Mechanisms underlying diabetes remission after weight loss surgery for morbid obesity: energy restriction, weight loss, gut hormones or adipokines?

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by

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August 2016

I declare that the thesis entitled:

Mechanisms underlying diabetes remission after weight loss surgery for morbid obesity: energy restriction, weight loss, gut hormones or adipokines?

is entirely my work performed whilst registered as a candidate for the degree of Doctor of Medicine at the University of Liverpool. No part of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or Institute of learning.

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Table of contents Page

Acknowledgements

Firstly, I would like to sincerely thank my supervisor Prof John Wilding for his valuable guidance and support; his constant encouragement kept me going until the end.

I would like to thank the entire research team at the Obesity and Endocrinology Research Unit at Aintree who have helped with various aspects of this project. In particular thanks to Jackie Cleator, Barbara Martin and Janet Cashen, who were very patient in guiding me during my early research days.

I would like to thank Mr David Kerrigan and his bariatric team who helped me with recruitment of patients for this study.

Sincere thanks to Andy Cross who helped me with laboratory work and assays.

There are so many clinical and academic colleagues who I do not have space to mention here who have constantly provided me with support and encouragement to complete this project.

Special thanks to all my patients who participated in this study.

Lastly and most importantly I would like to thank my wife Lalita for her ongoing support and understanding when I have needed time for research work and thesis writing. Also my daughter Anya and son Arush who were both born during the project and had to put up with their Dad sitting in front of a computer during their holidays.

I want to express my deepest appreciation to my parents and brothers who have always been very supportive and proud of my achievements.

Abstract

Background: Obesity and type 2 diabetes are closely linked both epidemiologically and pathophysiologically. Calorie restriction and weight loss improve diabetes and insulin resistance. Weight loss surgery, in particular malabsorptive procedures, causes durable weight loss and leads to early diabetes remission. The exact mechanisms by which diabetes undergoes remission even before weight loss occurs is not fully understood.

Objectives: Firstly I aimed to study diabetes remission rates after various weight loss procedures and to compare outcomes between diabetes and non-diabetes subjects. I then aimed to study the mechanisms which lead to diabetes remission after RYGB with particular focus on the effects of energy restriction, appetite regulating gut hormones, incretins, changes in weight and body composition, adipokines and eating behaviour.

Methods: 986 patients who had undergone a weight loss procedure in Aintree were identified from the database and their weight outcomes were studied. The results were compared between 216 patients who had type 2 diabetes and 770 patients who did not have type 2 diabetes. Diabetes remission rates following the four procedures were studied. In the prospective study, 8 obese patients who were given a calorie restricted diet of 1200 kcal/day and 22 patients who underwent RYGB were studied. In the RYGB group changes to glucose, insulin and GLP1 in response to a 330 kcal liquid meal was studied at baseline, 2 weeks post-surgery, at 4 and 12 months. Calorie restriction group were studied at baseline and after 4 weeks. Longitudinal changes to weight, body composition, eating behaviour, PYY and adipokines were studied.

Results: Patients with type 2 diabetes had comparable weight loss to non-diabetes subjects 3 years after LAGB (EWL 44.8% vs 55%, p = 0.33) and 2 years after RYGB (71.4% vs 77.6%, p = 0.08). Diabetes remission rates using the ADA consensus criteria were: LAGB (15%), RYGB (42.2%), LDS (62%) and SG (33.3%). In the prospective study there was no difference in the weight loss achieved between them at 2 weeks, 4 or 12 months. Weight loss after RYGB was significant at 4 and 12 months but not 2 weeks. Fasting glucose and AUC glucose fell significantly in the surgery (RYGB) group at all the time points. Fasting insulin was also reduced significantly but there was no change in the AUC for insulin. Early insulin response rose significantly within 2 weeks mirroring the changes seen in post-prandial GLP1 response. Fasting PYY did not change significantly. Hunger and disinhibition reduced and restraint improved immediately after RYGB. Adiponectin rose and Leptin, IL6. sTNF RII and hs-CRP fell after RYGB in keeping with the degree of weight loss. Insulin sensitivity improved significantly as shown by the fall in HOMA2 IR and rise in Matsuda index. None of the markers for insulin secretion from OGTT (HOMA %B, AUC insulin/glucose, Insulinogenic index or Disposition index) except the early insulin response (ΔIns 30 – ΔIns 0) reflected the improved insulin response seen after RYGB.

Conclusion: Patients with type 2 diabetes achieved similar weight loss to non-diabetes subjects in the long term. Improvements in glycaemia were seen soon after RYGB before weight loss had occurred. Glycaemic improvements were due to improved insulin sensitivity caused by calorie restriction and improved insulin production driven by incretins in the early phase. Later this was maintained by better appetite regulation, weight loss and reduced fat mass which led to favourable changes in adipokines.

Publications and presentations from this thesis

Book

Aditya BS, Wilding JPH, Obesity: An Atlas of Investigation and Management. ISBN: 978 1 84692 027 1. Clinical Publishing 2011

Publication

Aditya BS, Wilding JPH, Modern management of obesity. Clinical Medicine, 2009; 9(6): p. 617-21.

Poster presentation

Aditya BS, Wilding JP, Kerrigan D, Outcomes after Bariatric Surgery in a Diabetes Population. British Journal of Surgery 2007; 94(S5): 57

Oral presentation

Aditya BS, Magee C, Pipara L, Kerrigan D, Wilding JPH, How effective is bariatric surgery in diabetes? Diabetic Medicine 2008; 25 (S1): 26

Chapter 1

Introduction and review of literature

1.1. Overview

The prevalence of obesity is increasing alarmingly worldwide [1]. Obesity is closely linked to the pathogenesis of insulin resistance and type 2 diabetes [2, 3], leading to estimates that the prevalence of type 2 diabetes, will rise worldwide to over 220 million by 2010 and to over 300 million by 2025 [4]. Although severe obesity (BMI>40 kg/m2) only affects 1-2% of the population, about 8% of patients with type 2 diabetes have a BMI > 40, and these patients tend to have poor glycaemic control [5].

Although long-term lifestyle changes leading to reduction in calorie intake and increase in physical activity is the cornerstone of treatment, conservative methods including dietary modification and significant increases in physical exercise have largely been unsuccessful in causing meaningful or clinically significant weight loss long term or preventing co-morbidities like diabetes [6]. There have been vast amount of research into anti-obesity drugs but the current available options are very limited. Lack of efficacy and increased risk of undesirable side-effects have limited the success of pharmacological treatment of obesity.

Weight loss surgery, popularly known as bariatric surgery, has proven to be the only effective and durable treatment option for some patients who are very obese and have obesity-related co-morbidities and/or complications. Restrictive surgeries such as laparoscopic adjustable gastric band are less effective than malabsorptive procedures such as Roux-en-Y gastric bypass [7].

Of particular interest is the remission of type 2 diabetes which happens within days of malabsorptive procedures [8, 9]. The exact mechanisms for this change are still not very clear [10]. This thesis is a small step in trying to understand the mystery of how and why diabetes undergoes remission after weight loss surgery.

Understanding the mechanisms further may help us unravel potential newer treatments for type 2 diabetes and obesity, where weight loss surgery may not be suitable.

1.2 Obesity

1.2.1 Definition of obesity

In simple terms obesity can be defined as excessive amount of body fat which is associated with increased risk of medical illnesses and premature death. It is the result of a complex process of undesirable positive energy balance leading to accumulation of fatty deposits and weight gain. Although the total body fat mass is important, it is now recognised that the localisation of excess fat particularly in the intra-abdominal and visceral areas have a stronger correlation with risk of diabetes, cardiovascular disease and death [11]. The risk of co-existing diseases and complications associated with obesity is also affected by a range of factors, including nature of the diet, age and rate of weight gain, ethnic group and physical activity level [12].

Body mass Index (BMI) is a simple index of weight-for-height; calculation of BMI is shown below. Clinically 'Obesity' is defined as a BMI of 30 kg/m2 and higher. 'Overweight' is defined as BMI between 25 and 30 kg/m2.

Calculation of BMI
Body Mass Index (BMI) = Weight in Kg / Height in meters^2
Body Mass Index (BMI) = Weight in pounds x 704 / Height in inches^2

1.2.2 Classification of obesity

The commonly used classification of obesity is based on the measurement of body mass index. This is shown in the table below [12].

Classification of obesity based on BMI			
	Obesity Class	**BMI (Kg/m2)**	**Associated risk**
Underweight		< 18.5	Low (but risk of other clinical problems increased)
Normal		18.5 – 24.9	Normal
Overweight	Pre-obese	25.0 – 29.9	Increased
Obesity	I	30.0 – 34.9	Moderate
	II	35.0 – 39.9	Severe
Extreme Obesity	III	≥ 40.0	Very severe

Although BMI measurements can sometimes be misleading (e.g. muscular individuals, old age, ethnic variations), there appears to be a good correlation between BMI and the percentage of body fat [13]. There is also a strong correlation between BMI and mortality [14].

1.2.3 Measurements in obesity

Body Composition – Body fat content and fat distribution

Whilst weight and BMI are useful in classification of obesity, there are several limitations; they do not accurately estimate the body fat content or give information about fat distribution. Several techniques such as underwater weighing, air displacement

plethysmography using the Bodpod®, bioelectrical impedance and DEXA analysis are often used in research settings to measure total body fat content whereas imaging using CT and MRI are used to study fat distribution. However most of these are expensive and impractical for routine clinical use. Simple additional anthropometric measurements can be used in clinical practice for this purpose.

Anthropometric measurements

Waist circumference

Waist circumference is a simple means of estimating overall adiposity and also correlates better with intra-abdominal fat content than BMI alone [15]. It is measured (preferably after an overnight fast) directly over the skin at the end of normal expiration, horizontally, midway between the lower costal margin and the iliac crest with the arms relaxed at the sides. High waist circumference is associated with increased diabetes and cardiovascular risk independent of BMI, age and ethnicity [16].

Waist Hip Ratio

This has been studied as a further measure of fat distribution. Hip circumference is measured horizontally over the widest parts of the gluteal region. Waist to Hip Ratio (WHR) is then calculated. WHR > 0.95 in men and > 0.80 in women is said to be indicative of central obesity. Although it correlates well with disease risk [17, 18], the change in waist hip ratio is less remarkable than waist circumference with changes in weight and therefore is not always useful in clinical practice.

Abdominal sagittal diameter

Abdominal sagittal diameter is measured with a subject in supine position as the distance between the examination table and the highest point of the abdomen. Although the sagittal diameter shows the strongest relationship to intra-abdominal fat mass amongst all the anthropometric measurements [19], there are currently insufficient data to support its general use. It may not be useful in monitoring of serial changes in adiposity.

Measurements used to assess body composition

Skin fold thickness

Subcutaneous fat is measured at several sites using standard callipers, and can predict total fat mass with reasonable accuracy. The commonly used sites are triceps, biceps, anterior thigh, sub-scapular and supra-iliac areas. These measurements are age and gender specific and can be used to predict body density and total body fat [20]. However these are prone to inter- and intra-observer variations and therefore not recommended for routine clinical use. They can be used in research studies along with other measures of adiposity and to monitor progressive changes within individuals.

Bio-electrical Impedance

This method is based on the principle that fat is a poor conductor of applied current whereas fat-free tissue due to its water and electrolyte content is a good conductor. A small current is passed through the body to measure the body impedance. The bioelectrical impedance analysis of body water is used to estimate total body fat and fat-free (lean) mass. Body fat percentage is then calculated. In spite of several limitations, these techniques offer better estimation of fat content and have less observer error than anthropometric measurements and can be useful for monitoring changes within individuals in clinical practice [21].

Air displacement Plethysmography (BOD POD)

The BOD POD uses patented 'air displacement technology' for determining percent fat and fat-free mass in adults and children. The simple, 5-minute test consists of measuring the subject's mass (weight) using a very accurate electronic scale, and volume, which is determined by sitting inside the BOD POD chamber. From these two measurements, the subject's body composition is calculated [22].

The BOD POD consists of two chambers. The front, or test chamber, is where the subject sits and a reference chamber. A diaphragm is mounted on the common wall, which oscillates during testing. This causes small changes in volume inside the chamber, of which the pressure response to these small volume changes is measured. This is done by

measuring the interior volume of the empty BOD POD chamber, then measuring it again when the subject is seated inside. By subtraction, the subject's body volume is obtained.

Once the subject's mass and volume are determined, body density is calculated and the relative proportions of fat and fat-free mass are determined. This procedure is more user-friendly than UWW, can accommodate heavier patients than DEXA, CT or MRI machines and is there is no radiation exposure. However the equipment is too expensive for routine clinical use.

Dual Energy X-ray Absorptiometry (DEXA/DXA)

DEXA uses an x-ray beam with two energy peaks (high and low) in combination with a whole body scanner. This method is able to differentiate fat mass, fat-free mass, and bone mineral mass for the total body for specific anatomic regions through the differential absorption of the high and low-energy x-rays by these various tissues. There is a small radiation exposure but measurements are very precise and reliable. DEXA scanners are widely available in healthcare settings as they are frequently used to measure bone mineral density to asses risk of osteoporosis; an additional software package is required to assess body composition [23]. It is also very useful in assessment of adiposity in children [24] but is not recommended for routine clinical use.

Imaging techniques - Computerised tomography and Magnetic resonance

These can provide a direct measure of fat distribution, typically in the abdominal region. It is possible to discriminate between sub-cutaneous, omental, mesenteric and retroperitoneal deposits which may be associated with independent metabolic effects. There is good correlation between the fat areas measured in a single CT/MRI slice at the level of L4 to L5 with total visceral fat volume ($r > 0.95$) [25]. Their use is limited by availability, cost, radiation exposure (CT) and capacity of machines to accommodate very obese subjects.

Multi-compartment models

Classical methods to measure body composition are based on two compartment model, in which the body is assumed to be composed of fat & fat free tissue. Under water weighing, total body water, bioelectrical impedance or skin-fold thickness are all based on the assumption that the composition of fat-free mass is constant. However, this is not reliable in certain groups such as children, the elderly and physically fit individuals. Multi-compartment models have been used to overcome these limitations.

The multi-compartment model used to measure body composition requires a combination of measurement methods [26]. The four components used are as follows:

1. Determination of total body water (TBW) by using deuterium or 18O labelled water
2. Body mass
3. Body volume by air displacement (BOD POD) or underwater (UWW) weighing
4. Bone mineral content by dual-energy X-ray absorptiometry (DEXA)

1.2.4 Epidemiology of obesity

The prevalence of obesity is increasing at an alarming rate in both developed and developing countries. Traditionally obesity was seen as a sign of affluence but now, in both developed and developing countries, obesity is particularly marked in the lower socio-economic classes [27]. Throughout the world women in general have a higher prevalence of obesity, but more men are in the overweight category. Of particular concern is the increase in obesity and overweight rates in children [27]. The increase in prevalence of obesity across the world seems to be closely related to the increases in prevalence of cardiovascular disease, type 2 diabetes and mortality. Obesity could be regarded as 'The Millennium disease' and the epidemic may continue to rise, with serious health consequences, if appropriate action is not taken to reverse current trends.

Recent estimates from 2014 suggest that globally, there are more than 1.9 billion overweight adults, at least 600 million of them obese [27]. Childhood obesity is already epidemic in some areas and on the rise in others. An estimated 42 million children under

five are estimated to be overweight worldwide. Obesity accounts for 2-6% of total health care costs in developed countries; some estimates put the figure as high as 7%. The true costs are undoubtedly much greater as not all obesity-related conditions are included in the calculations.

