duodenum. Both the plasma GLP-1 levels (Figure 23) and the plasma PYY levels (Figure 24) were higher in the bile in ileum group than in the bile in duodenum group (p <0.05).

Figure 23. Plasma GLP-1 levels in rats with bile draining into their duodenum or bile draining into their ileum. * p < 0.05.
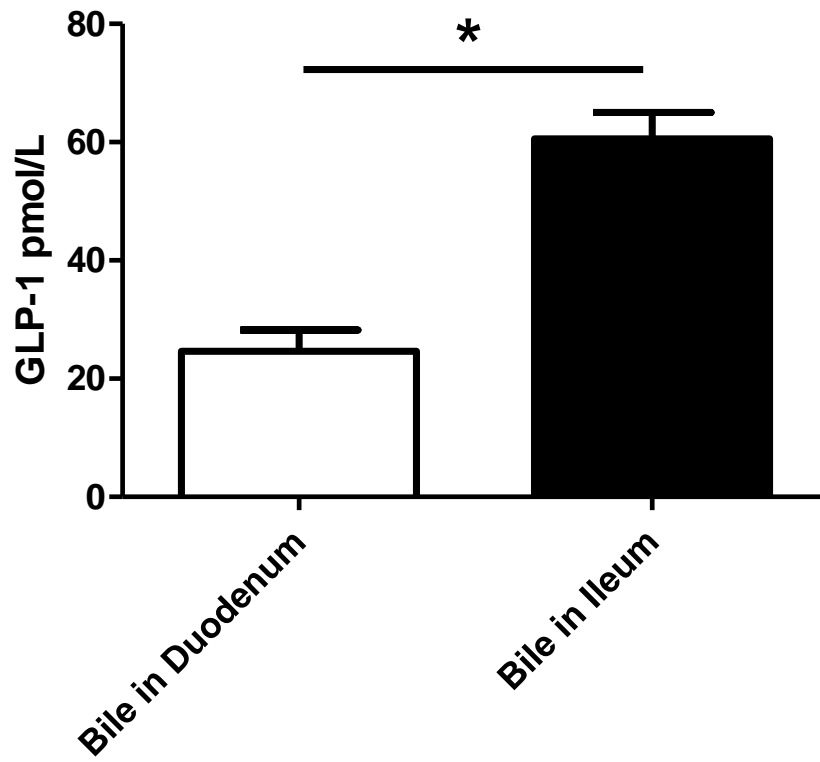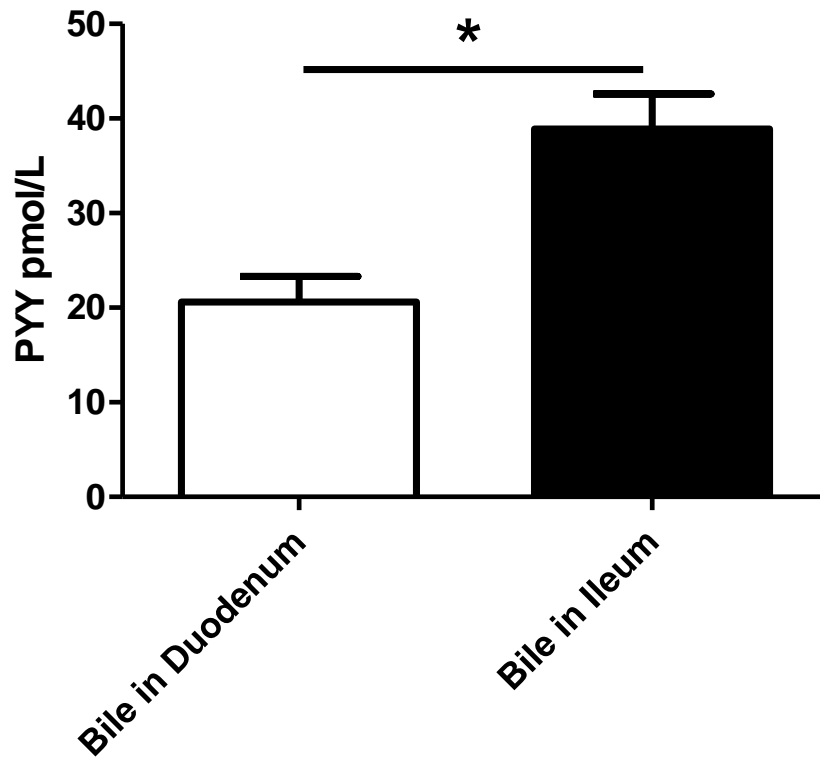
Figure 24. Plasma PYY levels in rats with bile draining into their duodenum or bile draining into their ileum. * p < 0.05.

Both the bile in ileum and bile in duodenum groups lost a similar initial amount of weight during the first four days while recovering from surgery as seen in Figure 25. The bile in duodenum group however reached their pre-operative weight within 8 days. The bile in ileum group weighed significantly less than the bile in duodenum group on day six (p <0.05) and continued to have a lower bodyweight for the duration of the study (p <0.05). Furthermore Figure 26 shows the rats in the bile in ileum group ate significantly less than the bile in duodenum group (p <0.05).

Figure 25. The weight of rats in bile in duodenum (solid line) and bile in ileum (broken line) groups before and 28 days after surgery. * p < 0.05.
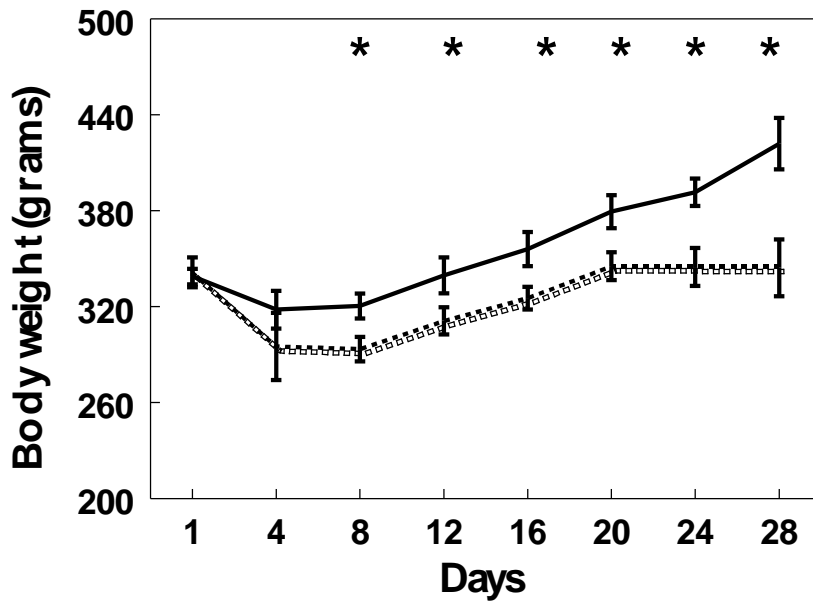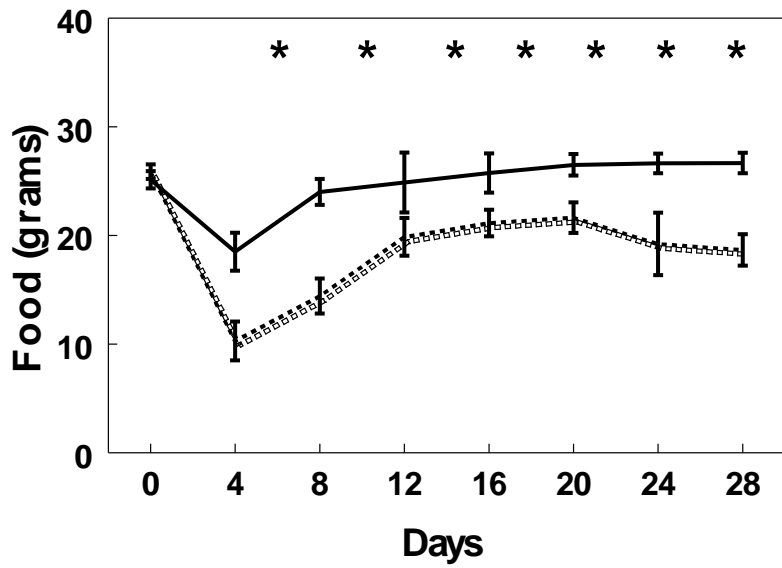
Figure 26. **Food intake of bile in duodenum (solid line) and bile in ileum (broken line) rats before and 28 days after surgery. * p < 0.05.**

Faecal parameters 25 days postoperatively did not reveal differences between the bile in the ileum and the bile in the duodenum groups at the end of the experiment in dry weight (4.12 g $\pm$ 0.20 vs. 4.28 $\pm$ 0.18, $p = 0.55$) or in calorific content (3.58 faecal kcal/24 h $\pm$ 0.4 vs. 3.58 $\pm$ 0.4, $p = 0.91$). No increase in inflammation in the bile in the ileum group was detected compared with the bile in the duodenum group as evidenced by the similarity in the white cell count (11.5 $\pm$ 0.32 x1000/microlitre vs. 11.64 $\pm$ 0.42, $p = 0.79$) or CRP levels (370.88 microg/litre $\pm$ 26.03 vs. 378.5 $\pm$ 21.71 microg/litre, $p = 0.83$).

4.4 Discussion

Fasting FGF 19 and plasma total bile acids were increased after gastric bypass, but not after gastric banding.

Food and bile alone lead to the release of GLP-1 and PYY and this was demonstrated in study 2. Food and bile together (before surgery or after surgery) resulted in a greater PYY response compared to food alone. Furthermore a trend for increased GLP-1 levels when food and bile were given together was also observed but this did not reach statistical significance.

In study 3, in the rodent model, draining endogenous bile and pancreatic fluid 10 cm proximally to the terminal ileum was associated with increased GLP-1 and PYY, reduced food intake and body weight.

These data allow me to hypothesise that one of the mechanisms by which a gastric bypass leads to the modification of the satiety gut hormone response could be due to the passage of undiluted bile via the biliopancreatic limb, a non-physiological phenomenon, or the altered delivery of bile to the terminal ileum, or a combination of both.

Exogenous bile salts have been shown to stimulate the release of gut hormones from the endocrine L-cells such as PYY in rabbit colon explants, in vivo in conscious dogs and in humans (Ballantyne, Longo, Savoca, Adrian, Vukasin, Bilchik, Sussman and Modlin 1989; Izukura, Hashimoto, Gomez, Uchida, Greeley and Thompson 1991; Adrian, Ballantyne, Longo, Bilchik, Graham, Basson, Tierney and Modlin 1993). The question of what arrives first in the terminal ileum after gastric bypass; bile, nutrients or both remains to be answered.

Näslund el showed an increase in CCK after jejuno-ileal bypass (Näslund, Grybäck, Hellström, Jacobsson, Holst, Theodorsson and Backman 1997). This may lead to increased flow of bile from the gallbladder or the liver (Näslund, Grybäck, Hellström, Jacobsson, Holst, Theodorsson and Backman 1997).

Bile may flow in the biliopancreatic limb reaching the distal L-cells in an undiluted state. Activation of TGR5 by bile acids leads to the stimulation of GLP-1 production in vitro and may explain the early and exaggerated release of incretin gut hormones such as GLP-1 and subsequently insulin (Katsuma, Hirasawa, and Tsujimoto 2005).

Bile acids may also affect glucose metabolism by weight loss. This could be attributed the enhanced satiety facilitated by the increase in satiety gut hormones as seen in Chapter 3, studies 2 and 3. Furthermore bile acids increase energy expenditure in brown adipose tissue, thus preventing obesity and insulin resistance via induction of the cyclic-AMP-dependent thyroid hormone activating enzyme type 2 iodothyronine deiodinase (Watanabe, Houten, Mataki, Christoffolete, Kim, Sato, Messaddeq, Harney, Ezaki, Kodama, Schoonjans, Bianco and Auwerx 2006). This is achieved via the TGR5 and is consistent with studies in rat models showing that gastric bypass prevented the decrease in energy expenditure after weight loss (Bueter, Löwenstein, Olbers, Wang, Cluny, Bloom, Sharkey, Lutz and le Roux 2010; Stylopoulos, Hoppin and Kaplan 2009).

Activation of the FXRα may also mediate the effects of bile acids on energy homeostasis via FGF-19 released from ileal enterocytes, leading to improved metabolic rate and decreased adiposity (Inagaki, Choi, Moschetta, Peng, Cummins, McDonald, Luo, Jones, Goodwin, Richardson, Gerard, Repa, Mangelsdorf and Kliewer 2005; Holt, Luo, Billin, Bisi, McNeill, Kozarsky, Donahee, Wang, Mansfield, Kliewer, Goodwin, Jones 2003). FGF-19 inhibits hepatic gluconeogenesis and works subsequent to insulin as a postprandial regulator of hepatic carbohydrate homeostasis (Potthoff, Boney-Montoya, Choi, He, Sunny, Satapati, Suino-Powell, Xu, Gerard, Finck, Burgess, Mangelsdorf and Kliewer 2011).

A model of studying bile acid metabolism is the use of bile acid sequestrants in patients with type diabetes. Bile acid sequestrants have been shown to be effective in improving glycaemic control in patients with type 2 diabetes (Garg and Grundy

1994; Fonseca, Rosenstock, Wang, Truitt and Jones 2008; Goldberg, Fonseca, Truitt and Jones 2008; Staels and Fonseca 2009). Garg et al conducted a randomised, double blind, crossover study of cholestyramine compared with placebo with the objective to assess clinical efficacy and tolerability of cholestyramine in patients with dyslipidaemia and type 2 diabetes (Garg and Grundy 1994). An unexpected finding was improved glycaemic control with lower mean plasma glucose, median reduction in urinary glucose excretion and a trend for lower glycated haemoglobin (Garg and Grundy 1994). Following this observation a randomised, double blind, placebo controlled, multicentre study was conducted with the objective to assess the efficacy and safety of a new bile acid sequestrant, colesevelam in patients with type 2 diabetes with inadequate glycaemic control on sulfonylurea therapy (Fonseca, Rosenstock, Wang, Truitt and Jones 2008). Colesevelam improved glycaemic control in this study and reduced LDL cholesterol levels (Fonseca, Rosenstock, Wang, Truitt and Jones 2008). A similar study with identical design was conducted in patients with type 2 diabetes and inadequate glycaemic control on insulin therapy alone or in combination with oral hypoglycaemic agents (Goldberg, Fonseca, Truitt and Jones 2008). Colesevelam was effective in these patients for both glycaemic control and lipid management (Goldberg, Fonseca, Truitt and Jones 2008).

In fact the bile acid sequestrant colevesham has been approved for the treatment of hyperglycaemia in type 2 diabetes (Rodbard, Jellinger, Davidson, Einhorn, Garber, Grunberger, Handelsman, Horton, Lebovitz, Levy, Moghissi and Schwartz 2009). Thus, some of the beneficial metabolic effect of Roux-en-Y gastric bypass on glycaemic control may be attributed to changes in bile acids. In study 1, the human

model, the previous cross sectional observation that fasting total plasma bile acids are elevated after gastric bypass surgery is confirmed in an experiment with prospective design (Patti, Houten, Bianco, Bernier, Larsen, Holst, Badman, Maratos-Flier, Mun, Pihlajamaki, Auwerx, and Goldfine 2009). Furthermore this observation was recorded at an earlier time point of 42 days after surgery reducing the bias of the effect of weight loss and reduced food intake. The changes facilitated by bile may increase satiety and improved glycaemic control via gut hormones as well as a direct effect on insulin resistance. Therefore bile may be one of the key products of the proximal gut which transfers a message to the distal gut and to other metabolically active tissues.

A limitation of study 1, the human model, is fact that the two groups were not randomised. However the groups were well matched for preoperative patient characteristics. In study 2, the canine model, migration the feeding tube or the Foley catheter may have lead to chemical peritonitis and the resulting sepsis may have affected metabolic pathways. Post-mortem examination of all subjects confirmed the correct position of both the feeding tube and the Foley catheter. In study 3, the rodent model, the differential severity and insult of the two surgical procedures, and perhaps the subsequent dissimilar response to trauma may have led to a different inflammatory response. This possibility can be excluded due the reported biochemical and histological markers of inflammation which were similar between the two groups.

Gastric bypass leads to altered bile flow which subsequently leads to an increase in fasting total plasma bile acids levels and fasting FGF19. These changes are

associated with enhanced postprandial GLP-1 and PYY responses. Changes in the bile flow may be responsible for some of the metabolic effects observed after gastric bypass surgery.