The UK Government's Foresight programme, aimed at tackling obesity submitted its project report in October 2007 [28]. The impact of obesity on the incidence of coronary artery disease, stroke and diabetes in the future was assessed using a micro-simulation that imposed the known association between BMI and health risks from the present day to 2050. The analysis indicates that the greatest increase in the incidence of disease would be for type 2 diabetes (a >70% increase by 2050) with increases of 30 % for stroke and 20% for IHD.

1.3 Etiology of Obesity

1.3.1 Obesity Pathophysiology

Obesity is a state of undesired positive energy balance. When energy intake exceeds energy expenditure over long periods of time (usually years), it leads to accumulation of adipose tissue with a corresponding increase in lean body mass. Even a small positive energy balance on a daily basis can lead to weight gain; a daily excess of 100 Kcal leads to an approximate increase of 5 kg of fat over 12 months or 50 kg over 10 years. The resulting weight gain is also dependent on a complex interaction of genetic, biochemical, hormonal and environmental influences [29].

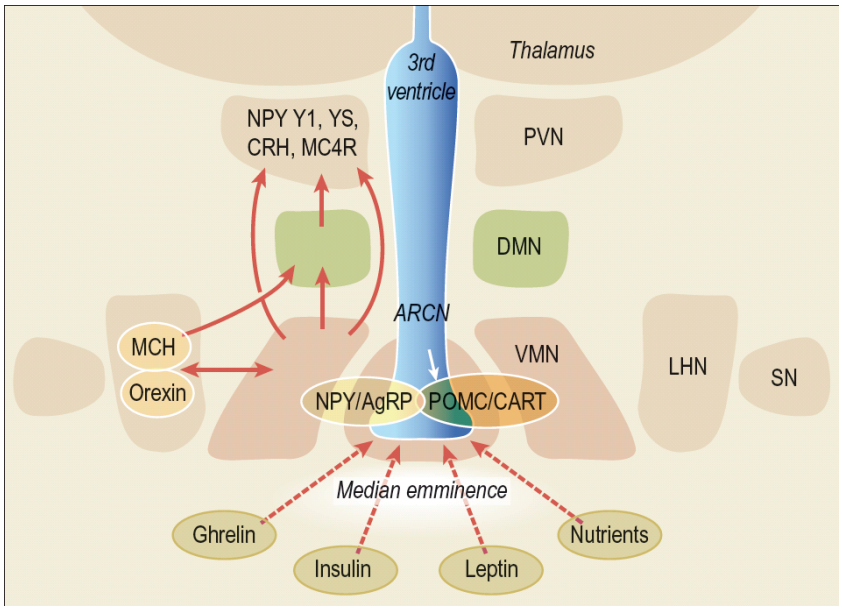
Obesity developing at a young age is more likely to be influenced by gene related changes in energy balance, whereas adult onset obesity is more likely to have a strong environmental component. The degree of fat accumulation and the adverse metabolic outcomes secondary to excess fat are significantly influenced by hereditary predisposition, lifestyle and ethnicity. The distribution of fat is pathophysiologically significant; for example, excess fat in the abdomen is associated with increased risk of metabolic diseases such as cardiovascular disease and diabetes [17].

An individual is said to be in a state of 'energy balance', if the energy intake is equal to energy expenditure. A 'positive energy balance' occurs when the energy expenditure does not match intake and the excess energy is stored in the body primarily as fat. The quantity and pattern of energy intake in modern society has changed progressively as a result of alterations in the availability and palatability of food. There has also been a significant change in the amount of physical activity which is an important component of energy expenditure but the principles of energy balance remain the same [30]. Energy balance is also tightly regulated by several neural and hormonal pathways [31]. The tendency to gain weight gradually throughout adult life probably reflects the fact that the regulatory systems have evolved to protect against weight loss rather than prevent weight gain.

1.3.2 Appetite regulation

Much of research into control of energy intake focuses on the following aspects: hunger & satiety control centres in the brain, brainstem–hypothalamic neurotransmitters involved in feeding regulation, hunger & satiety signals from the periphery especially the gut–brain axis and peripheral adiposity signals. It is important to understand the definition of hunger, appetite and satiety to understand the complex appetite regulation pathways. Hunger is a 'demand for calories' (e.g. after starvation) whereas appetite refers to 'a demand for a particular food' which may or may not be proportional to the body's need for nutrition. Hunger and satiation are controlled by neural centres and related neurotransmitters; appetite and feeding patterns are strongly influenced by psychological, economic, social and environmental factors.

Hypothalamic circuits implicated in the control of food intake



AgRP, *Agouti-related peptide;* ***ARCN,*** *arcuate nucleus;* ***CART,*** *cocaine and amphetamine-regulated transcript;* ***CRH,*** *corticotrophin-releasing hormone;* ***DMN,*** *dorsomedial nucleus;* ***LHN,*** *lateral hypothalamic nucleus;* ***MC4R,*** *melanocortin-4 receptor;* ***MCH,*** *melanin- concentrating hormone;* ***NPY,*** *neuropeptide-Y;* ***POMC,*** *pro-opiomelanocortin;* ***PVN,*** *paraventricular nucleus;* ***SN,*** *supra-optic nucleus;* ***VMN,*** *ventromedial nucleus.*

Image adapted from Aditya BS, Wilding JPH, Obesity: An Atlas of Investigation and Management. ISBN: 978 1 84692 027 1. Clinical Publishing 2011

Centres localised in hypothalamus that are involved in control of feeding behaviour are the arcuate nucleus (ARCN), paraventricular nucleus (PVN), ventromedial hypothalamic region (VMH), lateral hypothalamic region (LH) and perifornical area. These centres integrate neural (vagal) and circulatory (via nutrients and hormones) signals related to control of food intake. Neuropeptide-Y (NPY) and Agouti-related peptide (AgRP) expressed by NPY/AgRP neurons stimulate food intake. The pro-opiomelanocortin (POMC) is cleaved into alpha-melanocyte stimulating hormone (αMSH) which acts via melanocortin-4 receptors (MC4R). Activation of the POMC/CART neurons reduce food intake via the effects of αMSH, CART (cocaine and amphetamine regulated transcript) and neurotensin. All these neurons project to other regions in the hypothalamus, particularly in the LH area, which contains neurons expressing MCH (melanin concentrating hormone) and orexins A and B which stimulate food intake [32].

The changes in appetite regulation pathways in relation to obesity and the improvements after weight loss surgery are discussed later in detail.

1.3.3 The Gut – Brain axis in appetite regulation

The passage of food through the gut initiates a number of satiety signals. The vagus nerve carries afferent signals from stretch receptors and the chemoreceptors to the hindbrain [32]. Endocrine signals from the gut that play a part in appetite regulation and some of the common peptides are summarised in the table below. Several other peptides such as secretin, vasoactive intestinal peptide (VIP), orexin, apolipoprotein A-IV, bombesin-like-peptides, enterostatin, pancreatic polypeptide (PP), GLP-2, obestatin, gastric leptin have been reported to play some part in appetite regulation and further research into their actions is needed. Changes in appetite regulating hormones following weight loss surgery are discussed later.

The Gut – Brain axis in appetite regulation

Gut peptide	Site of production	Main actions related to appetite	Levels in obesity
Gastrin	Stomach & duodenum G cells	⇩ food intake	<—>
Cholecystokinin (CCK)	Proximal small intestine I cells	⇩ food intake, delay gastric emptying	<—> fasting <—> or ⇩ postprandial
Glucagon like peptide (GLP-1)	Ileum L cells	⇩ food intake, delay gastric emptying	<—> or ⇩
Glucose-dependent insulinotropic polypeptide (GIP)	Ileum K cells	⇩ food intake	<—>
Peptide YY (PYY)	Ileum, colon L cells	⇩ food intake, ⇩ gastric emptying & intestinal motility	⇩
Ghrelin	Stomach & pancreas	⇧ food intake, ⇧ gastric emptying and intestinal motility	<—> or ⇩ fasting and postprandial
Amylin	Pancreatic β cells	⇩ food intake, delay gastric emptying	⇩
Oxyntomodulin	Ileum L cells	⇩ food intake	<—>

1.3.4 Skeletal muscle and Adipose tissue metabolism in obesity

Skeletal muscle which makes up 30-40% of body weight is a major reservoir of carbohydrate and plays a significant role in glucose, amino acid and lipid metabolism. Impairment of muscle oxidative metabolism can lead to accumulation of body energy stores. The most prominent defects noted in skeletal muscle metabolism in obesity are reduced utilisation and storage of glucose, development of insulin resistance and impaired fatty acid oxidation.

Metabolic consequences of increased fat deposition and fat distribution play a significant role directly and indirectly in almost all major systems and biochemical pathways. There has been a great interest in the role of non-esterified fatty acids (NEFA) in development of insulin resistance. Last few decades have seen the discovery of newer adipokines which influence metabolic pathways in liver, skeletal muscle, gut and brain (appetite). The role of leptin, adiponectin, resistin, visfatin and pro-inflammatory cytokines such as interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP) and tumour necrosis factor-α (TNFα) are being studied extensively. These have been discussed in detail in chapter 6. They not only help us understand the pathophysiology of obesity but also its consequences such as cardiovascular disease and diabetes and provide likely therapeutic targets.

Peripheral signals involved in energy homeostasis [32]

Insert figure

Circulating gastrointestinal and adipocyte hormones and neuronal circuits involved in energy homeostasis: solid lines represent net stimulatory effect; dashed lines represent net inhibitory effect)

Image adapted from: ***Clinical Endocrinology***
Volume 60, Issue 2, pages 153-160, 18 NOV 2003 DOI: 10.1046/j.1365-2265.2003.01839.x
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2265.2003.01839.x

1.3.5 Lifestyle and environmental factors influencing obesity

Altered lifestyle of the population influenced by behavioural and environmental changes of the modern society have been blamed for the current worldwide increase in obesity and overweight [33] as the genetic and metabolic influences of an individual's susceptibility to obesity and the gene pool of the population have remained constant. Ethnic variations in susceptibility to obesity and population migration alone cannot explain the obesity epidemic. In fact, data from migration studies of some ethnic populations from developing countries have shown that the obesity risk increases dramatically when they are exposed to westernised lifestyles [34].

Studies show that in recent decades, rather surprisingly, that there has been no increase in the calories we eat per day. The data from the UK National Food Survey (NFS) shows that the average daily food intake in Britain has not changed significantly but the physical activity levels have dropped significantly [33]. However there is increase in high calorie (energy dense) food intake and fall in household food consumption. There has been a profound increase in the proportion of dietary fat consumption at the expense of carbohydrate intake. There appears to be a strong correlation between dietary fat consumption and obesity risk [35]. Experimental studies show that consumption of energy-dense foods that are high in fat and sugar content, produce a less powerful satiety response than food that are high in complex carbohydrates, leading to the phenomenon of passive overconsumption. Studies that estimate food consumption are moreover greatly confounded by under-reporting by obese subjects [36].

The levels of physical activity have dropped significantly over the last 50 years. The availability of labour saving devices, better transportation facilities, changes in work patterns and physical environment, lack of outdoor activity facilities and sedentary leisure-time pursuits such as television and computer games have all been implicated in declining physical activity levels. There is some observational data to support that time spent viewing television particularly with children and availability and use of cars are linked to increase in obesity risk [33].

Secular trends in diet (left) and activity (right) in relation to obesity in Britain (Data for diet from National Food Survey; data for body mass index from Office of Population Censuses and Surveys and historical surveys; data for television viewing and car ownership from Central Statistical Office).

Image adapted from: Prentice AM, Jebb SA. Obesity in Britain: gluttony or sloth? BMJ 1995; ***311****: 437–9.*

Insert figure

Changes in the fat/carbohydrate ratio of the British diet (source: National Food Survey)

Image adapted from: Prentice AM, Jebb SA. Obesity in Britain: gluttony or sloth? BMJ 1995; 311: 437–9.

Insert figure

1.3.6 Other causes of obesity

a) **Genetics** – As discussed earlier environmental factors and modern lifestyles and often quoted as the most likely explanation for the recent obesity epidemic across the globe. This does not mean that hereditary factors do not play a significant role in determining the risk of weight gain. Family, adoptee and twin studies confirm that the contribution of genetic and heritable factors to the tendency to develop obesity is about 45 – 75% [37]. Progress in identifying single-gene defects leading to obesity had been slow until recently, but significant progress has been made in recent years in this area [38-40]. It is clear that obesity is strongly influenced by environmental factors, but the susceptibility to these factors is influenced by genetic variations of an individual or a population subset. Although the amount of weight gain associated with individual genetic variations are very small, it is possible that several genetic variations come together in an individual to produce clinically significant weight gain over a number of years [41].

b) **Foetal and Infant origins of obesity** – Impaired foetal and infant growth have always been suspected as a factor in developing obesity and many other chronic diseases in adult life. Numerous animal studies and some recent human observational studies have provided compelling evidence that the quantity and distribution of adipose tissue may be influenced during intrauterine and early postnatal life [42]. High BMI during infancy, childhood and adolescence has been shown to predict adult obesity in longitudinal studies. There is evidence that weight at birth also correlates to adult weight [43]. Although increasing birth weight correlated with increased risk of adult obesity, it was noticed that people with lowest birth weight (under 5.5 lbs) also had increased obesity prevalence. In particular low birth weight is shown to be associated with increased truncal fat distribution [42].

c) **Intestinal flora** – There have been some animal and human studies that propose a link between gut flora and obesity [44]. Intestinal flora in obese mice and humans are rich in firmicutes species and relatively deficient in bacteroidetes. Transplantation of gut flora from obese mice to lean mice promoted weight gain. In obese humans who lose weight, the gut flora changes to lean-type. These may be primary or secondary to changes in weight or diet or both. Newer techniques in assessing the gut microbiota have led to many useful studies in this area, which are hinting that we should be considering this as a possible factor in pathophysiology of obesity.

d) **Endocrine disease** – Hypothyroidism, Cushing's syndrome, Acromegaly and Polycystic Ovarian syndrome (PCOS) are all rare but recognised causes of weight gain. They are usually diagnosed before causing significant weight gain due the presence of multiple signs and symptoms. Ruling out these conditions forms an important part of assessment of a subject presenting with obesity.

e) **Hypothalamic obesity** – Tumours in the hypothalamic region particularly craniopharyngiomas and pituitary macroadenomas with suprasellar extension can damage the ventromedial hypothalamic areas that regulate energy intake (appetite regulation) and expenditure. This can also be caused by trauma, surgery and radiation. These subjects can exhibit marked hyperphagia and have autonomic imbalance leading to hyperinsulinaemia, which can exacerbate weight gain by promoting abnormal fat deposition [45]. Associated pituitary hormone imbalances and somnolence leading to reduced physical activity may also contribute to their metabolic risk.

f) **Drugs** – Many drugs promote weight gain due to central effects on appetite and/or peripheral metabolic actions. Patients who are already overweight or at a risk of weight gain need to be aware of the side-effects of the drugs that they take. Alternatives should be considered wherever possible or adequate measures must be taken to prevent weight gain.

Drugs commonly associated with weight gain are:

- Anticonvulsants
- Antipsychotics
- Antidepressants
- beta-blockers
- Antihistamines
- Steroids
- Oral hypoglycaemic agents (sulphonylureas and thiazolidinediones)
- Insulin
- Protease inhibitors
- Sex hormones and contraceptive hormone preparations

1.4 Obesity, Insulin resistance, Type 2 diabetes and Cardiovascular risk

Obesity is characterised by a metabolic process associated with low-intensity chronic inflammation, which is evidenced by an increased concentration of circulating inflammatory mediators. More than fifty inflammatory proteins have been linked to obesity and its co-morbidities [46], and the mechanisms by which these proteins influence the manifestation of these diseases seem to involve the attenuation of insulin activity, fat mobilisation, endothelial dysfunction and oxidative stress.

Increasing BMI, in particular increased intra-abdominal and visceral fat, has been clearly shown to increase the risk of developing type 2 diabetes in both men and women [2, 3]. Epidemiological data show that the increase in prevalence of obesity closely mirrors the increases in prevalence of type 2 diabetes. It has been estimated that the risk of developing T2DM is increased 93-fold in women and 42-fold in men who are severely obese compared to those with a normal weight [2, 3]. The patho-physiological links between obesity, type 2 diabetes and metabolic syndrome are well established [47]. Increasing weight worsens insulin resistance and this leads to progressive beta cell failure and the risk of developing type 2 diabetes. This process is influenced by genetic and environmental factors.

Metabolic syndrome is characterised by a cluster of medical disorders that often occur together in one individual, which increases the risk of cardiovascular disease and diabetes.

Several definitions and criteria exist but the important components and associations that are thought to represent metabolic syndrome are summarised below.

Metabolic syndrome: proposed components and associated findings [47]

1. Insulin resistance*
2. Hyperinsulinaemia*
3. Obesity: visceral (central), but also generalized obesity*
4. Dyslipidaemia: high triglycerides, low high-density lipoprotein, small dense low-density lipoprotein*
5. Adipocyte dysfunction
6. Impaired glucose tolerance or type 2 diabetes mellitus*
7. Fatty liver (non-alcoholic steatohepatosis, steatohepatitis)
8. Essential hypertension: increased systolic and diastolic blood pressure*
9. Endothelial dysfunction
10. Renal dysfunction: micro- or macro-albuminuria
11. Polycystic ovary syndrome
12. Inflammation: increased C-reactive protein and other inflammatory markers
13. Hypercoagulability: increased fibrinogen and plasminogen activating inhibitor 1
14. Atherosclerosis leading to increased cardiovascular morbidity and mortality*

**Most widely incorporated into the definition of metabolic syndrome.*

In 1998, the American Diabetes Association (ADA) issued a consensus statement identifying "glucose intolerance, central obesity, dyslipidaemia (increased triglycerides, decreased HDL, increased small dense LDL), hypertension, increased pro-thrombotic and anti-fibrinolytic factors, and a predilection for atherosclerotic vascular disease" as components of the metabolic syndrome associated with insulin resistance . The ADA statement did not provide diagnostic cut-points or criteria for the syndrome.

Obesity has been shown to be linked strongly and independently to all the components of metabolic syndrome described above [48]. The NCEP – ATP III and AHA/NHLBI guidelines did not include abdominal obesity as a requirement for diagnosis because lesser degrees of abdominal girth are often associated with other components [49, 50]. However the IDF consensus statement includes central obesity as a prerequisite for diagnosis [51].

Most of the above definitions recognise central obesity (categorised by waist circumference or waist-to-hip ratio) as the essential component of metabolic syndrome rather than generalised obesity as the intra-abdominal fat content has a stronger correlation with insulin resistance and hyperinsulinaemia [52]. The relationship between insulin resistance and cardiovascular disease has been well described [53].

Visceral fat is an active endocrine organ producing a number of factors (adipokines) that may contribute to local and systemic inflammation in obesity. Some of these factors are also involved directly and indirectly in control of vascular tone, endothelial function, coagulation and insulin sensitivity. Local production and circulatory concentrations of most adipokines are increased in obesity, although adiponectin (generally considered as a protective factor) decreases as BMI rises. Increased levels of TNFα and IL-6 may promote insulin resistance in obesity whereas adiponectin reduces insulin resistance. Adipose tissue is also a source of PAI-1 and haptoglobin which increases blood coagulability; of angiotensinogen (raising blood pressure), ICAM-1 & RBP-4 may contribute via increased oxidative stress leading to endothelial dysfunction [54].

Insert figure

Visceral fat deposits and the associated insulin resistance contribute significantly to increased cardiovascular disease in obesity [54].

Excess visceral fat leads to endothelial dysfunction and inflammation through the direct and indirect effects of adipokines (adiponectin and TNF-α). Fat accumulation, insulin resistance, liver-induced inflammation, and dyslipidaemia may all lead to the premature atherosclerotic process.

CRP, C-reactive protein; **ICAM-1,** intercellular adhesion molecule-1; **IL-6,** interleukin-6; **LDL,** low-density lipoprotein; **LDL-ox,** oxidised low-density lipoprotein; **MCP-1,** monocyte chemo-attractant protein-1; **NEFA,** non-esterified fatty acids; **PAI-1,** plasminogen activator inhibitor-1; **RBP-4,** retinol-binding protein-4; **ROS,** reactive oxygen species; **TNF-a,** tumour necrosis factor-alpha; **VLDL,** very low-density lipoprotein

Figure adapted from:
Van Gaal, L.F., I.L. Mertens, and C.E. De Block, Mechanisms linking obesity with cardiovascular disease. Nature, 2006. 444(7121): p. 875-880 doi:10.1038/nature05487

1.5 Non-surgical management of obesity

1.5.1 Dietary changes, Exercise and Eating behaviour

The education of an obese patient about diet and eating habits is an essential component of any weight loss treatment. Commercial diets and diet based weight loss programmes have gained a lot of popularity and acceptance in the recent years. However, the general perceptions about dietary intervention for weight loss are often skewed from the basic principles of dietary management. These dietary interventions are usually effective in the short term but their lack of effectiveness in the long term is well documented [55, 56]. The diet cycle [57] described below is a common problem faced by obese subjects and ways to break the 'yo-yo' dieting culture should be part of the management plan.

Insert figure

The Diet cycle [57]

Adapted from: Kopelman, P.G., I.D. Caterson, and W.H. Dietz, ***Clinical obesity in adults and children.*** *3rd ed. 2010, Chichester, West Sussex ; Hoboken, NJ: Wiley-Blackwell. x, 502 p.*

Calorie restriction and reduced portion sizes are important to achieve weight loss but dietary advice should also include the importance of a well-balanced diet. The 'Eatwell Plate' recommended by the Food Standards Agency (UK) highlights the importance of a balanced diet and the essential components of a healthy diet [58]. An appropriate dietary management plan is essential for the success of any weight loss program even with the use

of anti-obesity drugs or bariatric surgery. Dietary interventions are very important in long term weight loss maintenance. It is also worth remembering that the success of dietary management depends on careful assessment of every subject particularly the recognition of behavioural problems (e.g. eating disorder) and readiness to change.

Physical exercise

Exercise is an essential adjunct to successful management of obesity. 'Energy expenditure' is as important as 'energy restriction' in achieving 'energy balance'. There are also weight loss independent beneficial health effects of exercise in obesity [59]. Several comorbidities related to obesity are also improved and obesity induced complications can be avoided by modest exercise. The role of physical exercise in obesity prevention and long term weight loss maintenance cannot be over emphasised. Obese subjects should be educated on the importance of improving cardiovascular 'fitness' rather than just focussing on measures of 'fatness' such as body weight and dress size [60].

The amount and type of exercise required depends on goals of the program. Aerobic exercises are usually recommended but the intensity of exercise will depend on several factors including the functional ability of the individual and presence of limiting comorbidities.

Behavioural therapy and Eating disorders

Behaviour therapy for weight management focuses on modification of people's eating habits and level of physical activity. Careful assessment of a particular individual's causes of weight gain or inability to lose weight or maintain weight loss should identify the need for behavioural treatment [61, 62]. Failure of weight management with lifestyle modifications or drugs should alert the physician to investigate the presence of eating disorder or compliance problems. Subjects with clear-cut eating disorders are best managed in specialised centres but successful weight management plans should involve at least some degree of behavioural modification [63].

Eating disorders related to overweight and obesity

1. Bulimia Nervosa (Atypical type sometimes associated with overweight)

   (DSM-IV and ICD-10 definitions for diagnostic criteria)

   - Recurrent episodes of overeating (at least twice a week for 3 months)
   - Persistent preoccupation with eating (strong desire or sense of compulsion to eat)
   - Inappropriate compensatory habits – self-induced vomiting; misuse of laxatives, diuretics, enemas or other drugs; periods of starvation; excessive exercise
   - Self-perception of being fat and dread of fatness

2. Binge Eating disorder

   - Recurrent episodes of binge eating (large amounts in discrete period of time with lack of control over eating during the episode)
   - Associated with at least three of the following
     - Eating more rapidly than normal
     - Eating until feeling uncomfortably full
     - Eating large amounts even when not hungry
     - Eating alone because of embarrassment
     - Feeling disgusted, depressed or very guilty after overeating
   - Marked distress regarding binge eating is present
   - Occurs, on average, at least 2 days a week for 6 months
   - Not associated with the regular use of inappropriate compensatory behaviour

3. Night Eating Syndrome
4. Anxiety, Depression related eating disorders
5. Addictive disorders affecting eating habits
6. Personality and eating disorders

1.5.2 Drug treatment of obesity

Over the years numerous agents have been used to treat obesity. Most of these drugs are rarely used now, due to lack of efficacy or undesirable side effects. Some agents are approved by the Food and Drug Administration (FDA) in the United States of America for short term use only and others have failed to show durable weight loss and therefore not yet approved in Europe. There is some evidence of their efficacy but very little data supports their long-term benefits or improvements in co-morbidities.

Rimonabant (Acomplia), which is a CB1 receptor antagonist, was previously approved for long-term use in Europe and was withdrawn in 2009 due to increased risk of psychiatric side-effects [64, 65]. Sibutramine (Reductil, Meridia) was withdrawn by the European Medicines Agency (EMA) in January 2010 and subsequently from the USA in October 2010 due to increased risk of adverse events such as myocardial infarction, stroke, cardiac arrest and cardiovascular death compared with placebo-treated patients [66]. It was a centrally-acting inhibitor of both serotonin and noradrenaline reuptake with little effect on dopamine receptors. It limited food intake by enhancement of the natural satiety process. This highlights the need for strong long-term research into efficacy and safety before approving weight loss agents.

Finding anti-obesity agents with good efficacy and acceptable side-effect profile continues to be elusive. The drugs that are currently licenced for long-term use in Europe are discussed below. Newer agents that have been approved in the United States but not yet in Europe are also discussed briefly.

Orlistat (Xenical, Alli)

It inhibits pancreatic and intestinal lipases, resulting in inhibition of absorption of about 30% of dietary triglycerides. It is not absorbed systemically and does not affect systemic lipases. The recommended dosage is 120 mg three times daily (with or up to 1 hr after each meal) combined with lifestyle and behavioural therapy. Lower dose of 60 mgs three times daily has recently been approved for 'over the counter' sales in many countries. It is indicated in the treatment of obesity when BMI > 30 or BMI > 28 with associated risk factors. It is contraindicated in chronic malabsorption, cholestasis, breast feeding and

hypersensitivity. The known adverse effects are gastrointestinal side effects such as oily spotting, flatus with discharge, faecal urgency, fatty or oily stool and faecal incontinence. The absorption of fat soluble vitamins and beta-carotene may be impaired. Withdrawal rate due to GI side effects can be lowered by appropriate dietary advice; people should be advised to avoid high fat meals as the incidence of GI adverse events appears to be related to the dietary fat content. Current evidence supports use for up to 48 months duration but may be considered for weight maintenance if there is a risk of weight regain on completion of therapy. There is evidence of mean weight loss (over placebo after 2 years of treatment) of 3.5 kg (10.3 kg vs. 6.1 kg); 5% weight loss is achieved in 58% (vs. 32%) and 10% weight loss is achieved in 39% (vs. 18%) [67].

Liraglutide (Saxenda)

Liraglutide was approved for use by the European Medicines agency (EMA) in March 2015 (but not yet marketed) [68]. It is a human glucagon-like peptide-1 (GLP-1) analogue produced by recombinant DNA technology in Saccharomyces cerevisiae. It acts in the same way as GLP-1 by improving insulin production in response to glucose, decreasing the production of glucagon, delaying gastric emptying, lowering hunger and increasing satiety.

It is indicated as an adjunct to a reduced-calorie diet and increased physical activity in adult patients with an initial BMI of ≥ 30 kg/m² (obese), or ≥ 27 kg/m² in the presence of at least one weight-related comorbidity such as pre-diabetes or type 2 diabetes mellitus, hypertension, dyslipidaemia or obstructive sleep apnoea. The starting dose is 0.6 mg subcutaneously daily. The dose should be increased to 3.0 mg daily, in increments of 0.6 mg, with at least one week intervals to improve gastro-intestinal tolerability. If escalation to the next dose step is not tolerated for two consecutive weeks, discontinuing treatment should be considered. Daily doses higher than 3.0 mg are not recommended. Treatment with Saxenda should be discontinued after 12 weeks on the 3.0 mg/day dose if patients have not lost at least 5% of their initial body weight. It is not recommended for use in patients with severe renal impairment (creatinine clearance <30 mL/min) including patients with end-stage renal disease. Common side-effects are nausea, vomiting, diarrhoea, constipation, injection site reactions, cholelithiasis, dyspepsia and dizziness. Uncommon adverse events are pancreatitis, cholecystitis, allergic reactions and acute kidney injury.

The four randomised, double-blind, placebo-controlled trials which included a total of 5,358 patients shown a mean weight loss (over placebo after 56 weeks of treatment) of 4.2 to 5.9 kg over placebo; 5% weight loss is achieved in 49.8 to 63.5% and 10% weight loss is seen in 22.4 to 32.8% (EMA/143005/2015) [69-71].

Naltrexone/Bupropion (Mysimba)

This combination product was also approved by the EMA in March 2015. Naltrexone is a mu-opioid antagonist and bupropion is a norepinephrine and dopamine reuptake inhibitor. Both compounds affect two key appetite regulating areas of the brain; the arcuate nucleus of the hypothalamus, an area of the brain that plays a critical role in the control of food intake and energy expenditure and the mesolimbic dopaminergic reward system, a region of the brain that is important for processing the rewarding aspects of food and food related stimuli. Both bupropion and naltrexone act in the mesolimbic reward system to influence eating behaviour.

Each tablet contains 8 mg naltrexone hydrochloride, equivalent to 7.2 mg of naltrexone, and 90 mg bupropion hydrochloride, equivalent to 78 mg of bupropion. It is indicated as an adjunct to a reduced-calorie diet and increased physical activity for the management of weight in adult patients (≥18 years) with an BMI of ≥ 30 kg/m2 or ≥ 27 kg/m2 in the presence of one or more weight-related co-morbidities (e.g., type 2 diabetes, dyslipidaemia, or controlled hypertension). The maximum recommended daily dose of Mysimba is two tablets taken twice daily. The starting dose is 1 tablet in week 1, 2 tablets in week 2, 3 tablets in week 3 and then starting the full dose in week 4. Treatment with Mysimba should be discontinued after 16 weeks if patients have not lost at least 5% of their initial body weight. The most frequent adverse reactions for naltrexone/bupropion are nausea, constipation, vomiting, dizziness, and dry mouth. Unfortunately there are numerous serious side-effects reported with the use of this treatment such as (but not limited to) rising blood pressure, palpitations, seizures, suicides & psychiatric disorders, hypersensitivity reactions, CNS disorders and cardiovascular side-effects.

In four double-blind, placebo-controlled studies involving 4,536 subjects, the mean percent body weight loss of -5.4% was observed compared to -1.3% in placebo-treated subjects. 5%

weight loss was observed in 31% vs. placebo 12% (EMA/805547/2015). This product has also not yet been marketed in Europe.