5. Summary and conclusion

In this thesis the effect of metabolic surgery on glucose metabolism is investigated. First of all evidence on the weight loss independent effect of gastric bypass, the basis of the concept of metabolic surgery is provided. Then this effect is dissected further demonstrating that gastric bypass has a favourable effect on both insulin production and insulin resistance. The change in insulin production is attributed to enhanced postprandial GLP-1 production galvanising the concept that gastric bypass is a gut hormone modulation leading to behavioural (appetite) and glycaemic modifications. Finally in an attempt to explain the improved insulin resistance, which is unrelated to GLP-1, the role of bile acids is explored. Bile flow changes after bypass and leads to a change in plasma bile acids. Furthermore modifying the flow of bile has effects on the gut hormone response, on appetite and body weight.

This thesis does not explain all the mechanism by which gastric bypass works on appetite control and glycaemic control. However I have demonstrated that it is not malabsorption of calories and it is not weight loss alone. Body weight is reduced after gastric bypass and it is a welcomed change for this population. But weight changes alone cannot explain the dramatic effect on insulin resistance. The change in bile acids provides an attractive theory, but this needs to be explored further. Previous attempts to explain the effects of gastric bypass have focused on the foregut theory (exclusion of duodenum) versus the hindgut theory (early delivery of nutrients and bile to the terminal ileum leading to the production of gut hormones). The data presented here suggest an alternative hypothesis combining the two, in

which a product of the proximal gut (bile) acts as a messenger, a hormone even, to the distal gut.

This thesis also does not fully explain the increase in insulin production. An enhanced GLP-1 postprandial response is associated with the increase in insulin production, but other factors may play a role, particularly as this increase is marked. Changes in Glucagon, GIP and even somatostatin may be responsible and further work is needed to explore this.

This thesis on its own does not provide the evidence needed for metabolic surgery to become part of the treatment algorithm for type 2 diabetes. There are no data available at the moment regarding the effect of gastric bypass on hard endpoints such as mortality and macrovascular complications. And it will be the provision of this data that can only change the status quo of metabolic surgery.

But what this thesis does show is that gastric bypass is an effective modality for glycaemic control. And understanding how this operation works is the duty of the surgeon performing it. Most surgeons want to know how their operations work. And if they understand how gastric bypass works, they may be able to make it safer or more effective.

Exploring the mechanism of action of gastric bypass is also intriguing for the physiologist as this procedure provides an excellent model of the disease and the reversal of it. The role of the gut in glucose metabolism is coming into focus again, because it can now be studied appropriately, but more importantly understanding

how metabolic surgery works is useful for the diabetologists. Because they are the ones who face the patients who experience the burden of the disease. Understanding how a treatment modality works will make it more available, removing some of the barriers in the use of metabolic surgery.

The data presented in this thesis can be used to explore the role of the gut as a target for novel intervention for type 2 diabetes, pharmacological or not. Perhaps it could lead to the consideration of randomised control trials to explore the role of metabolic surgery for type 2 diabetes.

There is a distinct lack of level 1 evidence on any aspect of metabolic surgery. The Holy Grail seems to be "head to head" comparative studies of metabolic surgery and best medical treatment as this is the gold standard. These studies are more than welcomed and definitely very important. But level 1 evidence is needed for the use of these operation in different populations (adolescents, elderly, obese women with subfertility, lower or higher BMI) and also the timing of the operations (early surgery, length of optimisation, newly diagnosed diabetics). And the aim of this thesis is to provide a basis for discussion and collaboration.

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Obesity, Gut Hormones, and Bariatric Surgery

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Abstract Obesity is becoming the healthcare epidemic of this century. Bariatric surgery is the only effective treatment for morbid obesity. Gut hormones are key players in the metabolic mechanisms causing obesity. In this review we explore the role of these hormones as facilitators of appetite control and weight loss after bariatric surgery, and we describe the now established gut–brain axis.

Introduction

Obesity is a major cause of premature death, and its prevalence is accelerating worldwide. Advice to the population to reduce food intake and take more exercise has been manifestly unsuccessful. At present, surgical procedures are the only effective therapy for long-term weight loss [1]. Bariatric surgery also has profound effects on obesity-related co-morbidities, such as type 2 diabetes, hypertension, and sleep apnea [2]. The significantly improved glycemic control in patients with type 2 diabetes mellitus may have an even greater impact on morbidity and mortality than weight loss.

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The mechanism that leads to sustained weight loss as well as diabetes remission after bariatric operations remains to be elucidated. Gut hormones have been implicated to play an important role in both weight loss and diabetes improvement after weight loss surgery.

Energy homeostasis

Through the process of energy homeostasis, energy intake and expenditure are adjusted, leading to remarkable stability in body mass over time [3–5]. From an evolutionary standpoint, it is likely that survival in an environment with constraints on the availability of food provided selection bias toward homeostatic systems that respond to reduced energy intake rather than energy excess [4–7]. Weight loss leads to an increase in the perceived hunger and a decrease in the metabolic rate. Leptin and insulin are the key messengers of the status of energy stores from the periphery to the central nervous system [3].

The central melanocortin system, which plays a crucial role in the regulation of energy homeostasis, is influenced by signals mediated through peptides made in the gut and released into the circulation [8]. These gut hormones cause hunger and satiety effects and thus have an integral role in appetite regulation.

Bariatric procedures were designed to promote weight loss by the reduction of stomach volume (laparoscopic adjustable gastric banding [LABG], laparoscopic sleeve gastrectomy [LSG]), malabsorption of nutrients (biliopancreatic diversion [BPD], duodenal switch [DS]) or a combination of both (Roux-en-Y gastric bypass [RYGB]). It is now known that calorie malabsorbtion does not occur (with the exception of the biliopancreatic diversion); however the effects of bariatric procedures are not entirely due to the

reduced stomach volume. A number of studies have shown that changes in gut hormone concentrations may partially explain the weight loss following bariatric surgery. The purpose of this review is to explore this interaction.

Gut hormones

The discovery of appetite-signaling peptides, gut hormones, has led to the establishment of the gut–brain axis. In this article anorexigenic and orexigenic hormones are reviewed in order of the level of evidence supporting their role after bariatric surgery, namely: glucagon-like peptide-1 (GLP-1), peptide YY (PYY), ghrelin, cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin (OXM), and pancreatic polypeptide (PP).

Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1), together with PYY and OXM, is released postprandially by intestinal endocrine L-cells [9]. These peptides act synergistically and cause satiety. Both GLP-1 and PYY inhibit food intake additively [10]. The former plays an important role in glucose metabolism, acting as an incretin by augmenting the insulin response to nutrients and also slowing gastric emptying and inhibiting the glucagon secretion in a glucose-dependent manner [11]. Incretins are hormones that are secreted from the gastrointestinal tract into the circulation in response to nutrient ingestion that enhances glucose-stimulated insulin secretion [11]. In addition, GLP-1 also promotes satiety, and sustained GLP-1–receptor activation is associated with weight loss in both preclinical and clinical studies [11].

In animal models GLP-1 has been shown to expand islet mass by stimulating pancreatic β-cell proliferation and induction of islet neogenesis, and it also promotes cell differentiation, from exocrine cells or immature islet progenitors toward a more differentiated β-cell phenotype [12]. Furthermore GLP-1 exerts antiapoptotic actions in vivo, resulting in preservation of β-cell mass [12]. The postprandial GLP-1 response is enhanced after RYGB, but not after LAGB in a similar manner to the PYY response [13, 14]. GLP-1, both fasting and postprandial, is elevated 20 years after JIB [15].

A recent study by Laferrère et al. showed early after RYGB, the greater GLP-1 and GIP release and improvement of incretin effect are related not to weight loss but rather to the surgical procedure itself, suggesting that this could contribute to improved glycemic control after RYGB [16].

Peptide YY

Peptide YY is a 36-amino-acid peptide, and a member of the PP-fold peptide family. The letter Y is the abbreviation

for tyrosine. The peptide is released postprandially by intestinal endocrine L-cells in proportion to the calories ingested, but it is not altered by gastric distension [17, 18]. It is present throughout the intestinal tract, with higher concentrations in the distal segments [17], and it has an inhibitory effect on gastrointestinal mobility as well as the gastric, pancreatic, and intestinal secretion [19, 20]. It has been shown to induce satiety and reduce food intake in both the obese and the non-obese [21, 22]. Furthermore obese individuals have a PYY deficiency that would reduce satiety and could thus reinforce obesity [23].

Korner et al. showed an exaggerated postprandial PYY response after RYGB, which may contribute to weight loss and to the ability of an individual to maintain weight loss postoperatively [24]. Another study on both a human model of RYGB and a rodent model of jejuno-intestinal bypass (JIB) demonstrated an increased postprandial PYY response favoring enhanced satiety [13]. Mechanistic experiments on the animal model suggested an additional to food-intake effect of RYGB on weight loss, raising the possibility of enhanced energy expenditure [13]. A prospective study of patients undergoing RYGB confirmed an increased postprandial PYY response associated with increased satiety observed as early as one month after operation [25]. The authors suggested that a gut adaptive response occurs after RYGB, which promotes satiety and is partially responsible for the weight loss following RYGB [25].

A recent study demonstrated a causality link between the exaggerated PYY and GLP-1 response and the enhanced satiety after RYGB [14]. In that study increased postprandial PYY and GLP-1 responses were seen within days after RYGB, prior to any significant weight loss. Furthermore, in a comparison of good versus poor responders to RYGB in terms of weight loss, suboptimal PYY and GLP-1 postprandial responses were associated with the poor responders. Finally, in a randomized double-blind saline controlled study of patients after RYGB and LAGB, inhibition of the gut hormone response with octeotride (a somatostatin analog) increased food intake in the RYGB group but not in the LAGB group, suggesting that gut hormones might play a key role in the reduced food intake after RYGB [14].

Comparison of patients after LAGB with patients after RYGB showed a reduced PYY response in the LAGB group in a number of studies [13, 25, 26]. However a prospective study of patients undergoing vertically banded gastroplasty (VBG) compared to non-obese controls demonstrated significantly lower PYY levels in the preoperative, obese group [27]. This difference was eliminated after VBG as PYY levels gradually increased to non-obese levels [27]. In a recent double-blind comparison of LSG and RYGB, PYY levels, both fasting and postprandial, were equally increased following the two procedures [28].

Appetite suppression, as well as weight loss was greater in the LSG group, allowing the authors to hypothesize that the sustained ghrelin reduction after LSG acts additively to the PYY response to suppress appetite [28]. This is supported by a recent animal study reporting that ghrelin attenuates the anorectic effect of PYY and GLP-1 in a dose-dependent manner [29].

Ghrelin

Ghrelin is a 28-amino acid peptide produced from the fundus of the stomach and the proximal intestine [30, 31]. It is the only known orexigenic gut hormone. Central and peripheral administration leads to increased food intake [32, 33]. Ghrelin levels increase prior to meals and are suppressed postprandially in proportion to the amount of calories ingested, suggesting a possible role in meal initiation [34, 35]. The 24-h profile of ghrelin increases following diet-induced weight loss, supporting the hypothesis that ghrelin has a role in the long-term regulation of body weight [36]. Obese individuals have lower fasting ghrelin levels, and significantly reduced postprandial ghrelin suppression compared to normal weight individuals [37].

The gene that encodes ghrelin is also responsible for the encoding of another peptide named obestatin [38]. The role of obestatin is currently controversial, although it might have a role as an anti-appetite agent [38–40].

A landmark study by Cummings et al. showed a profound suppression of ghrelin levels (24-h profile) following RYGB [36]. However the data published since are heterogeneous, with studies showing decreased fasting and postprandial[41, 42], unchanged fasting and postprandial[14, 43, 44], and increased fasting ghrelin levels after RYGB [45, 46]. The reason for this inconsistency is unclear, although multiple theories have been proposed. A study that investigated the intraoperative changes in the ghrelin levels during RYGB showed that complete division of the stomach, forming a vertical pouch, contributes to the decline in circulating ghrelin levels [47]. It is known that an intact vagus nerve is required for ghrelin to have an appetite effect [48]. Technical differences in the procedure with regard to preservation of the vagus nerve might be responsible for the differing effects, as shown by a study that demonstrated a decrease in ghrelin levels on the first postoperative day after RYGB, followed by an increase to preoperative levels at 1 month and a further increase at 12 months [49]. An alternative explanation has been proposed, suggesting that the different construction of the pouch might be responsible: with a vertical pouch, ghrelin-producing cells are more likely to be excluded than with a horizontal pouch [50]. Finally, hyperisulinemia and insulin resistance are associated with ghrelin suppression in obese individuals [51]. Therefore preoperative differences in these parameters, as well as differences in the postoperative improvement, may cause this inconsistency.

A study of patients prior to and 5 days and 2 months after BPD showed a similar response, with an initial reduction in fasting ghrelin followed by a return to the preoperative levels when food consumption resumed to almost preoperative levels [52]. This finding supports the hypothesis that although the primary source of ghrelin is the gastric mucosa, small intestinal nutrient exposure is sufficient for food-induced plasma ghrelin suppression in humans, and gastric nutrient exposure is not necessary for suppression [53].

Schindler et al. showed an increase in fasting ghrelin accompanied by a paradoxical decrease in hunger after LAGB, suggesting that weight loss is independent of circulating plasma ghrelin and relies on changes in eating behavior induced by gastric restriction [54]. Comparative studies of RYGB and restrictive procedures (LAGB and VBG) demonstrated both increased fasting ghrelin [55] and a blunted postprandial suppression of ghrelin in the restrictive procedures [25, 56].

Laparoscopic sleeve gastrectomy is a relatively new bariatric operation that was designed as a restrictive procedure. However, recent studies challenge this classification, showing accelerated gastric emptying after LSG [57]. The fact that the fundus of the stomach, the main location of ghrelin-producing cells, is excluded in the LSG procedure led to speculation that ghrelin could play a role in the mechanism of action. Two studies confirmed a decrease in fasting ghrelin levels after LSG [58, 59]. A recent prospective, double-blind study comparing RYGB and LSG confirmed a significant postprandial suppression of ghrelin postoperatively, whereas there was no change in the RYGB group [28]. In the same study the marked suppression of ghrelin levels after LSG was associated with greater appetite reduction and excess weight loss during the first postoperative year compared to RYGB [28].

The role of ghrelin in the success of bariatric surgery remains to be further elucidated. However, a review of the available data by Aylwin showed no correlation between ghrelin suppression and the degree of success in terms of weight loss, suggesting its role is only partial [60].

Cholecystokinin

Cholecystokinin (CCK) was the first gut peptide investigated for its role in appetite control. It is secreted by I cells located in the mucosa of the duodenum, jejunum, and proximal ileum in response to a meal. The peptide has a key regulatory role in the gut function: It has been implicated in gastric emptying and distension, gallbladder contraction, pancreatic secretion, and intestinal motility [61]. In addition it is involved in the regulation of food intake by inducing satiety following a meal [62].

No changes in the CCK response to a meal have been detected after RYGB or VBG in some studies, suggesting that CCK is not a mediator of appetite control and weight loss after bariatric surgery [62, 63]. However, in a different study eight subjects were studied before and after VBG, and six healthy lean volunteers were used as controls. Although there were no differences between the two groups in terms of basal CCK levels, the peak of CCK after the meal was significantly higher in obese patients after VBG than before VBG and when compared with the control group [64]. These changes could contribute to the satiety effects of gastric restrictive operations [64].

Glucose-dependent insulinotropic peptide or gastric inhibitory peptide

Glucose-dependent insulinotropic peptide (GIP), also known as gastric inhibitory peptide, is like GLP-1 in that it has an incretin effect. The common actions shared by GIP and GLP-1 on islet β-cells occur through structurally distinct yet related receptors. In addition, GIP promotes energy storage via direct actions on adipose tissue and enhances bone formation via stimulation of osteoblast proliferation and inhibition of apoptosis [11].

Rubino et al. studied the GIP response in diabetic and nondiabetic patients undergoing RYGB preoperatively and 3 weeks postoperatively. The RYGB procedure reduced GIP levels in diabetic patients, whereas no changes in GIP levels were found in the nondiabetics [64]. A study of patients undergoing JIB demonstrated elevated postprandial GIP levels postoperatively, whereas another study demonstrated a reduction in GIP [15, 65]. A reduction in GIP levels has also been detected after BPD [66].

Enteroglucagon and oxyntomodulin

Oyyntomodulin (OXM) belongs to the enteroglucagon family of peptides [12]. In humans, intravenous OXM infusion acutely decreases hunger and single-meal food intake, without reducing the palatability of the meal or causing nausea [67]. Furthermore, in a 4-week, double-blind randomized human trial, repeated OXM injections decreased body weight by 0.5 kg/week more than placebo [68]. In another study, the enteroglucagon response to glucose increased markedly after RYGB [62]. This increase in enteroglucagon occurred at the same time as development of dumping symptoms, which occurred exclusively in RYGB patients after glucose intake. Therefore enteroglucagon was proposed as a marker of the dumping syndrome after RYGB. Furthermore enteroglucagon has been shown to be elevated following JIB and BPD [66].

Table 1 Summary of gut hormone changes after Roux-en-Y gastric bypass (RYGB)

Gut hormone	Basal level	Postprandial
GLP-1	Unchanged	Increased
PYY	Unchanged	Increased
Ghrelin	Inconclusive	Decreased
CCK	Unchanged	Unchanged
GIP	Decreased	Unknown
PP	Unchanged	Unchanged

GLP-1 glucagon-like peptide-1; *PYY* peptide YY; *CCK* cholecystokinin; *GIP* glucose-dependent insulinotropic polypeptide; *PP* pancreatic polypeptide

Pancreatic polypeptide

Pancreatic polypeptide (PP) is a gut hormone released from the pancreas in response to ingestion of food. It has been shown to be reduced in conditions associated with increased food intake and elevated in anorexia nervosa. Infusion of PP causes a sustained decrease in both appetite and food intake [69].

No changes in PP were seen after RYGB, LAGB, or JIB [13, 49, 65], suggesting that PP levels are not significantly influenced by bariatric surgery.

The changes in gut hormones after RYGB, the commonest bariatric operation are summarized in Table 1.

Conclusions

Gut hormones are affected by bariatric surgical procedures in multiple ways. It is impossible to approach bariatric surgery outside the context of gut hormones and vice versa. More importantly, on many occasions, the mode of action of the bariatric operations is associated with gut hormone pathways. Research on this interaction leads not only to better understanding of these operations, but also offers new insight into the regulatory systems of metabolism. Further research will lead to refinement of the current procedures, perhaps aiming at specific metabolic pathways.

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Remission of Type 2 Diabetes After Gastric Bypass and Banding

Mechanisms and 2 Year Outcomes

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Objective: To investigate the rate of type 2 diabetes remission after gastric bypass and banding and establish the mechanism leading to remission of type 2 diabetes after bariatric surgery.

Summary Background Data: Glycemic control in type 2 diabetic patients is improved after bariatric surgery.

Methods: In study 1, 34 obese type 2 diabetic patients undergoing either gastric bypass or gastric banding were followed up for 36 months. Remission of diabetes was defined as patients not requiring hypoglycemic medication, fasting glucose below 7 mmol/L, 2 hour glucose after oral glucose tolerance test below 11.1 mmol/L, and glycated haemoglobin (HbA1c) <6%. In study 2, 41 obese type 2 diabetic patients undergoing either bypass, banding, or very low calorie diet were followed up for 42 days. Insulin resistance (HOMA-IR), insulin production, and glucagon-like peptide 1 (GLP-1) responses after a standard meal were measured.

Results: In study 1, HbA1c as a marker of glycemic control improved by 2.9% after gastric bypass and 1.9% after gastric banding at latest follow-up ($P < 0.001$ for both groups). Despite similar weight loss, 72% (16/22) of bypass and 17% (2/12) of banding patients ($P = 0.001$) fulfilled the definition of remission at latest follow-up. In study 2, within days, only bypass patients had improved insulin resistance, insulin production, and GLP-1 responses (all $P < 0.05$).

Conclusions: With gastric bypass, type 2 diabetes can be improved and even rapidly put into a state of remission irrespective of weight loss. Improved insulin resistance within the first week after surgery remains unexplained, but increased insulin production in the first week after surgery may be explained by the enhanced postprandial GLP-1 responses.

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T ype 2 diabetes mellitus is exponentially increasing because of the current epidemic of obesity. Both these lethal conditions threaten to overwhelm healthcare resources.[1] The most effective treatment for both type 2 diabetes and obesity is metabolic surgery.[2,3] The 2 most commonly performed metabolic surgery operations are the Roux-en-Y gastric bypass described in 1967 and laparoscopic gastric banding described in 1993.[4] The vast improvement in glycemic control and the concept of remission of type 2 diabetes after metabolic surgery has been established, but the underlying mechanism remains unclear.[3,5]

A meta-analysis reported improved glycemic control and remission of type 2 diabetes in 83.8% of patients following gastric bypass and 47.8% following gastric banding.[3,6,7] However, data comparing the 2 operations in the same center are limited, and definitions of remission are inconsistent between studies. The likely reasons for this include strong surgeon, patient or cultural preference for one procedure over another. This may explain the lack of published randomized controlled studies comparing different types of operations.

The improved glycemic control after gastric banding depends on weight loss, but after gastric bypass surgery this improvement occurs before weight loss. Two mechanisms have been proposed to explain this rapid normalization of glucose control after gastric bypass. The first suggests that exclusion of the duodenum and proximal jejunum may reduce insulin resistance.[8,9] The second involves exaggerated responses from the distal small bowel to nutrients. In the latter hypothesis, gut hormones produced in the distal small bowel such as glucagon-like peptide 1 (GLP-1) may act as incretins stimulating the beta cells in the pancreas to restore normal first phase insulin responses.[10]

We aimed to investigate the improved glycemic control and rate of remission of type 2 diabetes after gastric bypass and gastric banding in a homogeneous population, using the same method for assessment after each operation. Moreover, to explore potential mechanisms, we measured changes in insulin resistance and insulin production in the first week after surgery to test the hypothesis that GLP-1 contributes to the improved glycemic control.

METHODS

All human studies were performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC Protocol Number: 05/Q2202/96). Exclusion criteria included pregnancy, substance abuse, more than 2 alcoholic drinks per day. Written informed consent was obtained from all participants.

Study 1: Glycemic Control After Gastric Surgery Study

Selection criteria for study 1 included patients with type 2 diabetes who chose to have either gastric bypass or gastric banding operations. This was not a randomized study, but data were collected prospectively on 34 consecutive patients with type 2 diabetes who had surgery by the same surgeon in 1 center over a 3-year period. All patients were given unbiased information about both procedures during the initial assessment by the surgeon. This was accompanied by a patient information leaflet highlighting the advantages and disadvantages of banding and bypass. Also, patients were encouraged to attend patient support groups and to review available information on the internet with the objective of ensuring adequate information about diabetes remission, complications, and long-term postoperative diet. Patients' own preferences, based on their under-under-standing of what life would be like after the surgery, determined their operation. Fasting glucose, HbA1c, and dosage of antidiabetic medication

were recorded preoperatively and during follow-up at 3, 6, 12, 18, 24, and 36 months. In our study, we defined remission of type 2 diabetes when all the following criteria were met:

1. Fasting plasma glucose below 7 mmol/L in the absence of medical treatment for at least 3 days.
2. A 2-hour plasma glucose below 11.1 mmol/L following an oral glucose tolerance test (OGTT) as specified by the World Health Organisation.[11]
3. Glycated haemoglobin (HbA1c) below 6% after 3 months of last hypoglycemic agent usage. This measurement was added as it is often used in the surgical literature.[3,6,7]

All operations were performed laparoscopically by 1 surgeon (R.W.) between January 2004 and January 2007. At submission, the data from the latest follow-up which ranged from 24 to 36 months were obtained. For the gastric bypass (n = 22), an isolated lesser curve-based, 15 to 20 mL gastric pouch was created, and a retrocolic antegastric Roux limb was made 100-cm long for patients with a body mass index (BMI) equal to or less than 50 kg/m² and 150-cm long for patients with BMI of more than 50 kg/m².[12] The biliopancreatic limb was 25 cm for all patients.[12] For gastric banding (n = 12), the Swedish Adjustable Gastric band (Ethicon Endo-Surgery) and the LAP-BAND (Allergan) bands were used, and the pars flaccida dissection technique with gastro-gastric tunnelating sutures was used.[6] No differences in outcomes such as weight loss, hospital stay, or complications were observed between the 2 band types in our overall series of 107 patients or in this series of 12 patients reported here. Using an enhanced recovery protocol, postoperatively, patients were allowed free fluids on return to the ward, and diet was recommended when tolerated for both operations. The recommended postoperative diet for the first week was the same for the patients with banding and bypass. These diets recommend approximately 700 to 1000 kcal per day. Following gastric banding, patients were seen monthly and adjustments were performed until optimal reduction in hunger or restriction was obtained. Patients were seen at least 6 times in the first year and then yearly thereafter.

Study 2: Mechanism of Glycemic Control Study

The selection criteria for these study groups included patients with (*a*) type 2 diabetes and obesity undergoing gastric bypass (n = 17), (*b*) type 2 diabetes and obesity undergoing gastric banding (n = 9), (*c*) type 2 diabetes and obesity undergoing very low calorie diet for 1 week (n = 15), and (*d*) obesity without insulin resistance undergoing gastric bypass (n = 5). A 2-week preoperative diet of 1000 kcal was used in all patients before surgery. For the patients with type 2 diabetes, the glycemic control was optimized with pharmacotherapy and lifestyle changes for 6 months before surgery. None of the patients was on insulin during the study, and there was no difference in the usage of hypoglycemic agents between the groups with diabetes. Patients were studied immediately preoperatively and at 2, 4, 7, and 42 days after surgery, with the exception of the very low calorie diet group. These patients were studied at day 0, 2, 4, and 7. Following a 12-hour fast, a venous catheter was placed and blood was obtained. In patients who had bypass surgery, further collections of blood in tubes containing Ethylenediaminetetraacetic acid (EDTA) and aprotinin were then taken at 15, 30, 60, 90, 120, 150, and 180 minutes after a 400 kcal standard meal. Samples were immediately centrifuged and stored in a −80°C freezer until analyzed with an established GLP-1 assay,[13] automated glucose analyzer (Abbott laboratories, Chicago, IL), and an automated insulin assay (Abbott laboratories, Chicago, IL). Delta insulin was defined as the difference between a 0 minute and 15 minute insulin measurement.[14]

Results were analyzed using SPSS statistical software (SPSS Inc, Chicago, IL). Data are either expressed as mean (standard deviation) or median (range). Fisher exact test was used for categorical data. Time taken to diabetes remission was compared between operative groups by log-rank test. ANOVA with post hoc Dunnett test was used for HOMA-IR, delta insulin, and GLP-1 responses. The Mann-Whitney U test was used for nonparametric demographic data. The unpaired t test was used for parametric demographic data. Results were considered significant if $P < 0.05$.

RESULTS

Study 1: Glycemic Control After Gastric Surgery

At Musgrove Park Hospital, similar numbers of diabetic and nondiabetic patients had gastric bypass (n = 109) and gastric banding (n = 107) between January 2004 and January 2007, supporting the premise that the surgeon did not have a preference. A total of 34 consecutive patients with type 2 diabetes requiring hypoglycemic medication were identified preoperatively (16%). Of the 34 patients, 22 patients underwent gastric bypass and 12 underwent gastric banding.

Surgery was performed between January 2004 and January 2007 and at submission, minimum follow-up was 24 months (range, 24–36 months), and no patient was lost to follow-up. There were no significant differences in the demographic characteristics, duration of diabetes, or pre- and postoperative BMI between the groups (Table 1). Patients remained obese postoperatively with BMIs of 33 (5.2) kg/m² for gastric bypass and 32.6 (5.1) kg/m² for gastric banding patients. There were no significant differences in weight at any time point during the 3-year period (Fig. 1). Twelve of the 22 gastric bypass patients (54%) and 4 of the 12 gastric banding patients (33%) required insulin therapy preoperatively ($P = 0.04$). There was one early complication in a gastric bypass patient who recovered after requiring a reoperation on postoperative day 1 for a small bowel enterotomy. The patient was discharged fully recovered after 114 days and the case was described elsewhere.[15] Diabetic gastric bypass and gastric banding patients achieved less weight loss compared with the nondiabetic patients in our overall series (data not shown). There was no difference in median length of stay for gastric bypass between patients with diabetes (n = 22, 4 days range, 1–114) or without diabetes (n = 87, 3 days range, 1–44) ($P = 0.47$). The nondiabetic patient that required hospital admission for 44 days was also described

TABLE 1. Study 1: Patient Characteristics Presented as Mean (Standard Deviation) Except Where the Median (Range) is Indicated

	Bypass	Banding	*P* *
Age (yr)	46.0 (9.6)	47.4 (10.9)	0.71
Preoperative weight (kg)	137.4 (22.9)	137.7 (31.8)	0.97
Preoperative BMI	47.4 (7.2)	47.1 (7.1)	0.90
Preoperative HbA1c	9.1 (1.9)	8.4 (1.7)	0.29
Duration of diabetes (yr)	7.0 (1–18)	5.5 (1–14)	0.61
Proportion on insulin preoperatively (%)	12/22 (54%)	4/12 (33%)	0.04
Follow-up (mo)	29.5 (8.0)	33.0 (7.5)	0.21
% Total weight loss	29.5 (8.4)	28.5 (10.0)	0.76
% Excess BMI loss	63.3 (30.8–103.2)	62.3 (45.1–105.9)	0.92
BMI at follow-up	33.0 (5.2)	32.6 (5.1)	0.80
HbA1c at follow-up	6.2 (1.2)	6.5 (1.2)	0.59

Fisher exact test was used for categorical data.
* $P < 0.05$ by t test; or Mann - Whitney where median is reported.
BMI indicates body mass index. HbA1c indicates glycated haemoglobin.

elsewhere and was discharged fully recovered following treatment for an anastomotic leak.[15] For gastric banding, there was no difference in median length of stay between patients with diabetes (n = 12, 1 day range, 1–2) or without diabetes (n = 95, 1 day range, 1–3) (P = 0.68). Diabetic and nondiabetic patients after bypass stayed longer in hospital than patients after banding ($P < 0.001$).

HbA1c improved by 2.9% after gastric bypass and 1.9% after gastric banding ($P < 0.001$ compared with preoperatively for both groups), reflecting weight loss, the additional effect of bypass, and the hypoglycemic medication, respectively. The fasting plasma glucose results were 6.6 (2.7) mmol/L (2 hour post-OGTT glucose 8.1 [5.8]) for the gastric bypass group and 7.3 (2.4) mmol/L (2 hour post-OGTT glucose 11.8 [3.4]) for the gastric banding group (P = 0.43 for fasting and 0.052 for post-OGTT glucose) at latest followup. The time to diabetes remission was significantly shorter for gastric bypass than gastric banding with a hazard ratio of 8.2 (P = 0.001, 95% confidence interval, 1.8–36.7). At 1-year follow-up (Fig. 2), 15 of the 22 gastric bypass patients (68%) were in a state indistinguishable from remission compared with no gastric banding patients ($P < 0.001$), whereas the weight loss at 1 year was not significantly different between bypass, 25.2% (8.2) and banding patients, 20.4% (9.1), (P = 0.14). At latest follow up, 16 gastric bypass patients (72%) were in remission compared with 2 gastric band patients (17%) (P = 0.01), whereas the weight loss was not significantly different between bypass and banding patients 29.5% (8.4) versus 28.5(10), P = 0.76.