Locarserin (Belviq)

Lorcaserin is a selective 5-HT2C receptor agonist, which activates pro-opiomelanocortin (POMC) production and consequently promotes weight loss through satiety. In 2012, the FDA approved lorcaserin for use in the treatment of obesity for adults with a BMI ≥30 or BMI ≥27 with at least one weight-related health condition such as high blood pressure, type 2 diabetes or high cholesterol (FDA/3151563/2012). Lorcaserin hydrochloride does not have a UK marketing authorisation.

Topiramate/Phentermine (Qsymia)

This was approved by the FDA in 2012 but not yet approved in Europe. Qsymia is a combination oral product comprising of immediate-release phentermine which is a sympathomimetic agent that suppresses appetite and extended-release Topiramate which is a sulfamate-substituted monosaccharide related to fructose antiepileptic drug which works by decreasing appetite and by causing feelings of fullness to last longer after eating (FDA/ 3634966/2012).

1.6 **Surgical management of Morbid Obesity**

Surgical treatments for obesity have been developing for more than 60 years but their use, until recently, have been very limited due to the risk of death and peri-operative complications, the invasiveness, costs, need for intensive post-operative management and lack of long-term outcome and safety data. The surgical techniques used today are less complicated and the use of minimally invasive laparoscopic approach has drastically cut down the incidence of peri-operative complications [72]. With improved surgical instruments, technique and overall improvement in standards of post-operative care, the field of bariatric surgery is gaining popularity. We are beginning to see the long-term benefits of surgery and the confidence in the effectiveness and safety profile of some procedures is improving. In the 80's and 90's there were numerous reports supporting the fact that in addition to causing significant weight loss, bariatric surgery lead to remission and improvement of various comorbidities (particularly type 2 diabetes) [73]. This created a lot of interest amongst health professionals in the last quarter of twentieth century and the popularity of surgical treatment for obesity has been on an upward trend ever since.

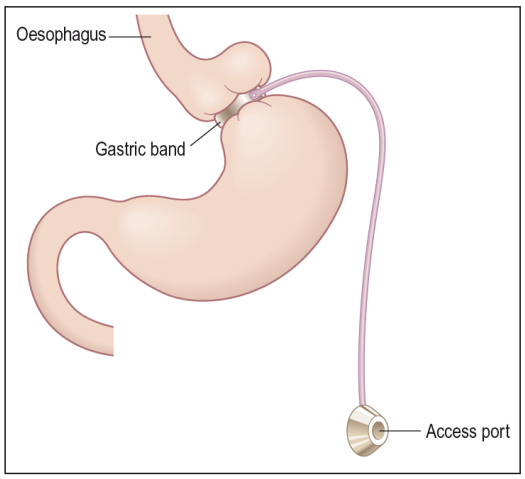
1.6.1 Types of weight loss procedures

Weight loss surgery is broadly classified by its mechanism of action. Procedures are either restrictive in nature leading to reduced food intake or malabsorptive or mixed involving both mechanisms of action. The procedures that are commonly performed in the UK are discussed below.

Mechanism of action	**Common surgical procedures** * Commonly performed in the UK
Restrictive	Laparoscopic Gastric Balloon insertion Vertical banded Gastroplasty (VBG) Laparoscopic Adjustable Gastric Banding* (LAGB) Sleeve Gastrectomy* (SG)
Malabsorptive	Biliopancreatic Diversion – Scopinaro procedure (BPD) Biliopancreatic Diversion with Duodenal Switch* (BPD-DS)
Combined	Roux-en-Y Gastric Bypass* (RYGB)

Laparoscopic Adjustable Gastric Band (LAGB)

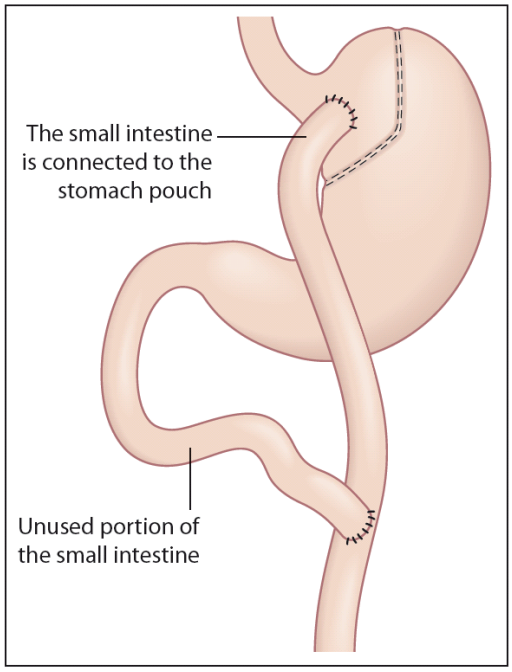
This was introduced in the mid 90's. It is a restrictive procedure which involves placing of an adjustable band in the upper part of the stomach, just distal to the gastro-oesophageal junction [74]. The amount of restriction can be altered by injecting or withdrawing saline from the band through a subcutaneous port (similar to that used for long-term venous access for chemotherapy patients). This is still common in commercial centres but their use has become very limited in many countries due to increased risk of post-operative complications, high re-operation rates and failure rates.



Laparoscopic Adjustable Gastric Band (LAGB)

Laparoscopic Roux-en-Y gastric bypass (RYGB)

This is the commonest procedure performed in the world today [75]. Open Roux-en-Y gastric bypass was introduced by Edward Mason in 1960 [76]. This procedure, although effective, was limited by significant peri-operative mortality and complication rates for several decades. The mortality and complication rates associated with this procedure have reduced significantly since the use of minimally invasive laparoscopic technique. In this procedure the stomach is reduced to a small upper gastric pouch which drained into a Roux-en-Y limb of proximal jejunum (variable lengths used between 40 & 150 cm). It is designed as a combined malabsorptive and restrictive procedure.

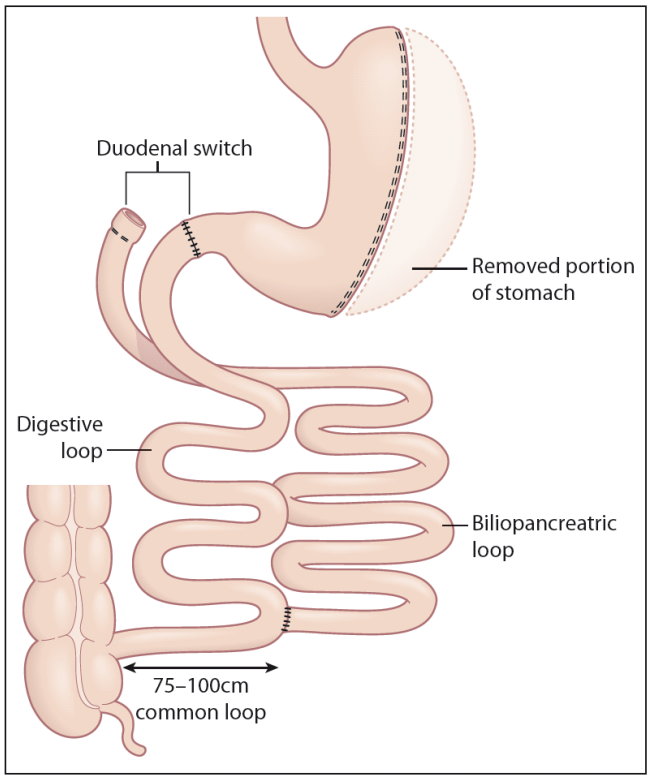


Laparoscopic Roux-en-Y Gastric Bypass (RYGB)

Laparoscopic Biliopancreatic diversion with Duodenal Switch (BPD-DS, LDS)

In this procedure, a sleeve gastrectomy is performed (rather than the original horizontal gastrectomy in the Scopinaro type) [77] leaving a gastric reservoir of 150 to 200mls [78]. The duodenum is closed about 2 cm distal to the pylorus and a duodeno-ileal anastomosis is performed. The gastric fundus is almost entirely resected, while the antrum, the pylorus and a short segment of duodenum are preserved along with vagus nerve. Moreover the 'common limb' is about 100 cm as opposed to 50 cm in the original procedure.

This procedure can be done in 2 stages in very obese subjects (BMI > 60) and those with a high Obesity Surgery Mortality Risk Score (OS-MRS) [79]. Initially a sleeve gastrectomy (SG) is performed to allow moderate weight loss. The rest of the procedure can be performed safely after a period of weight loss which is usually 12 to 24 months. As the two stage procedure, following the initial SG, the second stage could be RYGB or BPD-DS according to the preference of the surgeon and the patient.



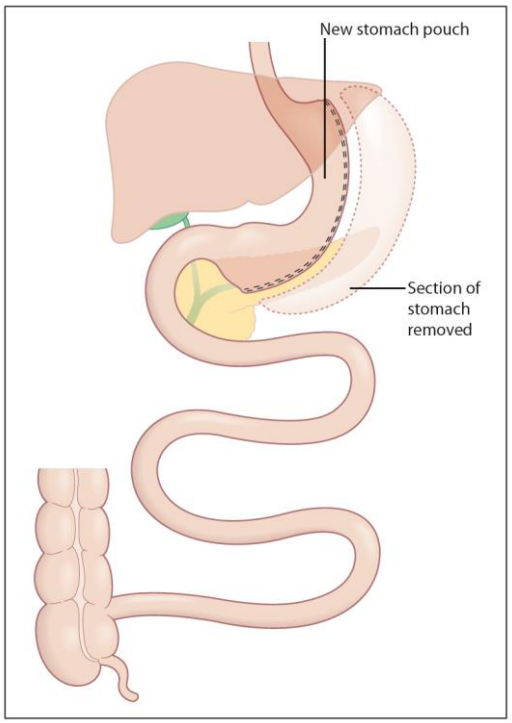
Laparoscopic Biliopancreatic diversion with Duodenal Switch (BPD-DS, LDS) *– this can be performed in a single stage or 2 stages.*

Novel Surgical procedures & techniques

Duodenal-Jejunal Bypass (DJB) – This was developed as an experimental anti-diabetic procedure. The proximal duodenum is anastomosed to the distal portion of small intestine (duodeno-jejunal type) or a pre-pyloric gastro-jejunostomy is performed. Long-term data is not yet available, but there appears to be significant remission /improvement of type 2 diabetes in addition to weight loss. This is now done in combination with a sleeve gastrectomy procedure with promising results [80].

Sleeve Gastrectomy (SG) – This was initially performed as the first stage of two stage procedure in very obese patients (BMI > 60) or those with a high mortality risk. After some weight loss following the procedure, patients were able to have either a duodenal switch procedure or even a Roux-en-Y gastric bypass as the second stage surgery. This has now

been acknowledged as an independent anti-obesity procedure particularly in very heavy patients or those with high risk of peri-operative complications. In the last few years this has become a popular procedure due to its simplicity and effectiveness and is now the second most common procedure performed in the world [81].



Laparoscopic Sleeve Gastrectomy (LSG)

Ileal Interposition (Ileal Transposition – IT) – A small segment of ileum with its vascular and nerve supply intact, is surgically interposed into proximal small intestine, which leads to increased exposure of the ileum to nutrients. This leads to exaggerated GLP-1 and PYY responses to nutrients, resulting in reduced food intake, weight loss and improved glucose homeostasis. Initial results are promising with regards to diabetes remission but the weight loss is not as expected in many subjects .

Mid jejunal resection & **Omentectomy** are some of the other experimental procedures that are promising in the short-term; further long-term data is awaited. They appear to be more efficient in improving metabolic consequences of obesity rather than cause significant weight loss.

Robotic surgery – Primary and revisional bariatric surgery can be performed with the help of robotic assisted systems (e.g. daVinci robot system). It helps the surgeon to increase precision and improves outcomes of complex procedures. They are shown to be 30% faster than even experienced laparoscopic surgeons.

Endoluminal procedures (NOTES – Natural Orifice Transluminal Endoscopic Surgery) – These are procedures performed via 'natural orifices' reducing perioperative risk and mortality. Several restrictive (e.g. endoscopically inserted intragastric balloon (IGB), intraluminal gastric partitioning techniques) and malabsorptive procedures (e.g. polyethylene endo-luminal duodeno-jejunal tube (EDJT)) are currently being studied in an attempt to develop a procedure which is safe as well as effective.

The **'**Endo-Barrier' Gastrointestinal Liner **(Duodenal-Jejunal Bypass Sleeve (DJBS)** is currently being studied in many centres. Although the weight loss is modest, exclusion of the proximal intestine improves glycaemia rapidly. Although the insulin secretion is not affected, the improvement in glycaemia is thought to be due to improved insulin sensitivity due to a reduction in hepatic glucose output [82, 83].

Criteria for surgical treatment of obesity

Weight loss surgery is self-funded in many countries but in publicly funded healthcare systems, several criteria for surgical treatment of obesity exist. In the UK weight loss surgery is gradually increasing in numbers over the last 10 years. Some salient points from the latest NICE guideline (CG189 – November 2014) are discussed below. However patient selection for weight loss surgery depends heavily on local policy, availability of resources, availability of experienced surgeons and bariatric units with a skilled multi-disciplinary team. The main aim is to identify those subjects who are most likely to benefit from surgery (risk of obesity and its complication versus the potential benefits of surgery) and reduction in peri-operative risk resulting from surgery.

NICE Clinical Guideline CG189 (2014)

Bariatric surgery is a treatment option for people with obesity if all of the following criteria are fulfilled:

- They have a BMI of 40 kg/m2 or more, or between 35 kg/m2 and 40 kg/m2 and other significant disease (for example, type 2 diabetes or high blood pressure) that could be improved if they lost weight.
- All appropriate non-surgical measures have been tried but the person has not achieved or maintained adequate, clinically beneficial weight loss.
- The person has been receiving or will receive intensive management in a tier 3 service.
- The person is generally fit for anaesthesia and surgery.
- The person commits to the need for long-term follow-up.

In addition to the criteria listed above, bariatric surgery is the option of choice (instead of lifestyle interventions or drug treatment) for adults with a BMI of more than 50 kg/m2 when other interventions have not been effective.

Offer an expedited assessment for bariatric surgery to people with a BMI of 35 or over who have recent-onset type 2 diabetes as long as they are also receiving or will receive assessment in a tier 3 service (or equivalent).

Consider an assessment for bariatric surgery for people with a BMI of 30–34.9 who have recent-onset type 2 diabetes as long as they are also receiving or will receive assessment in a tier 3 service (or equivalent).

Consider an assessment for bariatric surgery for people of Asian family origin who have recent-onset type 2 diabetes at a lower BMI than other populations as long as they are also receiving or will receive assessment in a tier 3 service (or equivalent).

Bariatric surgery in subjects with BMI less than 35

In recent years it has been proposed that bariatric procedures should also be considered in less obese subjects, due to the metabolic benefits seen following these procedures. Durable remission of type 2 diabetes and improvements in components of metabolic syndrome are extremely beneficial even to those with a BMI less than 35 but the current criteria would exclude them for accessing surgical treatments. There are a number of studies underway to assess the risk to benefit ratio of surgical procedures in the moderately obese population. Based on the promising results of metabolic surgery in people with BMI less than 35 and due to the improving safety and durability of the surgical procedures the joint statement by international diabetes organizations regarding the 'Metabolic Surgery in the Treatment Algorithm for Type 2 Diabetes' concluded that ''metabolic surgery should be recommended to treat T2D in patients with class III obesity (BMI ≥40 kg/m2) and in those with class II obesity (BMI 35.0–39.9 kg/m2) when hyperglycaemia is inadequately controlled by lifestyle and optimal medical therapy. Surgery should also be considered for patients with T2D and BMI 30.0–34.9 kg/m2 if hyperglycaemia is inadequately controlled despite optimal treatment with either oral or injectable medications. These BMI thresholds should be reduced by 2.5 kg/m2 for Asian patients'' [84]. There are also discussions that the selection criteria for surgery should move away from a BMI/weight based cut-offs to a more functional and metabolic improvement based assessment. The Edmonton Staging Criteria is a good example of this approach [85, 86].