Study 2: Mechanism of Glycemic Control Study

The demographic characteristics of the patients are shown in Table 2. None of the bypass, banding, or diet patients required insulin before or after the interventions. In patients with type 2 diabetes, insulin resistance (measured by HOMA-IR) improved within 7 days after gastric bypass, whereas after gastric banding or a 1000 kcal diet HOMA-IR remained unchanged (Fig. 3). Although the insulin resistance in the patients with diabetes and gastric bypass was reduced by 44% over the first week, the HOMA-IR remained unchanged at normal levels in those without diabetes who had gastric bypass (Fig. 3). In contrast, delta insulin increased in both groups of gastric bypass patients, with or without type 2 diabetes, as early as 2 days
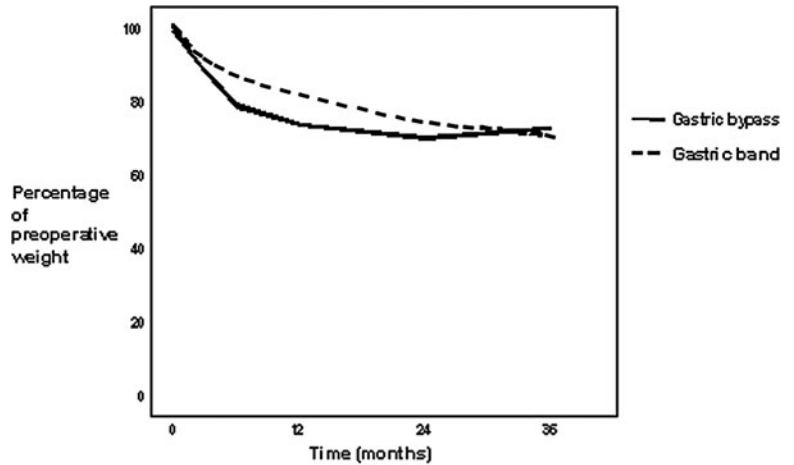

Months	0	3	6	12	24	36
Band n=	12	12	12	12	12	8
Bypass n=	22	22	22	22	22	11

FIGURE 1. Weight loss over time for gastric bypass (n = 22) and gastric banding (n = 12) patients over a 3-year period. No differences were detected in weight loss at 3, 6, 12, 24, and 36 months between bypass and banding.
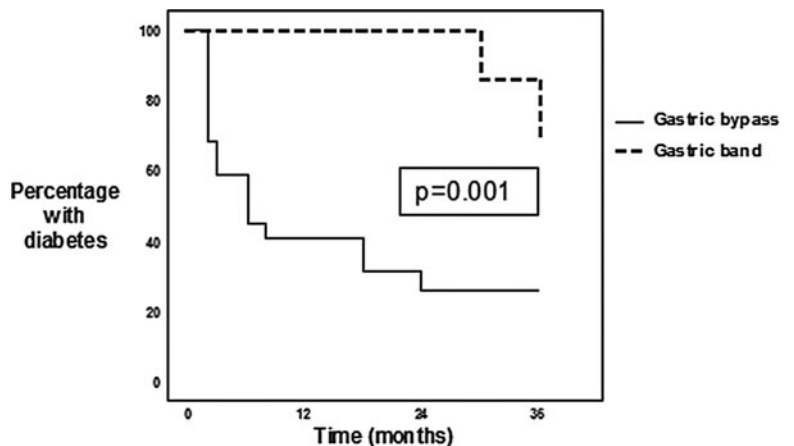

FIGURE 2. Kaplan-Meier curve for time to remission of type-2 diabetes in patients who had gastric bypass (solid line) or gastric banding (broken line) over a 3-year period. Follow-up at each time point was the same as in Figure 1.

TABLE 2. Study 2: Demographics of Patients Undergoing Standard Meal Tests Preoperatively and at Day 2, 4, 7, and 42

	Diet + DM	Band + DM	Bypass + DM	Bypass Non DM	P
Number	15	9	17	5	
Age (yr)	43.7 (10)	48.1 (8.7)	48.3 (8.6)	42.4 (6.9)	NS
Preoperative weight (kg)	139.0 (37.5)	129.5 (28.2)	138.9 (35.5)	143.8 (21.6)	NS
Preoperative BMI	46.9 (8.1)	43.5 (11.7)	48.0 (5.7)	52.2 (3.3)	NS
Preoperative glucose	5.9 (1.1)	6.1 (1.2)	7.1 (2)	5.9 (1.1)	NS
Preoperative HOMA-IR	7.1 (3)	8.8 (9.6)	9.2 (7.8)	2.1 (0.4)	<0.05

Comparisons are made between patients with diabetes (DM) who had very low calorie diet, gastric banding, gastric bypass or patients who did not have diabetes but underwent gastric bypass.

BMI indicates body mass index. NS indicates non-significant. HOMA-IR indicates homeostatic model assessment insulin resistance.
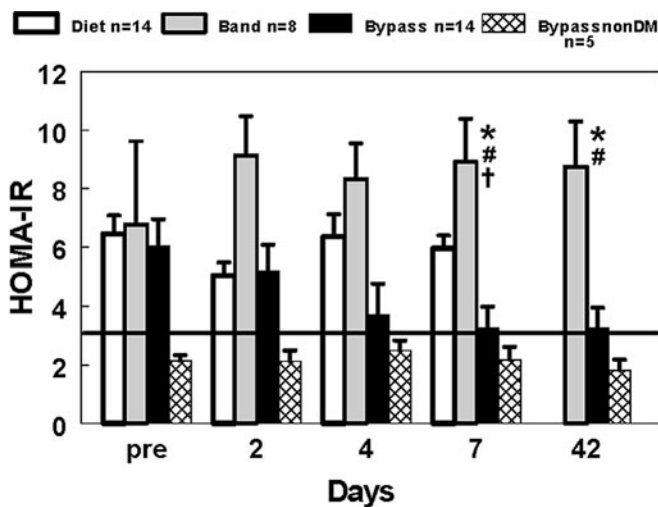

FIGURE 3. Progression of insulin resistance in patients with type 2 diabetes following a 1000 kcal diet with no surgery over 7 days (n = 15) and gastric band (n = 9), gastric bypass (n = 17) and nondiabetic patients following gastric bypass (n = 5) over a period of 42 days. The solid line indicates the level at which HOMA-IR is considered to indicate insulin resistance. $*P < 0.05$ compared with preoperative state. $\#P < 0.05$ compared with gastric banding at same time point. $\dagger P < 0.05$ compared with very low calorie diet at same time point.

FIGURE 4. Changes in delta insulin are shown after gastric bypass in patients with type 2 diabetes (n = 17) and without type 2 diabetes (n = 5), defined as the difference between fasting and 15 minutes postprandial insulin. $*P < 0.05$ compared with preoperative state for both groups.

after surgery (Fig. 4). This increase is mirrored for GLP-1 in both bypass groups (Fig. 5). Fasting levels of GLP-1 in the group with type 2 diabetes who had gastric bypass did not change over the first 42 days (data not shown, $P = 0.44$).

DISCUSSION

This study is a direct comparison of the improved glycemic control and "remission" rate for type 2 diabetes in the 2 commonest metabolic operations performed in a single center serving a homogeneous population. In study 1, the bypass and banding groups had similar age, sex, and BMIs pre- and postoperatively. The striking finding was that at latest follow-up, the HbA1c improved by 1.9% or more in both groups, whereas the proportion achieving fasting plasma glucose concentrations below 7 mmol/L (off all hypoglycemic medication) was much higher for gastric bypass (72%) than for gastric banding (17%). In addition, the rate of "remission" over time was markedly quicker for the gastric bypass. The latter 2 findings
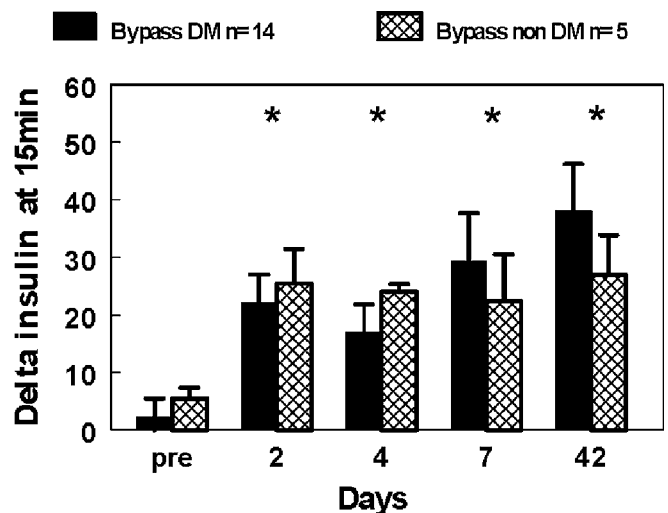
strongly suggest that the rapidly improved glycemic control cannot be attributed to weight loss alone.

In addition to these findings, in study 2 we found an unexpected improvement in insulin resistance of 44% within 7 days after gastric bypass. Insulin resistance after laparoscopic gastric banding or the very low calorie diet did not change within 7 days. Furthermore, patients without diabetes undergoing gastric bypass did not have any change in their insulin resistance.

Insulin production as measured by delta insulin between 0 and 15 minutes also increased after gastric bypass in all patients irrespective of whether they had type 2 diabetes. Both responses of GLP-1 and delta insulin reached significance within 2 days after surgery. Thus, it is possible that the changes in insulin production are associated with the enhanced GLP-1 responses. However, fasting GLP-1 levels remain unchanged within the first 42 days.

The patients participating in study 2, the mechanism of glycemic control study, did not require insulin therapy before surgery. The patients also had good glycemic control following a 6 month medical optimization program before the study and were thus less likely to be glucotoxic. This may explain why changes in diet and calorie consumption alone in the banding and very low calorie diet groups did not change HOMA-IR.
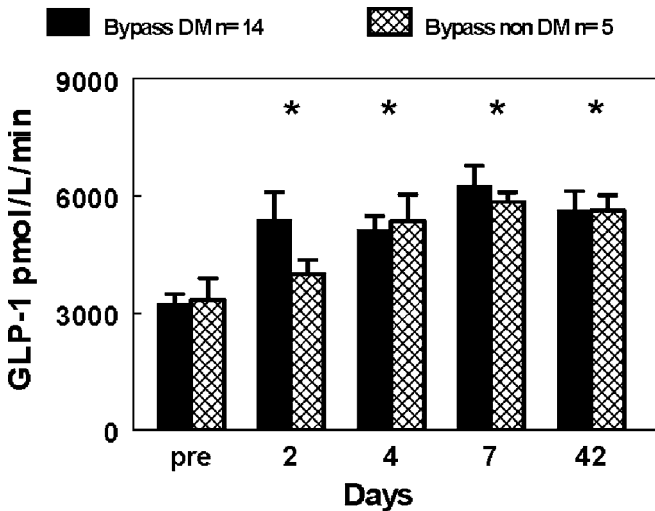
FIGURE 5. Area under the curve over a 3-hour period GLP-1 after gastric bypass in patients with type 2 diabetes (n = 17) and without type 2 diabetes (n = 5) after a 400 kcal meal. *$P < 0.05$ compared with preoperative state for both groups.

Other direct comparisons of glycemic control between early changes after gastric bypass and gastric banding also favored gastric bypass, suggesting that there may be a different mechanism, independent of weight loss, which explains diabetes remission after bypass.[16,17] Although in study 1, the glycemic control study, the remission rate for the gastric bypass group is comparable with other studies, the remission rate for the gastric banding group was lower.[4–7,16–20] The differences in our results could be attributed to the way we defined remission of type 2 diabetes as fasting plasma glucose below 7 mmol/L and 2 hour post oral glucose tolerance test plasma glucose below 11.1 mmol/L with a HbA1c below 6% off all hypoglycemic agents, which is more stringent than previous studies.[5,14,17–20] Moreover, study 1, the glycemic control study, only included patients with type 2 diabetes requiring medication of which a substantial portion required insulin. In addition, the duration of diabetes before surgery ranged up to 18 years with a median of 7 years in the bypass group and 5.5 years in the banding group. Prolonged duration of disease has been associated with poorer remission rates of diabetes after bariatric surgery.[7] Also, the smaller proportion of gastric banding patients requiring insulin in our study might be expected to favor the banding group.

Schauer et al reported inferior weight loss after gastric bypass in patients with diabetes compared with the overall cohort, for reasons that are not known.[3,7] This observation could explain the similar weight loss between our gastric bypass and banding groups. Furthermore, intensive follow-up with regular band adjustments has been shown to improve weight loss outcomes.[21] This is a possible explanation for the lack of difference in the weight loss between the 2 groups. Kim et al also reported no difference in weight loss between banding and bypass in nondiabetic obese subjects.[18]

Insulin resistance would usually be expected to increase after major abdominal operations where bowel manipulation and duration are similar to gastric bypass.[22] Previous reports suggested that the effect of gastric bypass on glucose metabolism may be partly because of endocrine mechanisms.[8,23] A recent study showed a reduction in insulin resistance following both band and diet in addition to bypass.[24] However, the earliest time point patients were studied was 4 weeks

postintervention, and therefore the insulin resistance changes were attributed to weight loss.[24]

Data published from our unit has shown that delta insulin was unchanged after 19 months following gastric banding.[14] Changes in responses of the incretin, GLP-1, are associated with the enhanced delta insulin.[25] However, the rapid improvement in insulin resistance after gastric bypass surgery is observed even if patients are kept nil by mouth[9] and thus is unlikely related to GLP-1, as fasting levels of GLP-1 did not change in our study.

A limitation of the glycemic control after gastric surgery study (study 1) is the fact that it was not randomized. Another limitation was the relatively small groups which may explain the nonsignificant difference in 2 hour post-OGTT glucose results between the patients with bands and bypasses. However, the groups were well-matched and patients selected their own operation without any bias from the surgeon. For the mechanisms of glycemic control study (study 2), HOMA-IR was preferred over more invasive techniques, as it has been used to track changes in insulin resistance over time.[26,27] Thus, our results can now be used to power new randomized controlled trials using more invasive measurements of insulin resistance. Another limitation is the number of calories consumed by the bypass and banding group in the first week postoperatively. These were in the same order as that of the very low calorie diet group, although it was not practical to achieve an identical match.

In conclusion, gastric bypass and gastric banding lead to vastly improved glycemic control, whereas type 2 diabetes is more likely to go into a state indistinguishable from remission after gastric bypass than gastric banding, irrespective of weight loss. Increased insulin production in the first week after gastric bypass may be explained by the enhanced postprandial GLP-1 responses. However, the mechanism of the rapidly improved insulin resistance after gastric bypass remains unclear. Even despite our imperfect understanding, the data suggest that sustained improvements in glycemic control and even remission of type 2 diabetes can now be considered a realistic option following metabolic surgery.

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CLINICAL RESEARCH

The Gut Hormone Response Following Roux-en-Y Gastric Bypass: Cross-sectional and Prospective Study

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Abstract

Background Bariatric surgery is the most effective treatment option for obesity, and gut hormones are implicated in the reduction of appetite and weight after Roux-en-Y gastric bypass. Although there is increasing interest in the gut hormone changes after gastric bypass, the long-term changes have not been fully elucidated.

Methods Thirty-four participants were studied cross-sectionally at four different time points, pre-operatively ($n=17$) and 12 ($n=6$), 18 ($n=5$) and 24 months ($n=6$) after laparoscopic Roux-en-Y gastric bypass. Another group of patients ($n=6$) were studied prospectively (18–24 months). All participants were given a standard 400 kcal meal after a 12-h fast, and plasma levels of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) were correlated with changes in appetite over 3 h using visual analogue scores.

Results The post-operative groups at 12, 18 and 24 months had a higher post-prandial PYY response compared to pre-operative ($p<0.05$). This finding was confirmed in the prospective study at 18 and 24 months. There was a trend for increasing GLP-1 response at 18 and 24 months, but this did not reach statistical significance ($p=0.189$) in the prospective study. Satiety was significantly reduced in the post-operative groups at 12, 18 and 24 months compared to pre-operative levels ($p<0.05$).

Conclusions Roux-en-Y gastric bypass causes an enhanced gut hormone response and increased satiety following a meal. This response is sustained over a 24-month period and may partly explain why weight loss is maintained.

Keywords Roux-en-Y gastric bypass · RYGB · Gut hormones · Peptide YY · Glucagon-like peptide-1 · GLP-1

Introduction

Surgical procedures are currently the only effective therapy for long-term weight loss [1]. There are also profound effects on obesity-related comorbidities, such as type 2 diabetes, hypertension and sleep apnoea, following bariatric surgery [2].

Gut hormones cause hunger and satiety effects and thus have an integral role in appetite regulation. The discovery of these appetite-signalling peptides, the gut hormones, has led to the establishment of the concept of the gut–brain axis. They have been implicated to play an important role in both weight loss and diabetes improvement after gastric bypass surgery [3].

Roux-en-Y gastric bypass (RYGB) was designed to promote weight loss due to a combination of reduced stomach volume and malabsorption of nutrients. It is now known that calorie malabsorption does not explain the weight loss; however the effects of RYGB are not entirely due to the reduced stomach volume. A number of studies have shown that changes in gut hormone concentrations may partly explain the weight loss following gastric bypass surgery.

We have recently demonstrated a causative link between the exaggerated peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) response and the enhanced satiety after RYGB [4]. In that study increased post-prandial PYY and

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Table 1 Demographic characteristics of patients in cross-sectional study and prospective study

Group	Number (n)	Age	Sex (no. of women)
Pre-operative	17	47.8±2.0	11
12 months post-operative	6	45.2±4.0	5
18 months post-operative	5	49.6±3.3	3
24 months post-operative	6	43.3±4.1	6
Prospective study	6	47.8±2.0	5

GLP-1 responses were seen within days after RYGB, prior to any significant weight loss. Furthermore in a comparison of good versus poor responders to RYGB in terms of weight loss, suboptimal PYY and GLP-1 post-prandial responses were associated with the poor responders. Finally, in a randomised double-blind saline controlled study of patients after RYGB and laparoscopic adjustable gastric banding (LAGB), inhibition of the gut hormone response with octeotride (a somatostatin analogue) increased food intake in the RYGB group, but not in the LAGB group, suggesting that gut hormones might play a role in the reduced food intake after RYGB [4].

Although there is increasing interest in the gut hormone changes after gastric bypass, the long-term changes have not been fully elucidated. The primary aim of this study was to evaluate the changes in the PYY and GLP-1 response in the first 24 months after gastric bypass.

Materials and Methods

All human studies were performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC Protocol Number: 05/Q2202/96). Exclusion criteria included pregnancy, substance abuse, more than two alcoholic drinks per day and aerobic exercise for more than 30 min three times per week. Written informed consent was obtained from all participants.

Thirty-four participants were studied cross-sectionally at four different time points, pre-operatively ($n=17$) and 12 ($n=6$), 18 ($n=5$) and 24 months ($n=6$) after RYGB. Another group of patients ($n=6$) were studied prospectively. Following a 12-h fast, a venous catheter was placed and blood was obtained. Further collections of blood in tubes containing EDTA and aprotinin were then taken at 15, 30, 60, 90, 120, 150 and 180 min after a 400 kcal standard meal. The 400-kcal meal macromutrient content was 48.8% carbohydrate, 10.2% protein and 41% fat. Samples were immediately centrifuged and stored in a −80°C freezer until analysis. Plasma levels of the gut hormones PYY and GLP-1 were compared at each time point. Visual analogue scales (VAS) were used to measure hunger and satiety immediately before consumption of the meal and at 60, 120 and 180 min later.

Surgical Technique

Laparoscopic Roux-en-Y gastric bypass was performed, creating an isolated, lesser curve based, 15–20-mL gastric pouch excluding the fundus [5]. A retrocolic antegastric Roux limb was made 100 cm long for patients with a body mass index (BMI) equal to or less than 50 kg/m^2 and 150 cm long for patients with BMI of more than 50 kg/m^2 [5]. The bilio-pancreatic limb was 25 cm for all patients. The subjects included in the study had similar outcomes in terms of excess weight loss compared with the rest of the surgical cohort as a whole (data not shown).

Hormone Assays

All samples were assayed in duplicate. PYY-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, which measures both the full length (PYY1–36) and the fragment (PYY3–36) [6, 7]. Plasma GLP-1 was measured in duplicate by established in-house radioimmunoassay [8, 9].

Statistical Analysis

Patient demographics, BMI and hormone levels are expressed as means ± standard error of the mean. Values
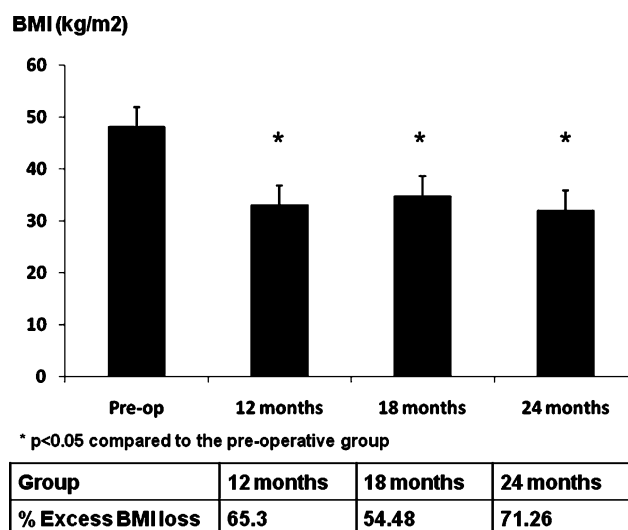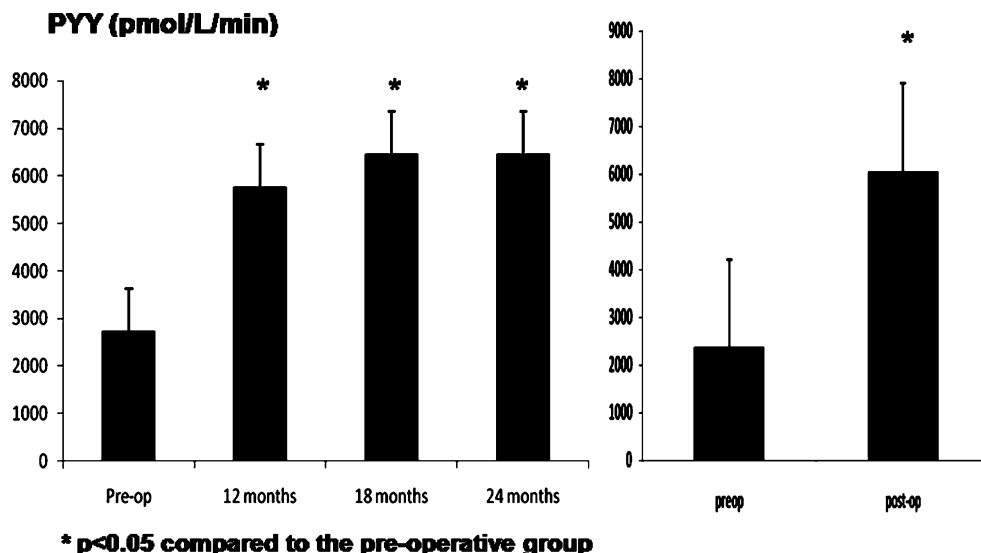
BMI (kg/m2)

* p<0.05 compared to the pre-operative group

Group	12 months	18 months	24 months
% Excess BMI loss	65.3	54.48	71.26

Fig. 1 BMI before and 12, 18 and 24 months after gastric bypass

Fig. 2 The PYY response in the cross-sectional (12, 18 and 24 months) and prospective studies (18–24 months combined)

for the area under the curve were calculated with the use of the trapezoidal rule. End points were compared with the use of two-tailed, paired Student t tests or analysis of variance. Results were analysed using SPSS statistical software (SPSS Inc., Chicago, IL, USA).

Results

Cross-sectional Study

The characteristics of the four groups can be seen in Table 1. The BMI in the post-operative groups is significantly lower than the pre-operative group as expected (Fig. 1). However there was no significant difference between the three post-operative groups. The PYY post-

prandial response, measured as area under the curve, was significantly enhanced in all the post-operative groups compared to the pre-operative group (Fig. 2). There was a trend for increased GLP-1 response in the post-operative groups, but this did not reach statistical significance (Fig. 3). Satiety as measured with the VAS was significantly increased in all post-operative groups (Fig. 4).

Prospective Study

The characteristics of the patients participating in the prospective study can be seen in Table 1. The PYY response was enhanced post-operatively in this group confirming the findings of the cross-sectional study (Fig. 1). However the GLP-1 response was not increased significantly post-operatively ($p=0.189$).

Fig. 3 The GLP-1 response in the cross-sectional (12, 18 and 24 months) and prospective studies (18–24 months combined)
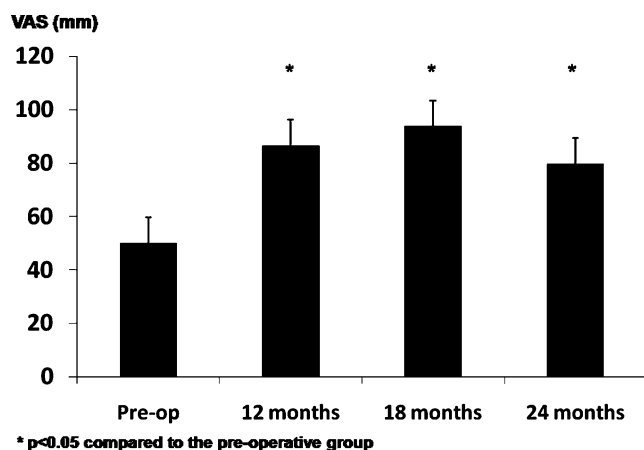
VAS (mm)

* p<0.05 compared to the pre-operative group
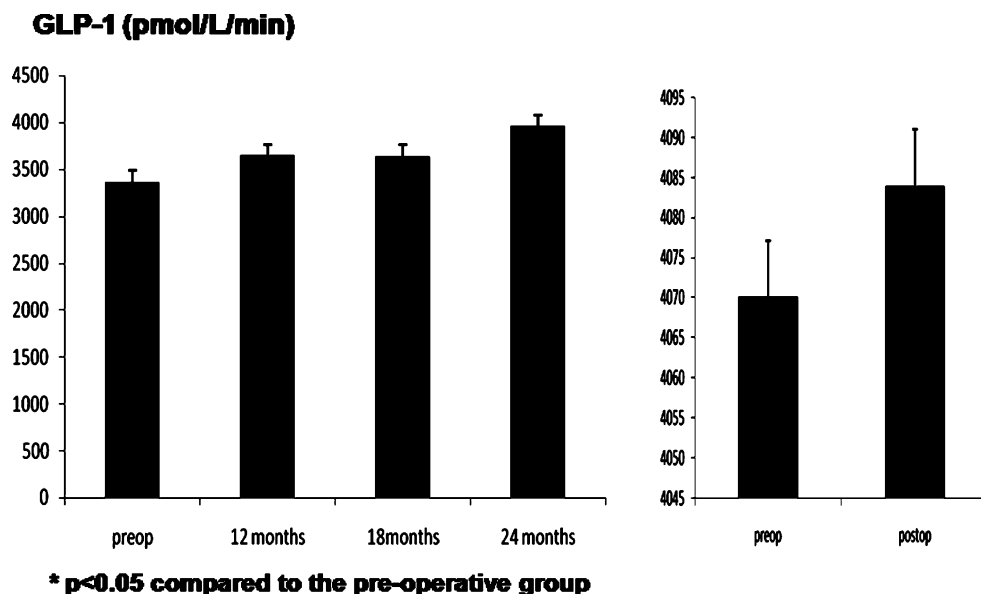
Fig. 4 The satiety measured with the VAS in all postoperative groups combined

Discussion

This study confirms that the enhanced PYY post-prandial response and the associated enhanced satiety following gastric bypass surgery are sustained for at least 24 months post-operatively. The findings of the cross-sectional study are further supported by the findings of the prospective study. The fact that the above cannot be confirmed for the GLP-1 response may be due to the small number of patients included in the prospective study.

This study is one of the very few studies investigating the PYY post-prandial response for longer than 6 months. Limitations include the low number of patients and the cross-sectional design. However the impact of the latter was minimised with the prospective study. The majority of participants were female, reflecting the higher number of females undergoing bariatric surgery.

Korner et al. showed an exaggerated post-prandial PYY response after RYGB in a study of 12 patients post-RYGB with a mean post-operative period was 35 months [10]. Chan et al. also showed an enhanced response in another cross-sectional study of six patients 18 months following RYGB [11]. Furthermore a recent study on eight patients 9–48 months following RYGB confirmed the above findings [12]. Two prospective studies showed an increase in the basal (starving) PYY levels following RYGB [13, 14].

Prospective studies measuring the post-prandial PYY response in the long term are limited. Borg et al. showed an increased post-prandial response in a prospective study with 6-month follow-up [15]. The same study suggested that gut adaptation might play a role in long-term changes in the gut hormone response. Three studies with 12-month follow-up showed similar results [16–18]. Our results are in accordance with the aforementioned studies with a minimum follow-up of 6 months extending up to a year. These results allow us to hypothesise that the enhanced PYY response is

sustained for at least 24 months and could explain the well-described long-term weight loss after gastric bypass surgery.

Similar studies investigated the changes in the GLP-1 response. Cross-sectional studies have indicated an enhanced post-prandial response following RYGB [10, 12, 19], while prospective studies with a follow-up extending to 12 months confirmed this [15, 20–22]. Our findings showed a trend for an enhanced GLP-1 response post-operatively, but this did not reach significance.

In conclusion, RYGB leads to an enhanced PYY post-prandial response and increased satiety. These changes are sustained over a 24-month period. These findings suggest a role for gut hormones in the maintenance of weight loss in the long term.

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Effect of the definition of type II diabetes remission in the evaluation of bariatric surgery for metabolic disorders

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Background: The American Diabetes Association recently defined remission of type II diabetes as a return to normal measures of glucose metabolism (haemoglobin (Hb) A1c below 6 per cent, fasting glucose less than 5·6 mmol/l) at least 1 year after bariatric surgery without hypoglycaemic medication. A previously used common definition was: being off diabetes medication with normal fasting blood glucose level or HbA1c below 6 per cent. This study evaluated the proportion of patients achieving complete remission of type II diabetes following bariatric surgery according to these definitions.
Methods: This was a retrospective review of data collected prospectively in three bariatric centres on patients undergoing gastric bypass, sleeve gastrectomy and gastric banding.
Results: Some 1006 patients underwent surgery, of whom 209 had type II diabetes. Median follow-up was 23 (range 12–75) months. HbA1c was reduced after operation in all three surgical groups ($P < 0·001$). A total of 72 (34·4 per cent) of 209 patients had complete remission of diabetes, according to the new definition; the remission rates were 40·6 per cent (65 of 160) after gastric bypass, 26 per cent (5 of 19) after sleeve gastrectomy and 7 per cent (2 of 30) after gastric banding ($P < 0·001$ between groups). The remission rate for gastric bypass was significantly lower with the new definition than with the previously used definition (40·6 *versus* 57·5 per cent; $P = 0·003$).
Conclusion: Expectations of patients and clinicians may have to be adjusted as regards remission of type II diabetes after bariatric surgery. Focusing on improved glycaemic control rather than remission may better reflect the benefit of this type of surgery and facilitate improved glycaemic control after surgery.

Introduction

Although the concept of remission of type II diabetes following gastric bypass and gastric banding surgery has gained acceptance, the definitions of remission and cure of diabetes have been controversial[1]. Recently a consensus group comprising experts in endocrinology, diabetes education, transplantation, metabolism, metabolic surgery and haematology–oncology proposed new definitions of partial and complete remission of type II diabetes[1]. There are two important changes. First, a standard metric is now proposed for reporting rates of diabetes remission, which may facilitate comparison of future reports. Second,

the new definitions rely on more stringent criteria for glycaemic control than previous criteria, so rates of diabetes remission are likely to be lower[2,3]. This has implications for treatment as it suggests that more patients are presumed to benefit from hypoglycaemic treatment after bariatric surgery. This study evaluated diabetes remission rates after gastric bypass, gastric banding and sleeve gastrectomy according to the 2009 consensus definitions.

Methods

Data were collected prospectively in three bariatric surgery centres, two in the UK and one in Norway[4]. For the UK

centres, data were also collected from the National Bariatric Surgery Registry, the result of a collaboration between the Association of Laparoscopic Surgeons of Great Britain and Ireland, Association of Upper Gastrointestinal Surgery and British Obesity and Metabolic Surgery Society. The analysis was retrospective. Permission was obtained from the Imperial College Healthcare NHS Trust Clinical Governance and Patient Safety Committee, and from the Norwegian Data Protection Agency. The study included patients with a preoperative diagnosis of type II diabetes who underwent laparoscopic bariatric surgery between August 2004 and July 2009. Patients were seen at 6 weeks, 6 months and 12 months after surgery.

Remission of type II diabetes was previously defined as being off diabetes medication with normal fasting blood glucose (5·6 mmol/l) or a HbA1c level of less than 6 per cent[2,3]. This definition was used in recent meta-analyses of the effect of bariatric surgery on type II diabetes[2,3]. In the 2009 consensus document, partial remission of diabetes was defined as hyperglycaemia (HbA1c less than 6·5 per cent and fasting glucose 5·6–6·9 mmol/l) at least 1 year after surgery in the absence of active hypoglycaemic pharmacological therapy or ongoing procedures[1]. Complete remission was defined as a return to normal measures of glucose metabolism (HbA1c less than 6 per cent, fasting glucose below 5·6 mmol/l) at least 1 year after surgery without hypoglycaemic pharmacological therapy or ongoing procedures[1]. Prolonged remission was defined as complete remission of at least 5 years' duration and was outside the remit of the study.

Statistical analysis

Continuous data, expressed as mean(s.d.), were compared using one-way ANOVA. Fisher's exact test and the Freeman–Halton extension of Fisher's exact test were used for analysis of categorical data. $P \leq 0.050$ was considered statistically significant. Data were analysed using SPSS® version 14 (SPSS, Chicago, Illinois, USA).

Results

A total of 1006 patients underwent bariatric surgery in the three centres between August 2004 and July 2009. The prevalence of type II diabetes before surgery was 26·5 per cent (36 of 136 patients) at Oslo University Hospital, Aker, 16·9 per cent (93 of 551) at Musgrove Park Hospital and 25·1 per cent (80 of 319) at Imperial College Hospitals. The 209 patients with type II diabetes before surgery were included in this study. Their mean age was 48(10) years and two-thirds were women. The preoperative body mass index (BMI) was 48(7) kg/m^2 and patients remained obese after surgery, with a postoperative BMI of 35(7) kg/m^2. *Table 1* shows patient characteristics and preoperative insulin use in relation to type of bariatric procedure. Two patients in the bypass group, and one in each of the sleeve gastrectomy and gastric banding groups were taking glucagon-like peptide receptor agonists.