1.6.2 Advantages and Adverse effects associated with bariatric procedures

Common peri-operative complications after abdominal surgery include infection, haemorrhage, perforation, intestinal obstruction, hernia and DVT. Other adverse events are described below for each surgical type.

Laparoscopic Adjustable Gastric Banding (LAGB)

Advantages	**Disadvantages**	**Adverse Effects**
Lower mortality rate	Not suitable for very obese	Nausea, vomiting, Reflux
Technically less complicated	Less efficacy than others	Stoma obstruction, dysphagia
Fully reversible	High failure rate	Altered bowel habits
Lower length of hospital stay	Patient dependent – diet, motivation	Band dislocation/slippage
Shorter recovery	High incidence of minor complications	Oesophageal dysmotility/dilatation
Adjustable restriction according	High reoperation rates	Band migration/erosion
to response	Gradual weight loss (variable)	Band/Port leakage
Nutritional deficiencies less	Slow improvement/changes in comorbidities	Band malfunction
common (no malabsorption)	requiring constant monitoring	Port displacement
	Long-term follow-up required	Port site discomfort, infection
	Skilled personnel to adjust band & diet advice	

Roux-en-Y Gastric Bypass (RYGB)

Advantages	Disadvantages	Adverse effects
Significant excess weight loss (more	Irreversible	Hernia
than LAGB)	Restricted intake (volume)	Anastomotic leakage
Remission/improvement of	Higher adverse effects	Stomal ulcers, stricture, obstruction
comorbidities particularly diabetes	Need for long-term monitoring	Nausea, vomiting
Less complication/mortality than BPD	Risk of weight regain	Dumping syndrome
	Nutritional deficiencies	Iron, B12, Vit D deficiency

Biliopancreatic diversion with Duodenal Switch (BPD-DS, LDS)

Advantages	Disadvantages	Adverse effects
Less diet restriction than bypass	Irreversible	Hernia
Less risk of dumping	Complicated procedure	Anastomotic leakage
Significant excess weight loss	Highest complication rate/mortality	Stomal ulcers, stricture, obstruction
(more than others)	Serious protein malnutrition	Nausea, vomiting
Remission/improvement of	Nutritional deficiencies	Bloating, flatulence, loose stools
comorbidities particularly diabetes	Requires intensive monitoring long-term	Protein malnutrition
Low risk of weight regain		Vitamin & mineral deficiencies
Can be performed in 2 stages		

In summary, the choice of anti-obesity treatment depends on a thorough assessment of an individual's problems; presence of comorbidities and obesity induced complications, motivation and willingness to change, past experiences and suitability for drug/surgical therapy tend to determine the level of intervention. Availability of specialist services and cost are likely to influence the choice of surgical treatment in most parts of the world. Lifestyle changes and behavioural therapy should usually be the first step before considering drugs or surgical procedure. Patient education regarding the likely outcomes of therapy (as compared to expectations) and risks will improve compliance. A detailed management plan needs to include weight maintenance strategies (after initial weight loss) for successful treatment of obesity in the long term.

1.6.3 Current trends in weight loss surgery

The latest global survey of weight loss surgery (also known as bariatric/metabolic surgery) based on 2013 data collected from the nations or national groupings of the International Federation for the Surgery of Obesity and Metabolic Diseases (IFSO) was published in 2015 [87]. A total of 468,609 bariatric procedures were performed with 95.7% of these carried out laparoscopically. The most commonly performed procedure in the world was Roux-en-Y gastric bypass (RYGB) at 45%. The prevalence of Sleeve Gastrectomy (SG) rose from 0 to 37% of the world total from 2003 to 2013, and LAGB fell from 68% from its peak in 2008 to 10% in 2013. SG is currently the most frequently performed procedure in the USA/Canada and in the Asia/Pacific regions. In Europe and Latin/South America regions RYGB is the commonest procedure followed by SG.

1.6.4 Outcomes after weight loss surgical procedures

Although the primary aim of undergoing bariatric surgery is to lose weight, it has become evident in the last three decades that there are many metabolic and cardiovascular benefits after weight loss surgery many of which are weight independent. Early and durable improvement in glycaemia often resulting in remission of diabetes, resolution of obstructive sleep apnoea and improvements in traditional CV risk factors such as hypertension and dyslipidaemia are prime examples. Other obesity related co-morbidities

and complications are also reported extensively to improve or resolve after weight loss surgery. The future risk of health problems such as type 2 diabetes and cancer also appear to be reliably reduced after weight loss surgery [88].

Weight loss

The weight loss achieved by bariatric surgery has been attributed to either a restriction of food intake, malabsorption or a combination of the two. A large meta-analyses by published by Buchwald et al. in 2004 and updated in 2009 showed that the excess weight loss percentage (%EWL) after weight loss surgery (all types) is 55.9% and the diabetes resolution rate is 78.1%, with improvements in diabetes control noted in 86.6% [7, 72]. The percentage excess weight loss after each type of procedure is reported as: LAGB 46.1%, RYGB 59.5% and LDS 63.6%. The observed peri-operative mortality rates are published periodically [89], reporting an acceptable mortality rate for all surgery types within 30 days of 0.28% (with 95% confidence interval of 0.22-0.34) in 475 treatment arms (n = 84,931). The 30 day peri-operative mortality rates for individual procedures were LAGB 0.07%, Lap RYGB 0.16% and LDS 1.11%. A more recent meta-analysis in 2014 reported that the %EWL after RYGB was 74.3% to 80% at 2 years with a perioperative mortality rate of 0.08% to 0.38% (≤30 days of surgery) [90].

The weight loss after purely restrictive procedures (e.g. LAGB) tends to be gradual over 12 to 24 months but the weight loss after malabsorptive (e.g. LDS) and mixed procedures (e.g. RYGB) tend to be quite rapid [88].

Hypertension, Dyslipidaemia and CV risk

Blood pressure improves after weight loss surgery but the reported resolution and improvement rates are quite variable. The hypertension resolution rates are approximately 50-55% and improvement in blood pressure control has been reported in 65-75% of subjects [91, 92]. It has been suggested that blood pressure drops quite rapidly in the first 10-12 months and then there is a slight increase before reaching a steady state.

Improvement in dyslipidaemia has been reported between 65% and 75% of patients in several meta-analyses [92]. In the large SOS study, the remission rates from diabetes, hypertriglyceridemia, low levels of high-density lipoprotein cholesterol, hypertension and hyperuricemia were more favourable in the surgery group than in the control group at 2 years and 10 years, whereas improvement of hypercholesterolemia did not differ between the groups. The surgery group had lower incidence rates of diabetes, hypertriglyceridemia and hyperuricemia than the control group, whereas differences between groups in the incidence of hypercholesterolemia and hypertension were not detectable at 10 years [93].

As a result of the risk factor improvements, there was a reported reduction in the 10-year coronary heart disease Framingham Risk Score from 5.9% to 3.3%. Traditional and novel markers of cardiovascular disease and cardiac function parameters have all been reported to be improved significantly with less CV events and mortality.

Other benefits of weight loss surgery

Weight loss surgery has been shown to resolve OSA in over 90%, reduce vascular events and stroke, improve traditional and novel CV markers and improve obesity associated co-morbidities like NASH, subfertility and Asthma [88, 94-97]. In addition, review of current literature shows that, improvements of numerous other co-morbidities and obesity-related complications have been reported [98-100]. Furthermore, bariatric surgery reduced all-cause mortality, cardiovascular events and mortality and the incidence of cancer [88]. Bariatric surgery was also effective in preventing type 2 diabetes [101] and in reducing the risk of micro-vascular and macro-vascular complications [102, 103]. Overall, bariatric surgery has been shown to improve the quality of life in obese subjects [104]. One recent analysis suggested that life expectancy of a 45 year old morbidly obese adult could be increased by an average of 6.7 years after successful weight loss surgery [105]. Also weight loss surgery has been shown to be a very cost-effective treatment in people with BMI greater than 40 [106]. There is also a lot of debate about the benefits of surgical procedures in those with a BMI below 40, particularly the improvements in glycaemia in people with BMI between 30 and 40, but concerns about long-term effects of surgery and the risk of peri-operative complications are to be considered before recommending weight loss surgery in this population [107, 108].

1.7 **Diabetes remission after weight loss surgery**

In 1992, Pories et al. noticed a dramatic improvement in insulin resistance and type 2 diabetes, in patients treated with RYGB for morbid obesity [73]. This has since been confirmed by others following all forms of bariatric surgery [7, 90]. Type 2 diabetes undergoes remission or improvement in the majority of patients undergoing RYGB surgery for morbid obesity and in non-diabetic subjects, glucose tolerance and insulin resistance improves and conversion rates to diabetes are lower than in comparable control groups. The mechanisms underlying this dramatic improvement in glucose tolerance and insulin resistance are however not fully understood. Weight loss may be important in the long-term, but dramatic changes in glucose metabolism occur within days of surgery, suggesting other mechanisms have a dominant role.

Diabetes remission rates were first reported in a large meta-analysis as 76.8% for all procedures [7]. However diabetes remission rates differed according to the type of surgical procedure – 83.8% after RYGB, 47.8% after LAGB and 97.9% after BPD or BPD-DS, which implies that the mechanism of diabetes remission is complex and encompasses a variety of anatomical, physiological, and molecular changes. However the criteria used to define diabetes remission in the early papers was not clearly defined or consistent. The American Diabetes Association published a consensus statement in 2009 [109] to define diabetes remission as below.

The American Diabetes Association – Diabetes Remission criteria [109]

Type of diabetes remission	**Definition**
Partial remission	Hyperglycaemia below diagnostic thresholds for diabetes At least 1 year's duration No active pharmacologic therapy or ongoing procedures
Complete remission	Normal glycaemic measures At least 1 year's duration No active pharmacologic therapy or ongoing procedures
Prolonged remission	Complete remission of at least 5 years' duration

Based on these criteria, the actual diabetes remission rate after weight loss surgery is thought to be lower than published before. In retrospective observational studies, about 35% patients had complete remission of diabetes, according to the new ADA definition; the remission rates were around 40% after gastric bypass, 25% per cent after sleeve gastrectomy and 7% after gastric banding [110].

In the randomised study called the 'STAMPEDE' - 'Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently' study the primary end point was the proportion of patients achieving HbA1c of 6.0% or less, 12 months after treatment. Out of 150 patients who were randomised to medical therapy or surgery (RYGB or SG), a total of 42% in the RYGB arm, 37% in the SG arm and 12% in the medical group achieved remission at 1 year [111]. At 3 years, 5% of patients in the medical group compared with 38% in the RYGB arm and 24% in the SG arm achieved remission [112].

Recent studies with longer duration of follow up have also highlighted the high incidence of recurrence of diabetes after initial remission. In the large SOS study half the patients who were in remission at 2 years were reported to have had recurrence of diabetes needing treatment at 10 years (72% in remission at 2 years and only 36% at 10 years). Patients with longer duration of DM (>5 years), those who suffered weight regain, were of older age, and those who required insulin before surgery were more likely to have diabetes recurrence [113, 114]. This makes it essential that patients with diabetes pre-surgery are followed up long term with regular biochemical tests to look for diabetes recurrence.

1.8 Mechanisms underlying diabetes remission after weight loss surgery

Possible short-term effects of surgery that may alter glucose metabolism include the malabsorptive effects of surgery, energy restriction that occurs in the immediate pre- and post-operative period, and changes in incretin hormones. Although malabsorption of vitamins and minerals is recognised following bypass procedures, energy malabsorption is minimal [115], and is not thought to contribute significantly to the weight loss or glucose homeostasis. Appetite is reported to be reduced post-operatively, but the potential mechanisms and the main contributors to reduced appetite have not yet been fully

understood. In the long-term, reduced energy intake leads to loss of adipose tissue; this is thought to improve insulin sensitivity via several mechanisms, including changes in fatty acids and favourable changes in adipokine hormones such as leptin, visfatin, resistin, tumour necrosis factor α (TNFα), IL6 and adiponectin.

1.8.1 Acute energy restriction

Glucose metabolism appears to improve as early as 6 days post RYGB [9]. Short periods of energy restriction are known to produce significant improvement in beta cell function with increased post-prandial insulin release and improvement in insulin sensitivity with reduction in hepatic insulin resistance, resulting in improved glucose tolerance in people with diabetes [116]. This may partly explain improvement in insulin sensitivity in the immediate postoperative period [117]. Taylor et al subjected 15 patients with type 2 diabetes of less than 4 years duration and 14 patients with type 2 diabetes of more than 8 years duration to a 8-week very-low-calorie diet (VLCD) and found that 87% of the short-duration group and 50% of the long-duration group achieved non-diabetic fasting plasma glucose levels at week 8. Clinically significant improvements in blood pressure and lipid profile were seen regardless of diabetes duration [118]. In another study, 30 subjects with type 2 diabetes were subjected to a 8 week VLCD followed by a 6 month weight maintenance program. In addition to significant weight loss (98.0 ± 2.6 to 83.8 ± 2.4 kg) they observed significant improvements in glycaemia in 12 of 30 subjects which was maintained at 6 months [119].

It is common practice to energy restrict patients for 10-14 days prior to surgery (pre-operative diet) and several days following surgery, and this has not always been taken into account when investigating the effects of surgical procedures on glucose metabolism. It is therefore necessary to compare the effects of energy restriction in matched obese controls to fully understand the factors that may influence glucose metabolism in surgical patients.

1.8.2 Role of incretins and gut hormones

Unger and Eisentraut in 1969 introduced the concept of 'enteroinsular' axis describing the connection between the gut and the pancreatic islets [120]. This axis encompasses nutrient, neural and hormonal signals from the gut to the islet cells. Incretins are gut hormones released by contact of nutrients with GI mucosa, which play a vital role in regulating the insulin secretory response to glucose after a meal. The predominant incretins in humans are glucagon like peptide-1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP), previously known as gastric inhibitory polypeptide. GLP-1 and GIP regulate nearly 50-60% of the postprandial insulin secretion [121]. Given that regions of intestine that produce hormones with important effects on insulin secretion are either bypassed (proximal) or exposed to an altered nutrient mix (distal) following RYGB, it has been suggested that changes in the entero-insular axis may mediate improvements in glucose metabolism [122].

Hyperinsulinaemic hypoglycaemia attributed to nesidioblastosis has been reported as a rare complication of gastric bypass surgery [123]. It has been suggested that this is mediated by the known (in-vitro) effect of incretins to stimulate islet cell hyperplasia. There is limited evidence to support a role for incretin hormones in modulating insulin sensitivity, which is of uncertain clinical relevance [124]. The role of GLP1, GIP and some of the relevant peptides that influence the incretin pathway is discussed later.

1.8.3 Changes in appetite-regulating hormones

Changes in circulating gut hormones such as ghrelin, Peptide YY 3-36 (PYY) and oxyntomodulin may partly explain reduced appetite following bariatric surgery [125]. Ghrelin produced in the oxyntic glands of stomach fundus increases food intake, but ghrelin concentrations are low in severe obesity [126]. The effects of weight loss surgery on total ghrelin and active (octanoylated) ghrelin levels have not been clear cut or consistent in previous studies [127, 128]. Interpretation of the role of ghrelin has been further confounded by the discovery that another hormone, obestatin, is produced from the same gene as ghrelin, but has opposite effects to ghrelin on energy intake [129]. PYY is produced postprandially by the gastrointestinal tract, concentrations are low in obesity and

it suppresses appetite in humans [130]. PYY response to food intake has been shown to be exaggerated post RYGB in some studies [128]. Although there is no clear evidence that these appetite-regulating hormones have major direct effects on glucose metabolism, studying changes that occur after bariatric surgery will enable better understanding of mechanisms that explain reduced appetite and subsequent weight loss. These peptides have been discussed in detail later.