Median follow-up after surgery was 23 (range 12–75) months. Based on the 2009 consensus criteria, the rate of complete remission of type II diabetes after all bariatric surgery procedures was significantly lower than when the previous definition was used: 34·4 per cent (72 of 209

Table 1 Patient characteristics and rates of remission of type II diabetes after bariatric surgery

	All patients (n = 209)	Gastric bypass (n = 160)	Sleeve gastrectomy (n = 19)	Gastric banding (n = 30)	P†
Age (years)*	48(10)	47(9)	53(14)	46(10)	0·041‡
Sex ratio (M : F)	137 (65·6)	105 (65·6)	11 (58)	21 (70)	0·695
Insulin use before surgery	63 (30·1)	51 (31·9)	6 (32)	6 (19)	0·457
BMI (kg/m^2)*					
Before surgery	48(7)	48(7)	50(8)	47(9)	0·390‡
After surgery	35(7)	34(6)	42(6)	36(8)	0·002‡
Fasting glucose (mmol/l)*					
Before surgery	9·6(3·6)	9·8(3·6)	8·9(4·2)	7·4(0·7)	0·144‡
After surgery	6·3(2·6)	6·0(2·1)	8·0(5·3)	6·5(2·1)	0·004‡
HbA1c (%)*					
Before surgery	8·0(1·9)	8·1(1·9)	7·5(1·5)	7·7(1·5)	0·388‡
After surgery	6·2(1·2)	6·2(1·2)	6·8(1·7)	6·3(0·7)	0·081‡
Complete remission based on 2009 criteria[1]	72 (34·4)	65 (40·6)	5 (26)	2 (7)	< 0·001
Partial remission based on 2009 criteria[1]	28 (13·4)	25 (15·6)	1 (5)	2 (7)	0·301
Remission based on previous definition[2,3]	103 (49·3)	92 (57·5)	6 (32)	5 (17)	< 0·001

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). Rates of remission of type II diabetes were determined a median of 23 (range 12–75) months after bariatric surgery. BMI, body mass index; Hb, haemoglobin. †One-way ANOVA, ‡except Fisher's exact test.
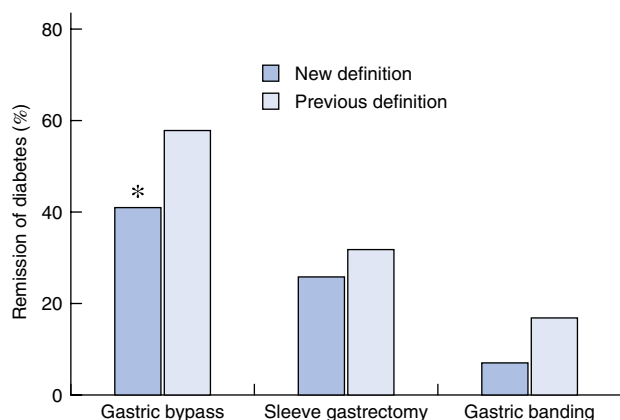
Fig. 1 Remission of diabetes after gastric bypass, sleeve gastrectomy and gastric banding based on the new (2009 consensus statement)[1] and the previous[2,3] definitions. $P < 0.001$ between groups. *$P = 0.003$ *versus* previous definition (one-way ANOVA)

patients) *versus* 49·3 per cent (103 of 209) ($P = 0.003$) (*Table 1*). Analysis by procedure showed no significant difference between remission rates based on new and previous definitions for either sleeve gastrectomy or gastric banding. However, for gastric bypass, the remission rate was significantly lower when the 2009 consensus criteria were used ($P = 0.003$) (*Fig. 1*, *Table 1*). The remission rate as defined with the new criteria was 43·9 per cent (61 of 139 patients) at 12 months and 40·6 per cent (65 of 160) at latest follow-up (median 23 months) after gastric bypass surgery.

Oral hypoglycaemic medication was used by 47 (29·4 per cent) of 160 patients after gastric bypass, 12 (63 per cent) of 19 after sleeve gastrectomy, and 25 (83 per cent) of 30 after gastric banding ($P < 0.001$). HbA1c was reduced after operation in all three surgical groups ($P < 0.001$). Mean HbA1c levels after surgery were 6·2(1·2), 6·8(1·7) and 6·3(0·7) per cent respectively ($P = 0.081$ between groups).

Preoperative insulin use was associated with a significantly lower remission rate following gastric bypass. Of 51 patients in the gastric bypass group who were taking insulin before operation, only eight achieved remission, compared with 57 of 109 on oral hypoglycaemic agents ($P < 0.001$).

Discussion

This study evaluated the effect of the proposed 2009 criteria on diabetes remission rates after bariatric surgery. At a median follow-up of 23 months after surgery, rates of complete remission of type II diabetes were 40·6 per cent

after gastric bypass, 26 per cent after sleeve gastrectomy and 7 per cent after gastric banding. These rates are substantially lower than previously reported remission rates of approximately 83 per cent for gastric bypass, 81 per cent for sleeve gastrectomy and 44 per cent for gastric banding[2–8]. Markers of glycaemic control such as HbA1c were no different between the groups, but there was a difference in additional hypoglycaemic agents used.

Although the antidiabetic effects of bariatric surgery are increasingly being recognized, there is scepticism regarding the role of surgical intervention in the treatment algorithm of type II diabetes[9,10]. Establishing realistic expectations among patients, clinicians and policy-makers may lead to a more rationalized and equitable use of bariatric surgery for the management of type II diabetes. Strategies to achieve this include standardizing definitions of the effect of surgery and avoiding the terms 'cure' and 'remission' unless they are defined carefully. Thus, the new criteria may emphasize bariatric surgery as the superior tool for achieving glycaemic control rather than as a tool for achieving remission from type II diabetes. The principal benefit of surgery, however, would not be to improve glycaemic control *per se* but rather to reduce microvascular and macrovascular complications associated with diabetes[11,12]. The findings of this study emphasize the need for intensive follow-up of patients with type II diabetes following bariatric surgery, in order to review pharmacological treatment, monitor for complications of diabetes and ensure that adequate glycaemic control is achieved.

Limitations of the study include the relatively small numbers of patients with type II diabetes in the gastric banding and sleeve gastrectomy groups. Another limitation is the lack of data on duration of diabetes, which was not part of the routine data collection in all of the centres. However, the 20·8 per cent prevalence of diabetes is similar to that in previously published series[2,3]. In addition, the proportion of patients on insulin therapy was similar to findings in the UK and Ireland National Bariatric Surgery Registry[13]. Both of these observations suggest that the results of this study could be applicable to other populations undergoing bariatric surgery.

Significant differences in age between the study groups may have contributed to differences in diabetes remission rates. Whether the results obtained after 23 months will remain similar 5 years after surgery remains to be determined. The remission rates in the present study using both the old and new definitions were lower than those in previous series[2,3]. The authors speculate that this could reflect a longer duration or an increased severity of type II diabetes among the participants in the present study.

Measurement of β-cell function was not routine in this population. Assessment of pancreatic function with c-peptide may give additional insight into the effects of bariatric surgical procedures on type II diabetes[14,15].

Further studies with longer follow-up are needed to determine the optimal management of patients with type II diabetes following bariatric surgery. In addition, future comparative studies of bariatric surgery for metabolic disorders and particularly type II diabetes should include hard endpoints such as changes in microvascular and macrovascular complication rates.

The 2009 consensus criteria are associated with lower type II diabetes remission rates after bariatric surgery than previous definitions, but the reported glycaemic control remains impressive. Using an agreed definition of diabetes and focusing on complications of diabetes as endpoints may remove some of the barriers to making surgery a more widely accepted treatment option for type II diabetes.

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Original article

Effect of bypassing the proximal gut on gut hormones involved with glycemic control and weight loss

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Abstract

Background: The reported remission of type 2 diabetes in patients undergoing Roux-en-Y gastric bypass has brought the role of the gut in glucose metabolism into focus. Our objective was to explore the differential effects on glucose homeostasis after oral versus gastrostomy glucose loading in patients with Roux-en-Y gastric bypass at an academic health science center.
Methods: A comparative controlled investigation of oral versus gastrostomy glucose loading in 5 patients who had previously undergone gastric bypass and had a gastrostomy tube placed in the gastric remnant for feeding. A standard glucose load was administered either orally (day 1) or by the gastrostomy tube (day 2). The plasma levels of glucose, insulin, glucagon-like peptide 1 and peptide YY were measured before and after glucose loading.
Results: Exclusion of the proximal small bowel from glucose passage induced greater plasma insulin, glucagon-like peptide 1, and peptide YY responses compared with glucose loading by way of the gastrostomy tube ($P < .05$).
Conclusions: Exclusion of glucose passage through the proximal small bowel results in enhanced insulin and gut hormone responses in patients after gastric bypass. The gut plays a central role in glucose metabolism and represents a target for future antidiabetes therapies. (Surg Obes Relat Dis 2012;8:371–374.) © 2012 American Society for Metabolic and Bariatric Surgery. All rights reserved.

Keywords: Gastric bypass; Bariatric surgery; Metabolic surgery; Diabetes remission; Gut hormones; Incretins; Glucagon-like peptide 1; GLP-1

Roux-en-Y gastric bypass improves glycemic control within days [1,2]. The initial increased insulin secretion and reduced insulin resistance appears to be independent of weight loss [3]. Increased glucagon-like peptide 1 (GLP-1) levels after gastric bypass are associated with increased insulin secretion but not reduced insulin resistance [1]. These findings, together with experiments on animal models, have brought the gut's role in glucose homeostasis into focus and novel endoluminal devices have been introduced in an attempt to mimic the metabolic effects of gastric bypass [4–6]. A remaining conundrum is the rapid weight loss and weight loss maintenance after gastric bypass, making it challenging to confirm the hypothesis that the effects on glycemic control are indeed weight loss independent. The aim of the present study was to investigate the effect of glucose loading into different gut segments in weight stable patients who have had their type 2 diabetes placed into remission after gastric bypass.

Methods

The study was approved by the Clinical Governance and Patient Safety Committee of Imperial College Healthcare National Health Service Trust (reference number 10/807) and was performed according to the Declaration of Helsinki. The 5 patients who had undergone gastric bypass provided informed consent. Surgery was performed as previously described [7]. All participants had had type 2 diabetes preoperatively and had had achieved normoglycemia without requiring any hypoglycemic medication by the time of the study. The mean body weight loss was 29.9% ± 4.6% resulting in a body mass index reduction from 43.2 ± 1.9 to 29.9 ± 2.4 kg/m^2 (P = .001). Because of surgical complications, the patients had been unable to maintain adequate calorie intake. To allow for enteral feeding, all patients had been provided with a functioning gastrostomy tube. The patients were treated conservatively or surgically and had fully recovered so that at the time of testing (14 ± 4 months postoperatively, range 9–24), all patients had normal nutrition status, tolerated oral liquids, and had a stable body weight.

All examinations were performed at 8 AM after an overnight fast on 2 different days 3–6 days apart. A 410-mL solution containing 75 g glucose, 287 kcal (Lucozade Energy Original, GlaxoSmithKline, Middlesex, United Kingdom) was given orally on day 1 (to verify the patients could tolerate the volume orally) and by gastrostomy on the second occasion. The duration of the solution administration was 10 minutes on both days. A schematic illustration of the gastrointestinal glucose route after oral and gastrostomy loading is given in Figure 1. Blood was obtained by a venous catheter using tubes containing ethylenediaminetetraacetic acid and aprotinin before and 15, 30, 60, 90, 120, 150, and 180 minutes after glucose loading. The samples were stored and peptide YY (PYY)-like immunoreactivity (full length, PYY1–36, and fragment, PYY3–36) and plasma total GLP-1 were measured, as previously described [8–10]. Gastric inhibitory polypeptide (GIP) was meas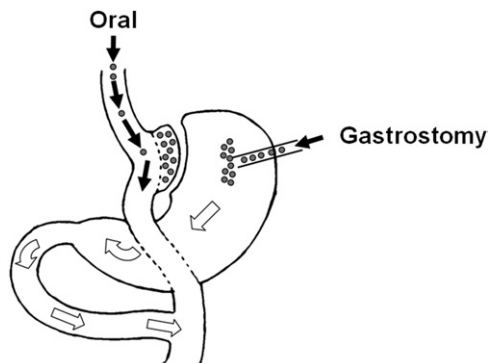ured using enzyme-linked immunosorbent assay (Millipore, Billerica, MA). Glucagon was measured by an in-house radioimmunoassay [11]. Glucose was measured with an automated glucose analyzer (Abbott Laboratories, Chicago, IL), and insulin was measured with an automated chemiluminescent immunoassay (Abbott Laboratories, Chicago, IL).

The results were analyzed using GraphPad Prism, version 5.00, for Windows (GraphPad Software, San Diego, CA). The data are presented as the mean ± SEM. The plasma levels of insulin, GLP-1, PYY, GIP, glucagon and glucose after the 2 glucose loadings were analyzed with a 2-way group (between subjects) × time (within subjects) analysis of variance, presented with the F test, and the numbers in parentheses are the degrees of freedom. Post hoc Bonferroni tests for each concentration were applied when there was a significant group × time interaction. P ≤.05 was considered significant.

Results

Figure 2 shows the plasma levels of glucose, insulin, GLP-1, PYY, GIP, and glucagon after oral and gastrostomy glucose loading. Two-way analysis of variance revealed a significant difference in plasma insulin, GLP-1, and PYY (all P <.01) levels between the oral and gastrostomy glucose loading. There was also a significant main effect of time and a significant group × time interaction for insulin, GLP-1, and PYY (all P <.001). The patients returned more quickly to the baseline glucose levels after oral glucose loading compared to patients who had received the glucose load by gastrostomy (P <.001), with a significant group × time interaction (P <.001) but no significant main group effect for glucose levels (P = .84).

No difference was found in the GIP postprandial response between the 2 routes used. The glucagon postprandial response was greater with the oral route (2-way analysis of variance, P <.01); however, there was no significant effect of time and group × time interaction. The values of the 2-way analysis of variance for glucose, insulin, GLP-1, PYY, GIP, and glucagon are summarized in Table 1.