1.8.4 Effect of weight loss on glucose metabolism

The relationship between BMI and insulin resistance is well documented and discussed previously. Weight loss improves insulin sensitivity and glycaemic control in type 2 diabetes, in particular with loss of visceral fat. RYGB results in significant loss of weight and reduction in fat mass and therefore improvement in insulin sensitivity, however the precise mechanisms mediating the effects of weight loss on insulin sensitivity are not known. Although increased non-esterified fatty acids (NEFA), have been proposed as an important link between adiposity and insulin resistance, fasting NEFA concentrations did not change significantly following RYGB in recent studies [131], suggesting that other mechanisms may be of greater importance. Although weight loss and loss of adipose tissue is the biggest contributor to improvements in glycaemia, the improvement of insulin response to food and insulin sensitivity occurs much before weight loss occurs after surgery. It is therefore important to identify the potential mechanisms that are independent of weight loss particularly in the early stages.

1.8.5 Role of adipokines and inflammatory markers

Adipose tissue is now recognised as a major endocrine organ [132]. Adipocytes produce a vast array of secretory products, with roles in the regulation of energy balance, metabolic, vascular and immune functions, collectively termed adipokines [133]. Increased production of TNFα, resistin, visfatin and IL-6 in obesity, have been implicated in the development of insulin resistance and type 2 diabetes, whereas adiponectin concentrations fall with increasing fat mass and this hormone improves insulin sensitivity [134]. Weight loss after bariatric surgery is associated with favourable changes in

adiponectin, which have been suggested to mediate the improvement in glucose metabolism after RYGB in the long-term [135]. Although leptin and insulin concentrations fall after RYGB, the relationship between changes in leptin levels and insulin resistance remains unclear. The relationship between resistin and BMI has been conflicting in various studies. Animal studies have shown a significant role for resistin in glucose tolerance [136], whereas in humans resistin levels do not appear to correlate with fat percent or insulin levels, or change significantly after bariatric surgery [137].

There is currently limited data on longitudinal changes in TNFα, IL-6 or hs-CRP after bariatric surgery [138] in relation to their influence on glycaemia. It will be essential to determine whether early changes in insulin sensitivity after surgery are directly or indirectly related to changes in adipokines.

1.9 Aims of study

Weight loss surgery is currently the only treatment that could lead to durable diabetes remission and reduction or remission of several cardiovascular risk factors and obesity-related complications. Understanding the mechanisms underlying diabetes remission could shed more light on the contribution of the several postulated theories which are discussed in chapter 5. This may also help us better understand the complex pathophysiology of type 2 diabetes and obesity and potentially lead to the development of more effective treatments for the majority of patients, for whom this intervention is not suitable. GLP1 mimetics & analogues and DPP4 inhibitors are good examples of where understanding the role of incretin hormones have contributed to new treatment options for type 2 diabetes [139, 140].

We aimed to use the Aintree Bariatric database to collect information about the observed weight- and diabetes-related outcomes after weight loss surgery. The weight loss results between the diabetes and non-diabetes subgroups could be compared to see if there is difference in the outcome. All patients, who were known to have type 2 diabetes before surgery, were also studied about the chances of diabetes remission after different weight loss procedures. The effect of the ADA guidelines, which clearly defined diabetes remission [109], was studied for impact on the diabetes remission rates, in comparison to the published literature.

The aim of the prospective study was to collect prospective, longitudinal data relating to the changes in glucose tolerance through improved insulin secretion and insulin sensitivity which are medicated by changes in incretin and gut hormones, regulation of appetite, changes in adipokines and inflammatory markers in obese patients who undergo weight loss surgery. We aimed to study the postprandial responses of insulin and gut hormones at different time points after surgery. We chose patients undergoing RYGB as this is the commonest procedure performed in our centre and RYGB has the most effect on the above parameters in relation to type 2 diabetes remission.

1.10 Hypothesis

Diabetes remission after bariatric surgery is mediated by changes in insulin secretion due to an enhanced incretin response and improved insulin sensitivity due to energy restriction in the initial stages and in the long-term by weight loss, reduced fat mass, improved appetite regulation and improved insulin sensitivity due to favourable changes in adipokines and inflammatory cytokines.

Chapter 2

Methods and Study design

2.1 Study design

As the first step, list of all the bariatric procedures performed in Aintree Hospital between 1999 and 2008 was obtained from the Aintree Bariatric database. Details about the type of procedure performed, weight loss outcomes and for those who were known to have type 2 diabetes pre-surgery, changes in the their diabetes status were recorded. Information about their glycaemic parameters and their co-morbidity status were obtained from the hospital or general practice records. This is discussed in chapter 3.

The prospective part of the study was initially planned to compare the effect of calorie restriction (control group) to all three procedures performed in our unit – LAGB, RYGB, and LDS (surgery group). However due to the changes in the specialist commissioning criteria for selection for bariatric surgery in England, the number of patients who were given gastric banding and duodenal switch procedures was very small. Therefore patients who underwent RYGB alone were included in the study.

All patients who successfully received NHS funding for bariatric surgery were invited to participate in this study. Participation in this study did not influence their funding approval, choice of procedure or their post-surgery care. Obese controls suitable for calorie restriction diet were recruited from the patients attending the weight management service at Aintree.

Power calculations were based on a study by Wickremesekera et al. who looked at the changes in insulin secretion and insulin sensitivity after bariatric surgery [9]. To assess changes in insulin sensitivity by HOMA score, at least 8 subjects will be required in each group to have greater than 90% power to detect a 50% difference in HOMA score. We aimed to study higher numbers in the surgery groups to allow for more detailed exploratory data analysis; based on data from our own laboratory, this will allow detection of 30-40% changes in area under the curve for incretin hormones after the test meal, and 50% changes in adipokines with 80% power. The overall numbers that we could recruit were however determined by the limits in the number of patients who were successful in obtaining funding for weight loss surgery before the current specialist commissioning criteria came into force in 2013.

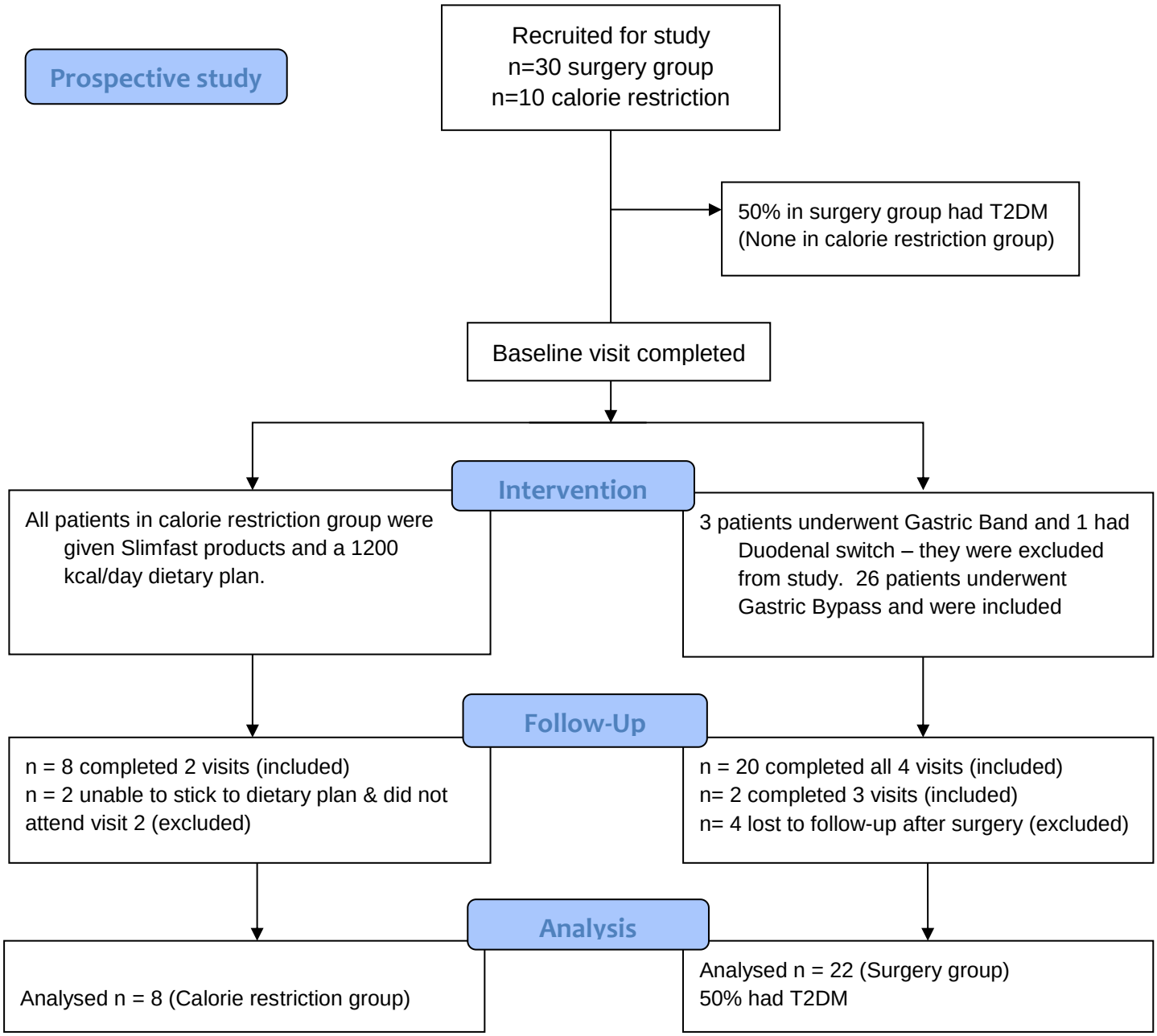
Ethical approval was obtained from Sefton Local Research Ethics Committee (06/Q1501/152) and the study was registered with the University of Liverpool and the Aintree University Hospital research department. All subjects were provided with written information leaflet and gave written informed consent. The study was performed in accordance with the principles of the Declaration of Helsinki.

2.2 Selection of subjects

The bariatric surgical unit at University Hospital Aintree is one of the largest NHS service in the UK, with five trained bariatric surgeons and had carried out more than 2400 procedures (excluding private surgery) between 1999 and 2008. Approximately 25% of patients have type 2 diabetes for whom the preferred procedure is the RYGB or LDS. In the last few years, the proportion of patients who have had LDS and LAGB procedures done had fallen significantly as discussed before. Majority of the patients are given RYGB and the rate of Sleeve gastrectomy (SG) is steadily rising in keeping with the national figures [87].

30 patients were recruited for the surgery group. Out of the 26 patients who had RYGB, 4 did not complete the study for personal reasons. 3 underwent LAGB and 1 had LDS. Their data is not included in this analysis. 11 (50%) of the surgery group had known type 2 diabetes and the rest were not known to have diabetes. None of the recruited patients were on GLP1 analogues or thiazolidinediones which could have affected the hormone or glycaemia measurements.

10 patients were recruited for the control group out of which two did not complete the calorie restriction diet for 4 weeks. These patients were recruited from the patients attending the Aintree weight management service and were planned to have conservative treatment with no plans to use anti-obesity drugs or surgery in the near-term. None of these patients were known to have type 2 diabetes and were not any treatment that had an effect on their weight, glucose regulation or appetite.


Prospective study
Recruited for study
n=30 surgery group
n=10 calorie restriction
50% in surgery group had T2DM
(None in calorie restriction group)
Baseline visit completed
Intervention
All patients in calorie restriction group were given Slimfast products and a 1200 kcal/day dietary plan.
3 patients underwent Gastric Band and 1 had Duodenal switch – they were excluded from study. 26 patients underwent Gastric Bypass and were included
Follow-Up
n = 8 completed 2 visits (included)
n = 2 unable to stick to dietary plan & did not attend visit 2 (excluded)
n = 20 completed all 4 visits (included)
n= 2 completed 3 visits (included)
n= 4 lost to follow-up after surgery (excluded)
Analysis
Analysed n = 8 (Calorie restriction group)
Analysed n = 22 (Surgery group)
50% had T2DM

2.3 Intervention

Visit	Calorie restriction group	Surgery group
1	Baseline visit	Baseline visit (before pre-op diet)
2	4 weeks low calorie diet Then visit 2	2-3 weeks after surgery
3	-	4 months after surgery
4	-	12 months after surgery

2.3.1 Calorie restriction group

All the subjects who were recruited in this group were invited for 2 visits. The tests and observations that were carried out in each visit were identical to the surgery patients as described in the study protocol in the next section.

After completing visit 1, they were seen by a dietitian or equivalent health professional and were asked to follow a standard low calorie diet for 4 weeks. We wanted to mimic the effect and duration of calorie restriction that the surgery patients would experience. Surgery subjects are routinely asked to follow a low calorie diet for 2 weeks pre-operatively and they usually need 2 weeks to start regular intake of calories following weight loss surgery. Although the calorie intake peri-operatively is a lot more restrictive, we chose 1200 kcal/day diet as appropriate for this group. Slimfast products and a clear diet plan equalling 1200 kcal/day was provided initially for 2 weeks and after getting weighed they were given a further 2 weeks supply. As stated earlier, 8 out of the 10 patients successfully completed the 4 week diet plan and completed the second visit.

2.3.2 Surgery group – RYGB diabetes

Out of the 22 patients who completed the study in the surgery group, 11 (50%) were known to have type 2 diabetes. Their change to glycaemia and diabetes treatment pre- and post-surgery was recorded. They were studied on 4 occasions – 2 weeks before surgery (before commencing pre-operative diet), 2 weeks post-surgery (within 3 weeks), 4 months (± 1 week) and 12 months (± 2 weeks) post-weight loss surgery.

2.3.3 Surgery group – RYGB no diabetes

11 patients in the surgery group did not have diabetes and they were invited for 4 visits similar to the diabetes group. The study protocol for each visit was similar in all groups as discussed below.

2.4 Study Protocol

The following protocol was used during the 2 visits for the calorie restriction group and the 4 visits for the surgery (RYGB) group.

During each visit subjects arrived at the Clinical Sciences Centre in Aintree Hospital in the morning at 8 AM after an overnight fast of at least 10 hours duration. In subjects with diabetes, oral hypoglycaemic agents were omitted in the evening prior to the study day, and insulin was reduced appropriately the previous evening to avoid hypoglycaemia and all diabetes treatment omitted on the morning of study. They were advised to monitor their BMs regularly and hypo- and hyper-glycaemia management was discussed before the study visit where appropriate.

Anthropometric measurements (height, weight, waist circumference) were made, together with bio-impedance measurements to determine body fat mass and fat distribution. Blood pressure and ECG were recorded. BMI was calculated using electronic scales. A detailed medical history was taken; their past medical history, medication history and changes to their health were recorded. Fasting blood samples were taken after insertion of a large bore cannula. They were asked to consume a standard 330 kcal liquid meal (Ensure plus) in 15 minutes. This was chosen rather than a mixed meal as the patients would struggle to

consume solid food, 2 weeks after surgery. Blood samples were then taken at 30, 60, 90, 120, 150 and 180 minutes post meal. Also at baseline and at 30 minutes intervals following the liquid meal, participants will be asked to record hunger, satiety, fullness and desire to eat using standard visual analogue scales (see appendix).