Discussion

The present study has demonstrated that an altered delivery of nutrients to the intestine, which excludes the proximal gut, results in improved postprandial glucose handling. In particular, excluding the distal stomach, duodenum, and proximal jejunum from nutrient transit reduces the duration of hyperglycemia and leads to enhanced insulin, incretin, and satiety gut hormone responses after glucose loading. In contrast, in weight stable patients, the restoration of the duodenal passage after gastric bypass by gastrostomy increases the duration of hyperglycemia and attenuates the incretin and insulin responses to glucose. Our observations support the hypothesis that endocrine changes

Fig. 1. Schematic illustration of gastrointestinal glucose route after oral (black arrows) and after gastrostomy load (empty arrows).
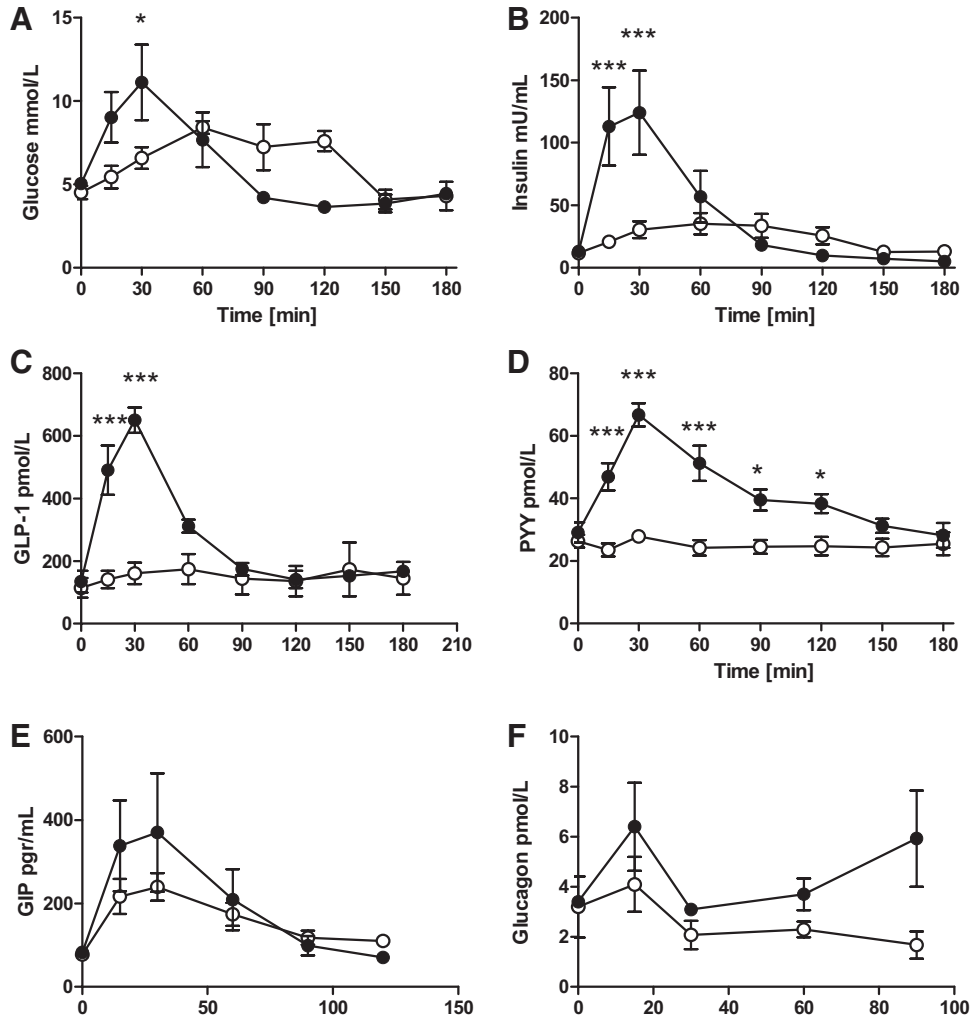
Fig. 2. Plasma levels of (A) glucose, (B) insulin, (C) GLP-1, and (D) PYY, (E) GIP, and (F) glucagon after oral (black circles) and gastrostomy (open circles) glucose load. Data presented as mean values $\pm$ SEM. When 2-way analysis of variance revealed a significant group $\times$ time interaction, post hoc Bonferroni test was used for point to point analysis between 2 groups (*P <.05, ***P <.001).

play an important role in the improvement of diabetes after gastrointestinal bypass surgery [3,4,12–18]. However, differentiating between the relative contribution of proximal versus distal small gut signals to glycemic control was outside the purpose of the present study.

Exclusion of the duodenum and jejunum in Goto-Kakizaki, spontaneously nonobese type 2 diabetic rats, can have a weight loss-independent effect on type 2 diabetes, suggesting that the proximal gut might be implicated in the pathogenesis of the disease [4,19]. Glucose tolerance was

Table 1
Two-way analysis of variance values (*F* test, numbers in parentheses are degrees of freedom) for comparison of gut hormones after oral and gastrostomy tube glucose load as a function of group and time

Variable	Time	Group	Time $\times$ group
Glucose	F(7,57) = 5.81; P <.001	F(1,57) = 0.04; P = .84	F(7;57) = 4.26; P <.001
Insulin	F(7,57) = 7.64; P <.001	F(1,57) = 8.88; P = .004	F(7,57) = 5.87; P <.001
GLP-1	F(7,58) = 10.51; P <.001	F(1,58) = 32.08; P <.001	F(7,58) = 9.10; P <.001
PYY	F(7,58) = 8.79; P <.001	F(1,58) = 96.77; P <.001	F(7,58) = 7.61; P <.001
GIP	F(6,43) = 3.98; P = .003	F(1,43) = 0.59; P = .45	F(6,43) = 0.52; P = .79
Glucagon	F(4,30) = 1.98; P = .12	F(1,30) = 7.88; P = .0087	F(4,30) = 1.12; P = .36

GLP-1 = glucagon-like peptide 1; PYY = peptide YY; GIP = gastric inhibitory polypeptide.

markedly improved postoperatively. Furthermore, in an another study of the same type of rats, duodenojejunal bypass led to the improvement of oral glucose tolerance in contrast to gastrojejunostomy, which had no effect [4]. Exclusion of the duodenum by reoperation of the rats with gastrojejunostomy improved glucose tolerance, and restoration of the duodenal passage in rats that had undergone duodenojejunal bypass caused the recurrence of impaired glucose tolerance [4]. The available human data supporting the weight loss-independent effect of Roux-en-Y gastric bypass on glucose homeostasis and the "foregut hypothesis" are limited.

GIP responses after gastric bypass remain controversial, with most investigators showing a decrease [16], but others showing an increase [15]. We did not find a significant difference in our study, but our experiment was not powered to detect a difference in GIP.

A single case has been reported in which nutrient stimulation by oral feeding was compared with gastric tube feeding in a patient after gastric bypass [20]. However, by repeating the experiments in a series of weight stable patients in whom diabetes had gone into remission, we have shown a consistent threefold elevation of insulin, GLP-1, and PYY after oral glucose loading. Dirksen et al. [20] have also demonstrated no overall difference in GIP or glucagon, consistent with the result of our study.

One limitation of our study was the nonrandom allocation of oral and gastrostomy days; however, we had to ensure that the patients could tolerate the volume orally, before administering it through the gastrostomy tube. Moreover, each patient served as their own control, and all tests were performed within a 3–6-day period.

Conclusions

Exclusion of the proximal small gut by gastric bypass surgery resulted in weight-independent modifications of gut hormones and glucose homeostasis. Understanding the mechanisms by which gastric bypass alters the metabolism might lead to novel devices or therapeutic approaches for the treatment of type 2 diabetes.

Acknowledgments

Disclosures

The authors have no commercial associations that might be a conflict of interest in relation to this article.

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The Role of Bile After Roux-en-Y Gastric Bypass in Promoting Weight Loss and Improving Glycaemic Control

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Gastric bypass leads to the remission of type 2 diabetes independently of weight loss. Our hypothesis is that changes in bile flow due to the altered anatomy may partly explain the metabolic outcomes of the operation. We prospectively studied 12 patients undergoing gastric bypass and six patients undergoing gastric banding over a 6-wk period. Plasma fibroblast growth factor (FGF)19, stimulated by bile acid absorption in the terminal ileum, and plasma bile acids were measured. In canine and rodent models, we investigated changes in the gut hormone response after altered bile flow. FGF19 and total plasma bile acids levels increased after gastric bypass compared with no change after gastric banding. In the canine model, both food and bile, on their own, stimulated satiety gut hormone responses. However, when combined, the response was doubled. In rats, drainage of endogenous bile into the terminal ileum was associated with an enhanced satiety gut hormone response, reduced food intake, and lower body weight. In conclusion, after gastric bypass, bile flow is altered, leading to increased plasma bile acids, FGF19, incretin. and satiety gut hormone concentrations. Elucidating the mechanism of action of gastric bypass surgery may lead to novel treatments for type 2 diabetes. (*Endocrinology* 153: 0000–0000, 2012)

A link between bile acids and glycemic control has been suggested by studies showing improved blood glucose in patients with type 2 diabetes when receiving the bile acid sequestrants cholestyramine and colesevelam (1–5). Several possible mechanisms have been proposed, including the disruption of enterohepatic circulation of bile acids and a number of different effects through the farnesoid X receptor (FXR) pathway, which is the intracellular signaling pathway for bile acids (4, 6). Bile acids also activate the cell-membrane G protein-coupled receptor, TGR5, which has been shown to stimulate the incretin, glucagon-like peptide-1 (GLP-1) *in vitro* in an FXR-independent manner (7). GLP-1 in turn stimulates β-cells in the pancreas to release insulin (8).

Gastric bypass surgery is being used as a treatment for type 2 diabetes, although the mechanism of action remains largely unclear (9–11). The improved glycemic control in the immediate postoperative period is weight loss independent, because simultaneous improved insulin secretion and reduced insulin resistance have been observed between d 2 and 7 after gastric bypass (9). The early increase in insulin secretion was associated with an enhanced

Abbreviations: AUC, Area under the curve; CRP, C-reactive protein; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

GLP-1 response (9), which may partly be explained by L-cell stimulation from bile acids (12–14). Moreover, the decrease in insulin resistance may also be the result of increased fasting plasma bile acid levels after gastric bypass surgery (15, 16), because firstly, bile acids inhibit gluconeogenesis in an FXR dependent and independent manner (17–20) and bind to TGR5, leading to cAMP generation and activation of the intracellular type 2 thyroid hormone deiodinase (21). Secondly, bile acids also act via the phosphatidylinositol 3 kinase/serine-threonine kinase pathway directly promoting insulin signaling and glycogen synthase activation, thus aiding insulin-dependent control of glucose metabolism in the liver (22). Thirdly, possible effects of bile acids on fibroblast growth factor (FGF)19 could lead to enhanced mitochondrial activity, which improves insulin resistance (23). Recently FGF19 has been shown to regulate glycogen metabolism in an insulin-independent manner (24). Furthermore, FGF19 has been shown to correlate with nutritional status (25). Finally, tauroursodeoxycholic acid has also been shown to protect against the onset of insulin resistance in obese and diabetic mice by alleviating stress in the endoplasmic reticulum (26).

The prolonged improvements in glycemic control after gastric bypass are further aided by the substantial and maintained weight loss. The attenuated appetite may be partly explained by enhanced satiety gut hormones from the endocrine L cell, such as peptide YY (PYY), GLP-1, and oxyntomodulin (27, 28). Bile acids are also implicated in the release of these L-cell hormones.

We hypothesized that the altered anatomy after gastric bypass affects bile delivery to the terminal ileum and lead to elevated plasma bile acids. We postulated that changes in bile flow result in increased satiety gut hormone responses, reduced food intake, and weight loss. Our aim was to test this hypothesis in humans after gastric bypass surgery and to explore further the potential mechanisms involved in two animal models of altered bile flow.

Materials and Methods

The human studies were performed according to the principles of the Declaration of Helsinki. The Somerset Research and Ethics committee approved the study (LREC protocol no. 05/Q2202/96). The canine studies were approved by the ethics committee of Onderstepoort Vetinary School (University of Pretoria). The rat studies were approved by the Home Office United Kingdom (PL 70-6669).

Human studies

Written informed consent was obtained from all participants. Exclusion criteria included pregnancy, substance abuse, and more than two alcoholic drinks per day. Twelve gastric bypass patients (seven females and five males) with mean age of 45.2 ± 2.7 yr and body mass index 49.8 ± 1.5 as well as six gastric banding patients (four females and two males), with mean age 45.4 ± 2.6 yr and body mass index 44 ± 2.0 kg/m^2 were recruited. All patients were prescribed a 2-wk preoperative diet of 1000 kcal before surgery. Operations were performed laparoscopically by one surgeon. The technique for the gastric bypass has been described previously (29). For gastric banding, the Swedish Adjustable Gastric band (Ethicon Endo-Surgery, London, UK) and the LAP-BAND (Allergan, Marlow, UK) bands were used with the pars flaccida dissection technique and gastrogastric tunnelating sutures (30). Using an enhanced recovery protocol postoperatively, all patients were allowed free fluids on return to the ward, and diet was recommenced when tolerated. The recommended postoperative diet for the first week was the same for the patients with banding and bypass. After a 12-h fast, blood was obtained in tubes containing EDTA and aprotinin. Samples were immediately centrifuged and stored in a -80 C freezer until analysis.

Canine studies

Four male and four female Beagles were fasted overnight and then given a standard 400 g of test meal of dog chow (Husky, Purina, South Africa). The composition was 7.5% protein, 2% fat, 1% fiber, 7.5% crude ash, and 82% moisture. Five milliliters of blood were collected (in tubes containing EDTA and aprotinin) every 30 min from 30 min before the meal up to 150 min postprandially.

The next day, the dogs were prepared for theater by placing an overnight fentanyl patch and withholding food overnight. The common bile duct was transected. An 8 French Foley catheter was placed into the gall bladder. An 8 French feeding tube was advanced through the pylorus to the duodenum with the most distal point being 5–8 cm distal to the pylorus, close to the level of the ampulla of Vater (Fig. 1A). For the first 12 h after the surgery, the dogs were given *ad libitum* access to water but no food.

Only one dog was terminated after showing signs of jaundice and infection. The dogs received a standard meal of 400 g of normal chow at the start of the light phase. At this time, as much bile as possible was aspirated from the Foley catheter and injected through the gastrostomy tube, followed by a 5-ml flush of saline. This prevented the dogs from becoming jaundiced and allowed normal digestion.

On d 4–6, the dogs were randomized to a 180-min crossover designed protocol of venous blood collection every 30 min after 1) a standard meal of 400 g of dog food only without bile; 2) bile only, without food; or 3) 400 g of dog food and bile in combination.

Rodent studies

Sixteen male Wistar obese rats were randomized to a sham operation, which maintained the normal bile delivery to the duodenum or to an operation that would deliver bile to the ileum. The bile-in-duodenum group underwent transections 1 cm proximal and distal to the drainage point of the common bile duct and reanastomosis to maintain the normal anatomy but allow for a similar surgical insult. The bile-in-ileum group had the same transection of the duodenum, but the proximal and distal ends of the transected duodenum were anastomosed end to end and con-
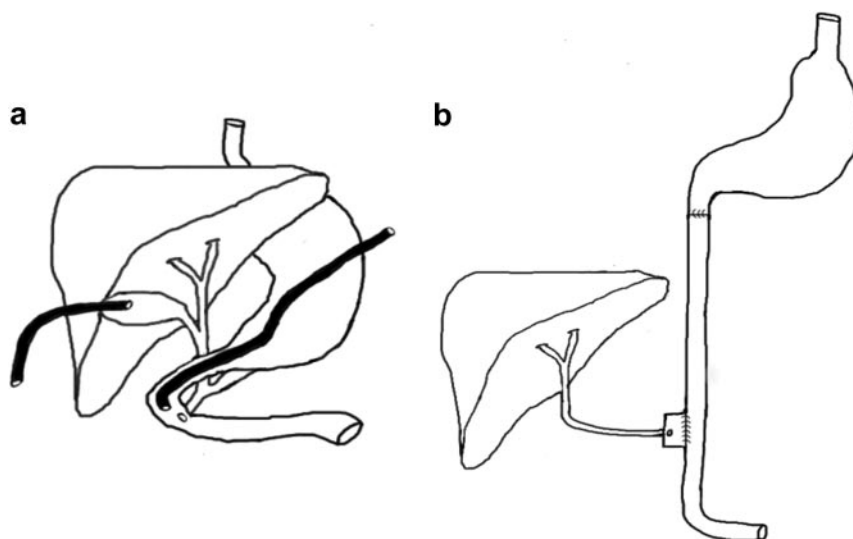
FIG. 1. A, Schematic illustration of the anatomy and canulation of the canine model. A gastrostomy tube was placed into the duodenum close to the ampulla of Vater. The common bile duct was ligated and the gallbladder canulated to allow drainage of bile. B, Schematic illustration of the functional anatomy of the bile in ileum group. Transections 1 cm proximal and distal to the drainage point of the common bile duct were performed. The proximal and distal ends of the transected duodenum were anastomosed end to end and continuity restored. The segment of the duodenum containing the common bile duct was anastomosed side to side to the distal jejunum, 10 cm proximally to the terminal ileum.

tinuity restored (Fig. 1B). The segment of the duodenum containing the common bile duct was anastomosed side to side to the distal jejunum, 10 cm proximally to the terminal ileum. This allowed bile and pancreatic juices to bypass the duodenum and most of the jejunum.

Body weight and food consumption was measured daily at the beginning of the light phase for 28 d. Feces were collected over a 24-h period on d 25. The rats were then fasted for 12 h before they were terminated, and blood samples were collected.

FGF19 assay

Plasma FGF19 concentration was measured using a quantitative sandwich ELISA technique (FGF19 Quantikine ELISA kit, catalog no. DF1900; R&D Systems, Minneapolis, MN).
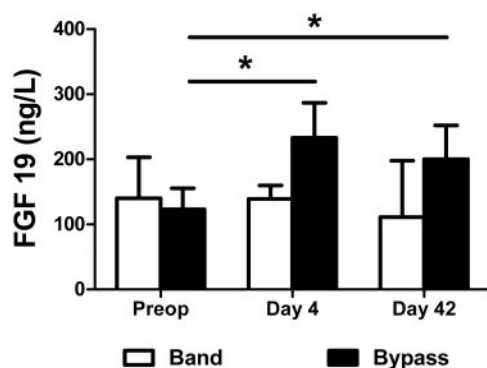

FIG. 2. Fasting plasma FGF19 concentrations (median and interquartile ranges) at d 0, 4, and 42 in six gastric banding patients (*white bars*) and 12 gastric bypass patients (*black bars*). *, $P < 0.05$ Mann-Whitney *U* test. Preop, Preoperatively.