At each visit, patients also completed the validated self-completion 'Three Factor Eating Questionnaire' (TFEQ) to measure expression of appetite (see appendix).

2.5 Measurements and rationale

2.5.1 Anthropometric measurements, blood pressure & bio-impedance

Height was recorded with the subject standing barefoot against a standard stadiometer to the nearest 0.1 cm and weight was recorded digitally in kilograms (to the nearest 0.1 Kg) at each visit using the same Tanita scales. BMI was then calculated as weight (kg) / height^2 (m). Blood pressure was recorded using an appropriate sized cuff, after at least 5 minutes of rest, in supine position, three times at one minute intervals, according to the British Hypertension Society guidelines [141]. We also measured waist circumference as it correlates better than BMI alone in assessing serial changes in intra-abdominal fat mass [11]. This was measured directly over the skin at the end of normal expiration, horizontally, midway between the lower costal margin and the iliac crest with the arms relaxed at the sides.

Body composition was assessed by bio-electrical impedance analysis using the Tanita Body Composition Analyser TBF – 310GS (Tanita Corp, Tokyo, Japan) which gives a reasonable measure of fat mass and fat-free mass. Despite its limitations this gives a better assessment of serial changes in a person's fat mass especially after the rapid changes seen after weight loss surgery. Subjects were asked to void urine before this test and stand barefoot on the metal platform. When set against gender, height and weight, the measurements highly correlate with the gold standard for measuring body composition, the four compartment model for percentage body fat ($r=0.89$, $p<0.05$) and fat mass ($r=0.93$, $p<0.05$) [142].

2.5.2 Medication changes including diabetes treatment

Any change to their prescribed medication was recorded along with relevant medical history. In particular the changes to number and dose of anti-hypertensives and lipid-lowering agents were noted. In diabetes subjects, detailed record of changes to their glycaemia was recorded with changes in treatment. Frequency and severity of hypoglycaemia was also discussed and recorded.

2.5.3 The Three Factor Eating Questionnaire (TFEQ)

The Three Factor Eating Questionnaire was developed by Albert Stunkard and Samuel Messick in 1984 as a psychometric instrument for the study of eating behaviour [143]. This validated questionnaire with 51 questions is used to measure three dimensions of human eating behaviour: cognitive restraint of eating, disinhibition and susceptibility to hunger. It has been used in several studies to measure and record changes in eating behaviour and appetite regulation [144, 145]. Shorter versions with 18 and 21 questions also exist and this questionnaire has also been modified to suit different populations and measured health conditions. A copy of the original questionnaire is included as an appendix. Part 1 has 36 closed questions with a True or False response and 15 scaled items (4 or 6 points per response). It has been used to assess changes in eating behaviour in a number of different patient groups such as obesity, eating disorders and following bariatric surgery [146, 147].

All subjects were asked to complete this at each visit reflecting the changes in their eating behaviour since the last visit.

2.5.4 Standard 330 kcal liquid meal

At every visit patients were asked to consume an 'Ensure plus' drink which has 330 kilocalories. We chose this rather than a mixed meal as the post-surgery patients would have found it difficult to tolerate anything other than liquids in the first few weeks. It is made of 220mls of milkshake style oral nutritional supplement with 1.5kcal/ml. It has 17% calories as fat and 26% of calories as protein.

2.5.5 Visual Analogue Scale (VAS)

Hunger and satiety was assessed using a Visual analogue Scale (VAS). A VAS consists of a question and a 100mm unmarked line anchored by descriptors acting as reference points of extreme sensations at the end. The scales may be unipolar ('Not at all hungry' vs. 'Very hungry') or bipolar ('Hungry vs. Sated') depending on the question asked. Unipolar scales are considered more sensitive and accurate [148]. When VAS is measured only pre-meal, data tends to be clustered towards the end of the scale representing hunger. Although there may be weaknesses in reproducibility in some studies, serial changes in the same person following a test meal on repeated occasions in controlled research settings has been shown to be fairly reliable [149].

All patients in this study were asked to complete a 100mm unipolar visual analogue scale (see appendix 2) to measure expressions of hunger, satiety and fullness. Using the reference points they were asked to mark with a single vertical line the point on the line which best described their subjective sensation at that point of time. This allowed the conversion of their subjective sensation into an analysable numerical value. VAS scores were recorded at baseline (pre-meal) and at 30 minute intervals after the meal until 180 minutes post-meal.

2.5.6 Biochemical assays

Serum samples were collected in serum separator tubes and allowed to stand for 15 minutes prior to centrifugation at 4°C. Plasma samples for glucose were collected in fluoride oxalate tubes and frozen at -20°C within 30 minutes of collection. Samples were also collected in plastic EDTA tubes containing 0.07 mg Aprotinin [500 Kallikrein Inactivator Units (KIU)] and centrifuged immediately. All samples were then immediately stored at -80°C until assayed.

2.5.6.1 HbA1c and Fasting lipid profile

Glycated haemoglobin (HbA1c) and fasting lipid profile was measured by sending the baseline fasting blood sample (plasma and serum samples respectively) to the biochemistry

department in Aintree University Hospital. HbA1c was determined by using high performance liquid chromatography method (Ha 8140, Menarini Diagnostics, Berkshire, UK) by an experienced technician in the Department of Biochemistry, Aintree University Hospital. Fasting lipid profile (total cholesterol, HDL & triglycerides) was measured by homogeneous enzymatic, colorimetric method using the Roche/Hitachi Cobas C analyser (Roche Diagnostics USA). Serum LDL was calculated from the total cholesterol, HDL and triglyceride levels.

2.5.6.2 Glucose and Insulin

Plasma Glucose (mmol/L) was analysed by using the glucose hexokinase method with Roche Cobas analysers (Roche Diagnostics USA). Insulin was measured from the plasma sample and quantified using the Elecsys Insulin assay, a solid-phase, two site, electro chemi-luminescent enzyme-labelled immunometric assay (ECLIA) using the sandwich principle with an Roche Cobas E automated immunoassay analyser (Roche Diagnostics USA).

Markers of insulin sensitivity and secretion

Insulin resistance was measured from fasting Glucose and Fasting insulin values using the HOMA2 calculator (v 2.2.1) [150]. In addition to HOMA2 IR values, HOMA2 %B was also measured using this calculator. We also measured Matsuda index (ISI) as a marker of insulin sensitivity [151]. Although HOMA2-%B score, which is calculated from the fasting insulin and glucose values is useful, it is not considered to be an accurate marker of insulin secretion capacity [152]. Reviewing the current literature, there is no single measure of beta cell function that is shown to be accurate in measuring changes in insulin secretion after weight loss surgery using oral meal response curves of glucose and insulin [153]. Therefore we used HOMA2 %B scores, AUC insulin/glucose values (pmol/mmol 180 mins), Insulinogenic index (IGI) and Disposition index (IGI x ISI) as markers of insulin secretion for exploratory analysis [154, 155]. The calculation of these indices in described in the table below and discussed in chapters 5 and 7.

Index	Measure of	Calculation
HOMA2 IR	Insulin Resistance (IR)	Fasting blood glucose [mmol/l] x Fasting plasma insulin [μU / ml]) / 22.5
HOMA2 %B	Insulin secretion (IS)	Fasting plasma insulin [μU / ml] x 20) / Fasting blood glucose [mmol/l] – 3.5)
Matsuda Index (ISI)	Insulin Resistance (IR)	10000/SQRT(Fasting blood glucose[mmol/l] x Fasting plasma insulin [μU / ml] x Mean blood glucose [mmol/l] x Mean plasma insulin [μU / ml])
AUC Insulin/Glucose pmol/mmol 180 mins	Insulin secretion (IS)	AUC fasting plasma insulin [pmol/l] /AUC fasting blood glucose [mmol/l]
Insulinogenic Index (IGI)	Insulin secretion (IS)	30 min plasma insulin – fasting plasma insulin [μU/ml] / 30 min blood glucose – fasting blood glucose) [mmol/l]
Disposition index	Insulin secretion in relation to sensitivity	ISI x IGI
Early Insulin response ΔInsulin 30 - ΔInsulin 0	Early Insulin response	ΔInsulin 30 - ΔInsulin 0 [pmol/l]

2.5.6.3 GLP1, PYY

Total GLP1 was measured from plasma EDTA samples collected and stored with aprotinin as described above. Total GLP1 was measured using a commercially available ELISA kit (Multi Species GLP-1 Total ELISA, EZGLP1T-36K, Merck Millipore UK). This kit measures both the active (7-36) and inactive (9-36) forms of GLP-1. The antibody pair used in this assay is specific to GLP-1 (7-36) and (9-36) and has no cross-reactivity with GLP-2, GIP, Glucagon or Oxyntomodulin. The sensitivity of this assay is 3pmol/L with an intra-assay precision of <5% and inter-assay precision of <12%. Manufacturer's instructions were adhered to when performing the assay.

PYY was measured from plasma EDTA samples collected and stored with Aprotinin as described above. Total PYY was measured using a commercially available ELISA kit (Total

Human PYY ELISA, EZHPYYT66K, Merck Millipore UK). The sensitivity of this assay is 1.4pg/mL, with an intra-assay precision of 0.9 to 5.8% and inter-assay precision of 3.7 to 16.5%. Manufacturer's instructions were adhered to when performing the assay.

2.5.6.4 Adiponectin, Leptin

Adiponectin was measured from plasma EDTA samples collected and stored as described above. Adiponectin was measured using a commercially available ELISA kit (Human Adiponectin ELISA, EZHADP-61K, Merck Millipore UK). The sensitivity of this assay is 1.5ng/mL, with an intra-assay precision of 1.0 to 7.4% and inter-assay precision of 2.4 to 8.4%. Manufacturer's instructions were adhered to when performing the assay.

Leptin was measured from plasma EDTA samples collected and stored as described above. Leptin was measured using a commercially available ELISA kit (Human Leptin "Dual Range" ELISA, EZHL-80SK, Merck Millipore UK). The sensitivity of this assay is 0.5 ng/mL (for sensitive assay 0.125 ng/mL), with an intra-assay precision of 2.6 to 4.6% (for sensitive assay 1.4 to 4.9%) and inter-assay precision of 2.6 to 6.2% (for sensitive assay 1.3 to 8.6%). Manufacturer's instructions were adhered to when performing the assay.

2.5.6.5 IL-6, sTNF RII receptor, hs-CRP

Interleukin-6 (IL-6) was measured from plasma EDTA samples collected and stored as described above. IL-6 level was measured using a commercially available ELISA kit (Human IL-6 Quantikine ELISA Kit, D6050, R&D Systems UK). It is a solid phase sandwich ELISA immunoassay with a sensitivity of 0.7pg/mL, with an intra-assay precision of 1.6 to 4.2 CV% and inter-assay precision of 3.3 to 6.4 CV%. Manufacturer's instructions were adhered to when performing the assay.

Tumour Necrosis Factor Receptor II (sTNF R II, TNFRSF1B) was measured as a marker of TNF-α activity from plasma EDTA samples collected and stored as described above. sTNF R II level was measured using a commercially available ELISA kit (Human sTNF RII/TNFRSF1B Quantikine ELISA Kit, DRT200, R&D Systems UK). It is a solid phase sandwich ELISA

immunoassay with a sensitivity of 2.3pg/mL, with an intra-assay precision of 2.6 to 4.8 CV% and inter-assay precision of 3.5 to 5.1 CV%. Manufacturer's instructions were adhered to when performing the assay.

High sensitivity C-Reactive Protein (hs-CRP) was measured from plasma EDTA samples collected and stored as described above. hs-CRP level was measured using a commercially available ELISA kit (Human high sensitivity C-Reactive Protein ELISA Kit, CSB-E08617h, 2BScientific Ltd, UK). This assay employs the quantitative sandwich enzyme immunoassay technique with a sensitivity of 0.156ng/ml, with an intra-assay precision of CV <8% and inter-assay precision of CV <10%. Manufacturer's instructions were adhered to when performing the assay.

Chapter 3

Effectiveness of weight loss surgery in diabetes population and the effect of the revised definition of diabetes remission in the evaluation of weight loss surgery

Introduction

It has now become obvious that type 2 diabetes undergoes remission or improvement in a vast majority of patients undergoing weight loss surgery, with this effect more pronounced and quicker following malabsorptive procedures. The earlier studies used variable definitions and criteria for diabetes remission, which did not portray the true likelihood of diabetes remission after weight loss surgery. The mechanisms that are thought to underlie diabetes remission have been discussed in detail in chapter one. There is some evidence that weight loss outcome in patients with type 2 diabetes is not inferior to non-diabetic population [72] but diabetes remission rates are inversely proportional to the duration of diabetes [156].

Aims

1. To determine and compare the weight loss outcomes after weight loss surgery in a large cohort of morbidly obese patients with type 2 diabetes (n = 216) in comparison with patients who were not known to have diabetes before surgery (n = 770)
2. To determine the diabetes remission rates as per the definitions used before 2009 and to study the effect of the new American Diabetes Association consensus definition of diabetes remission on the diabetes remission rates.

Patients and Methods

Between 1999 and 2008, 2408 weight loss procedures were carried out in the Aintree Bariatric Unit. Only 1650 patients were attending one of the bariatric or weight management clinics in Aintree at the time of this study. A total of 986 adults with a BMI >35, who had at least 1 year follow up data, were included. Out of these, a total of 216 adults were known to have type 2 diabetes. Patients known to have any other health condition or treatment (e.g. steroid use or diagnosis of malignancy) that could influence their weight or glycaemic status were excluded from the analysis.

Clinical details of these patients were recorded in a database. The following details were analysed from their hospital records: date and type of bariatric procedure/s performed; serial weights and BMI; presence of type 2 diabetes. In patients known to have diabetes, their serial fasting glucose and HbA1c measurements were recorded if available. The weight change, BMI and percentage excess weight loss (EWL) up to 3 years post-surgery was compared in diabetes and non-diabetes subgroups following LAGB, RYGB, LDS and SG procedures.

Further analysis was conducted within the diabetes sub-group to determine the remission rate for each bariatric surgical procedure (n = 161). Diabetes remission was initially defined as a glycated haemoglobin level of <6.5% without the requirement for any hypoglycaemic agents or insulin based on definition used in the available literature [7]. The ADA definition for diabetes remission (HbA1c < 6.0 and Fasting glucose < 5.6 mmol/L, was then used to assess complete remission from type 2 diabetes [109].

Statistical analysis

In this study, the independent samples t-test was used to compare two groups of a categorical variable (i.e. patients with diabetes and patients without diabetes) on the basis of their distributions on a continuous variable (i.e. % EWL achieved). The null hypothesis associated with the independent sample t-statistic is that the means of %EWL achieved across two groups are equal. A two sided p value of less than 0.05 was considered significant and therefore led to the rejection of this null hypothesis and to the conclusion that the two groups achieved unequal mean %EWL. A confidence interval of the difference between the means of the two groups was calculated in order to measure the extent of this difference.

The Levene test was used to test the equality of variance between two independent samples. The test has the advantage of being less dependent upon assumptions of normality than other tests. The null hypothesis associated with the Levene statistic is that all different sample variances are equal. Hence, a significant statistic should lead to a rejection of this null hypothesis and therefore to the conclusion that the groups (patients with diabetes versus patients without diabetes) have unequal variances.

The one way Analysis of Variance (ANOVA) was used to evaluate whether there are statistically significant differences between the mean EWL achieved by the four different bariatric procedures included in this study. Fisher's exact test and the Freeman–Halton extension of Fisher's exact test were used for analysis of categorical data. Data were analysed using SPSS version 22 (SPSS, Chicago, Illinois, USA).