Bile acids assay

The measurement of fractionated plasma bile acids was performed with liquid chromatography tandem mass spectrometry (31). The method allowed 12 different bile acids [cholic acid (CA), chenodeoxycholic acid (CDC), deoxycholic acid (DC), glycocholic acid (GCA), glycodeoxycholic acid (GCD), glycochenodeoxycholic acid (GCDC), glycolithocholic acid (GLC), glycoursodeoxycholic acid (GUDC), lithocholic acid (LC), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDC), and taurodeoxycholic acid (TDC)] to be measured within the range of 0.1–10 μM.

GLP-1 and PYY assay

All samples were assayed in duplicate. Analysis was performed with an established GLP-1 RIA (7). PYY-like immunoreactivity was measured with a specific and sensitive RIA, which measures both the full length (PYY1-36) and the fragment (PYY3-36) (32).

Bomb calorimetry

To evaluate nutrient absorption, feces were collected over 24 h on postoperative d 25 from all rats. Feces were dried in an oven and weighed; calorie content was measured using an established ballistic bomb calorimeter technique (33).

Rodent C-reactive protein (CRP) assay

To assess inflammation serum CRP levels were measured (Rat Serum CRP ELISA kit catalog no. 1010; Alpha Diagnostics International, San Antonio, TX).

Statistical analysis

Results were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Data are expressed as means ± SEM when the data follow a Gaussian distribution. When the data did not follow a Gaussian distribution, the median (range) is used. Values for the area under the curve (AUC) were calculated with the use of the trapezoidal rule. End points were compared with the use of two-tailed, paired Student's *t* tests for parametric data and Mann-Whitney *U* test for nonparametric data. Results were considered significant if $P < 0.05$.

Results

Human study FGF19

Preoperative fasted plasma FGF19 levels did not differ between gastric banding and gastric bypass patients [median 140 ng/liter range (26–466) *vs.* 123 (41–406), respectively; $P = 0.37$]. However, these values were lower than those found in nonobese controls (34). In the gastric
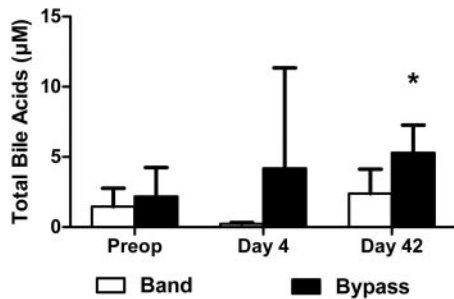
FIG. 3. Fasting total plasma bile acid concentrations at d 0, 4, and 42 in six gastric banding patients (*white bars*) and 12 gastric bypass patients (*black bars*). *, $P < 0.05$ Mann-Whitney *U* test. Preop, Preoperatively.

banding group, there was no significant change from preoperative values for fasting FGF19 at d 4 or 42 after surgery. In the gastric bypass group, fasting levels of plasma FGF19 were significantly increased as early as 4 d after gastric bypass compared with preoperative values ($P < 0.01$) (Fig. 2). The enhanced FGF19 level were sustained at 42 d postoperatively ($P < 0.05$).

Human study plasma bile acids

Fasting concentrations of total plasma bile acids measured in banding and bypass patients preoperatively were not different (Fig. 3). On d 4, fasting bile acids were increased after gastric bypass, and there was a significant difference compared with the banding group. By d 42 after gastric bypass fasting, total bile acid concentrations were higher compared with preoperative levels. There was no difference in the banding group.

A similar pattern of results (increase in the gastric bypass group but not in the banding group) was previously shown for GLP-1 and PYY in these same patients (9).
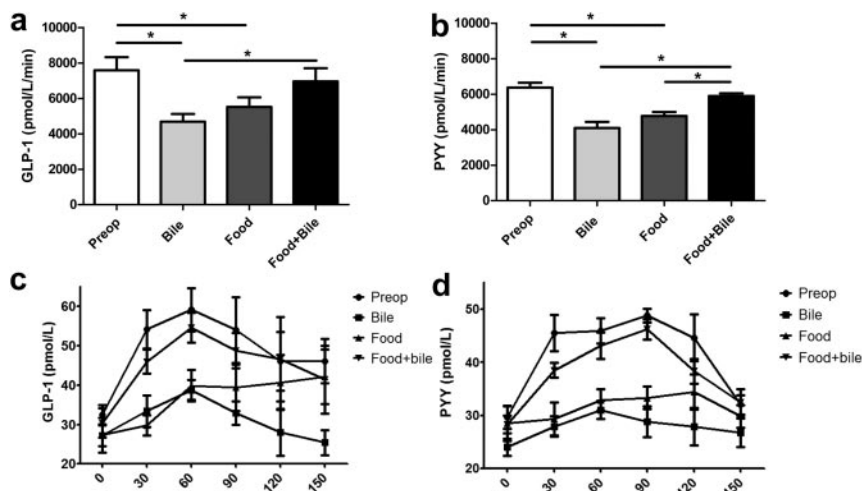
Canine studies

The responses of GLP-1 and PYY were studied in this model after stimulation with food or bile alone or a combination of both. Unfortunately, at the time of these experiments, there was no available assay for canine FGF19. Baseline GLP-1 and PYY levels were the same before and after the operation (6936 *vs.* 7290 for GLP-1 and 6386 *vs.* 5856 for PYY). Figure 4 shows the AUC and the time course over 150 min for the postprandial GLP-1 and PYY response after the standard meal of 400 g of dog food. In the operated dogs, both food alone and bile alone lead to a significant GLP-1 and PYY response from baseline, although the responses were attenuated. The response to bile and or food alone was inferior to the combination of food and bile either pre- or postoperatively.

Rodent studies

In this model, rats had bile draining into their ileum or duodenum. Both the fasting plasma GLP-1 levels (Fig. 5A) and the plasma PYY levels (Fig. 5B) were higher in the bile-in-ileum group than in the bile-in-duodenum group ($P < 0.05$).

Figure 6A demonstrates that both the bile-in-ileum and bile-in-duodenum groups lost a similar initial amount of weight during the first 4 d while recovering from surgery. The bile-in-duodenum group, however, reached their preoperative weight within 8 d. The bile-in-ileum group weighed significantly less than the bile-in-duodenum group on d 6 ($P < 0.05$) and continued to have a lower bodyweight for the duration of the study ($P < 0.05$). Figure 6B shows that the rats in the bile-in-ileum group ate significantly less than the bile-in-duodenum group ($P < 0.05$).

Fecal parameters at 25 d after the operation did not reveal differences between the bile-in-ileum and the bile-in-duodenum groups at the end of the experiment in dry weight (4.12 ± 0.20 *vs.* 4.28 ± 0.18 g, $P = 0.55$) or in calorific content (3.58 ± 0.4 *vs.* 3.58 ± 0.4 fecal kcal/24 h, $P = 0.91$). There was no evidence of increased inflammation in the bile-in-ileum compared with the bile-in-duodenum group either by white cell count ($11.5 \pm 0.32 \times 1000/\mu$l *vs.* $11.64 \pm 0.42 \times 1000/\mu$l, $P = 0.79$) or CRP (370.88 ± 26.03 *vs.* 378.5 ± 21.71 μg, $P = 0.83$).

FIG. 4. A, AUC for the postprandial GLP-1. B, AUC for postprandial PYY response after 400 g of food in dogs pre- or postoperatively either receiving food alone without bile (food), bile alone without food (bile), or food and bile in combination (food + bile).*, $P < 0.05$. The time course of the postprandial response for GLP-1 (C) and PYY (D).

Discussion

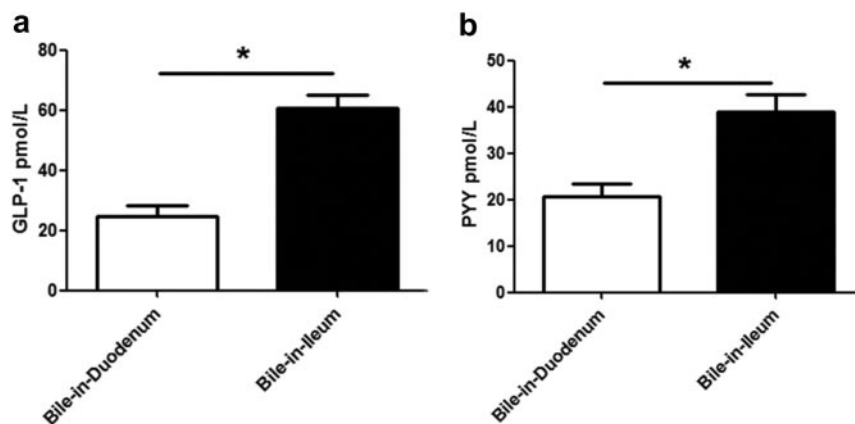We showed in obese patients that FGF19 and plasma total bile acids

FIG. 5. Plasma GLP-1 (A) and PYY (B) levels in rats that had bile draining into their duodenum or bile draining into their ileum.

were increased after gastric bypass but not after gastric banding. We have shown previously that GLP-1 and PYY are affected in the same way as FGF19 in these patients (9). How could the alterations in gastro-intestinal physiology produce these changes?

Both food and bile on their own can release GLP-1 and PYY as shown in the canine model, but the combination of food and bile (before surgery or after surgery) resulted in greater PYY responses compared with food alone, whereas GLP-1 also showed a trend to be higher after the combination of food and bile. Endogenous bile acids and pancreatic juices delivered in the rat model 10 cm proximally to the terminal ileum were also associated with elevated plasma GLP-1 and PYY, reduced food intake, and lower body weight. Taken together, these data suggest that one of the mechanisms by which a gastric bypass leads to beneficial elevations of GLP-1 and PYY may be through the undiluted flow of bile through the biliopancreatric limb and the altered delivery of bile to the terminal ileum.

Well-matched patients undergoing gastric banding were used as a control group, because they have a similar laparoscopic surgical insult and identical preoperative and immediate postoperative diets compared with bypass, but in the gastric banding operation, there is no change in the

anatomy of the gut that would affect bile flow. Exogenous bile salts have been shown to be the most potent stimulus of gut hormones from the endocrine L cells, such as PYY in rabbit colon explants (12), *in vivo* in conscious dogs (13), and in humans (14). GLP-1, PYY, and bile are released postprandially and in proportion to the amount of calories consumed. Although early arrival of nutrients at the terminal ileum still remains a possibility, the reduced gut motility and early peak GLP-1 and PYY responses postprandially suggest other mechanisms, which may involve bile in the release of L-cell hormones.

The gallbladder is not usually removed during Roux-en-Y gastric bypass, and there is no indication that gallbladder contraction is adversely affected after gastric bypass. Previous studies suggested an increase in cholecystokinin after jejunoileal bypass, which may result in enhanced bile flow from the gallbladder or liver (34). After gastric bypass, the length of small bowel from the ampulla of Vater to the terminal ileum is reduced by 100–150 cm, and bile may thus reach the terminal ileum before the ingested food, which triggers the release of bile. We postulate that due to the anatomical changes after gastric bypass, bile progresses down the biliopancreatic limb to the distal L cells in an undiluted state. This could lead to increased availability of bile acids in the distal intestine with the potential to engage TGR5 on L cells. Bile acid activation of TGR5 has been found to stimulate GLP-1 production *in vitro* and may explain the early and exaggerated release of incretin gut hormones, such as GLP-1 and subsequently insulin (8). Bile acids would normally be more bound up in micelles due to the presence of nutrients and therefore less likely to stimulate L cells for peptide secretion.

Although the canine studies should be interpreted with caution, especially because the anatomical changes are dissimilar to gastric bypass, we would suggest that both bile and food contribute to the postprandial gut hormone response. The role of bile can be attributed to the fact that bile conjugated with food facilitates better digestion of complex ingested fats by intestinal lipases into smaller lipid subunits, therefore leading to a more effective stimulation of L cells (35). Inhibition of intestinal
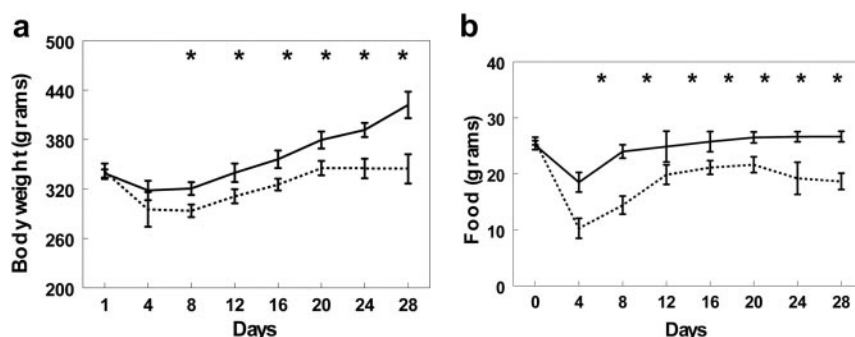

FIG. 6. A, Weight of rats in bile-in-duodenum (*solid line*) and bile-in-ileum (*broken line*) groups before and up to 28 d after surgery. B, Food intake of bile-in-duodenum (*solid line*) and bile-in-ileum (*broken line*) rats before and up to 28 d after surgery. *, $P < 0.05$.

lipases leads to attenuated postprandial GLP-1 and PYY associated with increased appetite (35). However, the results of the rodent studies do not support this hypothesis, because the distal intestinal delivery of bile should interfere with the intestinal digestion of fats and hence lead to reduced L-cell responses. As the opposite was demonstrated, the effect of bile on digestion of ingested fats cannot explain the enhanced gut hormone response, suggesting that bile acids may play a role as signaling molecules.

Bile acids may also influence glucose metabolism by altering body weight. Weight loss may result from enhanced satiety, which may be facilitated via L cell-derived gut hormones (9, 25). In addition, bile acids increase energy expenditure in brown adipose tissue, thus preventing obesity and insulin resistance via induction of the cAMP-dependent thyroid hormone-activating enzyme type 2 iodothyronine deiodinase (21). This is achieved via the TGR5 and is consistent with recent findings that in rat models, gastric bypass prevented the decrease in energy expenditure after weight loss (36, 37).

Activation of the FXRα may also mediate the effects of bile acids on energy homeostasis via FGF19 released from ileal enterocytes, leading to improved metabolic rate and decreased adiposity (38, 39). FGF19 has recently been shown to inhibit hepatic gluconeogenesis (40). FGF19 was only assayed in humans, because unfortunately there are no reliable assays for use in dogs or rats. FGF19 and the FGF15 ortholog in rodents are thought to provide feedback inhibition of bile acid synthesis in the liver. Why the increased levels of FGF19 after gastric bypass are associated with an increase in total bile acids is not immediately apparent, unless the changes in ileal bile acid absorption and FXR-mediated FGF19 production are much greater than the effects on the liver.

Some of the beneficial metabolic effect of Roux-en-Y gastric bypass on glycemic control may be attributed to changes in bile acids (15). Our prospective study confirms the previous cross sectional observation that total plasma bile acids are elevated after gastric bypass. We went on to show that this happens as early as 42 d after surgery. We propose an additional mechanism for the observed improvements in glycemic control involving altered bile flow after gastric bypass. The changes facilitated by bile may increase satiety and improved glycemic control via gut hormones as well as a direct effect on insulin resistance. Therefore, bile may be one of the key products of the proximal gut, which transfers a message to the distal gut and to other metabolically active tissues.

Limitations of our study include the lack of randomization in the human studies. However, the groups were well matched for preoperative patient characteristics. Portal vein levels of FGF19 and bile acids were not measured, and the relationship between portal veins and systemic levels

after gastric bypass is not currently known. Furthermore only fasting levels of FGF19 and bile acids were measured. Postprandial changes may also occur and are the subject of future studies. In the canine experiment, a dislodgement of either the feeding tube or the Foley catheter could have lead to chemical peritonitis affecting the results. However, the postmortem examination confirming the position of the tubes makes this unlikely. In the rat experiment, the difference in the severity of the operative procedures may have caused different inflammatory response, but the lack of difference in the biochemical and histological markers of inflammation mitigates against this.

In conclusion, Roux-en-Y gastric bypass causes changes in bile flow resulting in increased total plasma bile acids concentrations, FGF19, GLP-1, and PYY. Altering the flow of endogenous bile leads to an increase in gut hormone responses, which can be further enhanced by the synergistic delivery of food. Bile may partly explain the pleotrophic metabolic effects seen after gastric bypass surgery and could be a therapeutic target for novel surgical devices or pharmaceuticals.

Acknowledgments

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