Results

3.1 Outcomes after weight loss surgery in diabetes population when compared to non-diabetes subjects

The baseline characteristics of patients who had weight loss surgery in Aintree, between 1999 and 2008, and had at least 1 year follow up data are shown in Table 1. Out of 986 patients who were included in this study, 216 patients had type 2 diabetes and had a mean weight of 145.8Kg, a mean BMI of 51.7 and mean age of 47 years. The remaining 770 patients, who were not known to have diabetes, had a mean weight of 135.5Kg, a mean BMI of 48.7 and a mean age 42 years. Weight, BMI and %EWL up to three years is shown in Table 2 and 3. In all four surgery sub-groups, the baseline differences in weight and BMI between patients with diabetes and patients without diabetes were statistically insignificant.

At year 1, the mean %EWL achieved for all 4 bariatric procedures was 58% in patients with diabetes and 57% in patients without diabetes. By year 2 %EWL achieved was increased to 70% in both the diabetes and non-diabetes patients and by year 3 the %EWL was 61% in both groups. The differences in %EWL between the patients with and without diabetes were statistically insignificant, when combining the %EWL achieved for all 4 bariatric procedures included in this study.

Within the LAGB sub-group, the patients without diabetes achieved a significantly higher %EWL one year after surgery. The diabetes group achieved a %EWL of 27.8% whereas the non-diabetes group achieved 38.4% (p=0.006) (95% CI of the differences = -18.2 to -3.04).

Two years after LAGB the %EWL achieved by the non-diabetes group remained statistically greater than that achieved by patients with diabetes (53.4% vs 41.9%, p=0.043) (95% CI of the differences= 22.6 to 0.39)). By year 3, the weight loss achieved in patients without diabetes remained higher than that of patients with diabetes, although this difference was statistically insignificant.

Similarly, patients without diabetes that had undergone RYGB achieved a significantly higher % EWL than patients with diabetes in the first year post surgery (68.4 vs 62.4, p=0.02), (95% CI of the differences=11.5 to 0.59). By the second and third year the weight loss achieved between the two groups was statistically comparable. There were no differences in the weight loss achieved in patients with and without diabetes that had undergone LDS from year one through to year three. Although the number of patients that had undergone SG was considerably less than that of the other three bariatric procedures included in this study, our results indicated that there was no difference in the weight loss achieved in patients with and without diabetes one and two years post SG.

Table1: Baseline characteristics of all patients that underwent weight loss surgery

	Type 2 diabetes (n = 216)	No diabetes (n = 770)
LAGB		
N	30	301
Male	4	34
Female	26	267
Mean age (years)	48.5	41.7
Mean weight (kg)	123.4	117.7
RYGB		
N	124	350
Male	36	57
Female	88	293
Mean age (years)	46.6	42.1
Mean weight (kg)	140.1	138.4
LDS		
N	40	78
Male	15	30
Female	25	48
Mean age (years)	47.3	41.8
Mean weight (kg)	153.1	156.2
SG		
N	22	41
Male	18	22
Female	4	19
Mean age (years)	47	43.6
Mean weight (kg)	195.6	202.3
Total		
N	216	770
Male	69	143
Female	147	627
Mean age (years)	47	42
Mean weight (kg)	145.8	135.5

Table 2: Weight loss outcomes of patients with diabetes and without diabetes

	Baseline		Year 1		Year 2		Year 3	
	Diabetes	Non-Diabetes	Diabetes	Non-Diabetes	Diabetes	Non-Diabetes	Diabetes	Non-Diabetes
LAGB								
N	30	301	26	198	13	98	11	56
Weight (kg)	123.4	117.7	105.1	98.7	94.4	90.9	93.9	85.2
BMI (p value)	45.3	42.7	37.6	35.5 (0.19)	32.3	31.3 (0.75)	30.2	28.8 (0.71)
%EWL (p value)			27.8	38.4 (0.006)	41.9	53.4 (0.043)	44.8	55 (0.33)
RYGB								
N	124	350	91	266	42	141	24	60
Weight	140.1	138.4	94.2	88.4	79.8	80.9	78.7	73.3
BMI (p value)	50	50.2	33	31.9 (0.40)	26.8	28 (0.63)	26.7	22.9 (0.41)
%EWL (p value)			62.4	68.4 (0.02)	71.4	77.6 (0.084)	51.7	55.8 (0.62)
LDS								
N	40	78	36	61	18	36	11	27
Weight	153.1	156.2	89.7	94.8	82.6	82.5	80.9	79.2
BMI (p value)	54.4	54.7	33.8	33.4 (0.83)	28.9	28.3 (0.75)	26.9	26.4 (0.84)
%EWL (p value)			74.6	75.8 (0.81)	90.4	90 (0.94)	94.4	87.4 (0.34)
SG								
N	22	41	10	27	5	11		
Weight	195.6	202.3	155.7	148.3	92.4	118.5		
BMI (p value)	55.5	58.4	45.7	48.1 (0.56)				
%EWL (p value)			39.5	39.2 (0.96)	53.8	47.5 (0.48)		
Total								
N	216	770	163	552	78	286	46	143
Weight	145.8	135.5	99.6	96	84.1	86.1	83	80.5
BMI (p value)	51.7	48.7	35.3 (0.35)	34.4	28.1 (0.57)	29	27.6 (0.51)	26
%EWL (p value)			58.2 (0.62)	57.1	69.8 (0.99)	69.8	61 (0.99)	61.1

Weight, BMI and %EWL are expressed as mean values

Table 3: Weight loss outcomes of all patients irrespective of diabetes status

		Follow-up		
		Year 1	Year 2	Year 3
Mean %EWL by procedure	LAGB	37.2	52.1	53.3
	RYGB	66.9	76.2	54.6
	LDS	75.3	79.1	89.4
	SG	39.3	49.5	

Table 4: Diabetes Remission

	LAGB	**RYGB**	**LDS**	**SG**	**Total**	**p value**
n	20	97	29	15	161	
Age (mean yrs)	47.6	45.9	48	47	47.2	
Duration of DM (mean years)	7.6	8.6	6.5	7.2	7.9	
Duration of follow-up (mean months)	36.2	29.5	25.5	16.2	31.2	
Mean % EWL achieved (kg)	21.0	53.2	69.3	46.5	49.2	<0.0001
Mean BMI change (kg/m2)	-7.7	-14.8	-22.1	-14.8	-15.1	0.0002
Mean HbA1C change Mean HbA1C (pre-operation) Mean HbA1C (post-operation)	-1.06 7.58 6.52	-1.76 7.99 6.23	-2.42 7.48 5.06	-1.48 7.78 6.30	- 1.78 7.71 5.93	0.004
Diabetes remission HbA1c < 6.5%	n = 10 50%	n = 79 81.4%	n = 27 93.1%	n = 11 73.3%	n = 127 78.8%	<0.0001
Diabetes remission (at least 12 months) HbA1c < 6.0% FBS < 5.6 mmol/L	n = 3 15%	n = 41 42.2%	n= 18 62%	n= 5 33.3%	n= 67 41.6%	0.0004

3.2 Diabetes remission rate and the effect of using the ADA diabetes remission criteria [109]

Details regarding pre- and post-surgery HbA1C, post-surgery fasting glucose along with details of diabetes treatment with hypoglycaemic agent and insulin use were only available for 161 patients with type 2 diabetes. Table 4 shows their details. Of these 161 patients, 29 patients had undergone LDS, 97 had undergone RYGB, 20 had undergone LAGB and 15 had undergone SG. HbA1C at baseline was comparable between groups. The LDS group achieved the highest rate of diabetes remission, followed by RYGB, SG and LAGB based on the pre-2009 diabetes remission criteria of HbA1c less than 6.5%. This was in keeping with literature before the ADA diabetes remission criteria was published [7].

The diabetes remission rates applying the ADA criteria were 41.61% (n = 67/161) for all procedures and for each procedure as follows: LAGB 15% (3/20), RYGB 42.2% (41/97), LDS 62% (18/29) and SG 33.3% (5/15).

Discussion

In this analysis, which included almost 1000 participants, bariatric surgery appears to be an effective therapy for achieving durable weight loss in obese patients regardless of their diabetes status. A mean of 58 % EWL was achieved a year after bariatric surgery, which increased to 61% by year 3. Our results are slightly higher but comparable to the results reported by a large meta-analysis (%EWL of 53.8% <2 years post bariatric surgery and a %EWL of 59%> 2 years post-surgery) [7].

LDS is shown to be the most efficacious procedure, followed by RYGB, SG and LAGB, regardless of the diabetes status of the patient. These results concurred with the results of a meta-analysis by Buchwald et al [7].

Additionally, it is seen that patients with diabetes undergoing RYGB achieved significantly less weight loss than patients without diabetes a year after surgery. This observation was in line with the results by Carbonell A et al, who reported almost identical %EWL a year after RYGB (61% in patients with DM vs 68% in patients without DM) [157]. Other studies have

also observed that diabetes is a predictor of suboptimal weight loss up to one year after RYGB [158]. Interestingly, our study showed that the difference in weight loss achieved between patients with and without diabetes was only of statistical significance up to a year post RYGB. We have also identified that diabetes is associated with reduced weight loss for up to two years post LAGB. This observation is in line with those of other studies who also found a reduced %EWL in patients with diabetes up to two years post LAGB [159, 160]. Ponce et al reported a higher %EWL (39.2% in patients with diabetes) but this %EWL was lower than the non-diabetes cohort (41.2% in the non-diabetes group). Other studies did not identify a difference in weight loss achieved between patients with and without diabetes [161, 162]. However, these studies were limited by their smaller sample size and by the fact that their sample included only patients with diabetes, thereby basing their conclusions on the fact that their results were comparable to those of previous studies that included patients without diabetes. However, this methodology did not control for confounding factors such as variation in surgical techniques, thereby potentially limiting the validity of these results.

The reduced weight loss associated with the presence of diabetes in the early stages may be related to the lipogenic effects of insulin. Another possible explanation may be that patients with diabetes often increase their food intake to prevent hypoglycaemic events. A potential explanation for the fact that the effect of diabetes on weight loss did not extend beyond 2 years after LAGB and more than a year after RYGB, was that in most patients, diabetes remitted by that time. Once diabetes remission occurs, there is potentially no difference in the weight loss achieved between the groups. This in itself may be the reason we did not find any differences in the %EWL achieved by patients with and without diabetes after LDS.

Diabetes remission

Overall, the study has confirmed the beneficial effect of bariatric surgery on diabetes control. After an average follow up of approximately 2 years, diabetes remitted in 78.8% of patients (HbA1c <6.5%). These results are in line with those of a meta-analysis where a diabetes remission rate of 82% was reported [7]. In our study we showed that the rates of DM remission for each procedure appeared to be directly related to the %EWL achieved.

The clear criteria presented by ADA have made the understanding of literature about diabetes remission easier and it is possible to compare between published data from different countries and centres. Although the remission rates are lower than the data published before in most new studies [110], diabetes remission rate is still impressive and for selected patients weight loss surgery continues to be the treatment of choice. 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.

Chapter 4

The effect of Roux-en-Y Gastric Bypass on body composition, glycaemic status, blood pressure and lipid profile

Objectives

1. To study the longitudinal changes in weight and body composition in morbidly obese subjects after RYGB.
2. To compare the outcomes in the diabetes and non-diabetes subgroups.
3. To measure the serial changes in glycaemic control, blood pressure and lipid profile after RYGB and their respective treatments.

Patients and Methods

As described in Chapter 2, 22 subjects who had RYGB and 8 subjects who completed the 4 week calorie restriction were included in this study. 11 out of the 22 subjects who had RYGB were known to have diabetes. No subjects in the calorie restriction group were known to have diabetes.
Recruitment and study protocol is discussed in detail in chapter 2.

Statistical analysis

Statistical analysis was performed using the software R Version 3.2.1 and GraphPad Prism version 6; all statistical tests were performed at a two-sided significance level of $p < 0.05$. Data are expressed as mean ± 1 standard deviation from mean unless specified. Comparison of outcome with baseline was performed using paired t test. Comparison between groups were analysed using the Student's t test or Wilcoxon signed rank test as appropriate. Categorical data by groups was analysed using the ANOVA for multiple comparisons with baseline (Dunnett's method).

Results

The baseline characteristics of all patients are shown in Table 4.1. The calorie restriction group ($n = 8$) had significantly less weight ($p = 0.016$) and BMI when compared to the surgery group. The difference in weight and BMI, between the surgery subgroups, was not significant. The baseline measures of body composition, blood pressure, lipid profile, HbA1c and their treatments are also detailed in the table below.

Baseline characteristics of all subjects

	Calorie restriction	RYGB – No diabetes	RYGB - Diabetes
No of subjects (n)	8	11	11
Male: Female	2M:6F	3M:8F	6M:5F
Mean Age (years)	42.4 (11.6)	46.6 (10.5)	48.5 (8.8)
Mean weight (kg)	124.4 (24.5)	148.5 (22.4)	154.8 (25.9)
Weight range	95.8 – 162.6	125.6 – 194.2	115.2 – 194.2
Mean BMI (kg/m^2)	45.1 (5.1)	54.8 (9.5)	53 (6.7)
Mean Waist circumference(cm)	125.8 (14.1)	142.2 (15.1)	149.6 (15.4)
Mean Fat mass (kg)	64.8 (14.2)	72.5 (10.1)	76.7 (19.8)
Mean Fat free mass (kg)	62.5 (12.8)	71.6 (12)	74.5 (15.2)
Mean Fat %	51.4 (2.2)	50.4 (4.7)	50.5 (7.9)
Mean HbA1c (mmol/mol)	39.4 (1.4)	38.9 (2.0)	66.5 (17.3)
Mean HOMA-IR	2.9 (1.3)	2.6 (1.3)	3.5 (1.3)
Mean Duration of DM (years)	NA	NA	7 (2 – 18)
No of OHAs taken (= n)	NA	NA	0 OHA = 2 1 OHA = 5 2 OHA = 3 3 OHA = 1
No on insulin (units per day)	NA	NA	n = 4 (46, 82, 148, 250)
Mean blood pressure (mm of Hg)	148 (31) / 86 (15)	139 (26) / 85 (17)	151 (24) / 88 (19)
No on BP treatment	2/8	7/11	8/11
Mean Total cholesterol (mmol/L)	4.7 (0.7)	4.9 (0.9)	4.2 (1.3)
Mean HDL cholesterol (mmol/L)	1.0 (0.2)	1.0 (0.3)	0.9 (0.2)
Mean LDL cholesterol (mmol/L)	3.0 (0.6)	3.1 (0.7)	2.6 (1.1)
Mean triglycerides (mmol/L)	1.6 (0.6)	1.6 (0.6)	1.6 (0.4)
No on lipid lowering agent	0	4/11	7/11

Values are expressed as mean (± SD) unless specified

The subjects in the diabetes group were heavier and had a higher waist circumference when compared to the non-diabetes subjects. Rest of the anthropometric measurements and body composition measurements were consistent with the differences in weight. 9 out of 11 subjects in the diabetes group were on oral hypoglycaemic agents (OHA) and 4 were also on insulin along with oral agents. The daily insulin doses are detailed in the table above. The OHAs taken were mainly metformin and sulphonylureas and one patient was on sitagliptin. None of the patients were on GLP1 analogues or thiazolidinediones which could impact on the results. None of the patients in the calorie restriction group were known to have diabetes.

4.1 Changes in anthropometric measurements

4.1.1 Changes in weight and BMI

The table below summarises the changes in weight and BMI for the three study groups. Both in the diabetes and non-diabetes subgroups, the weight loss at 2 weeks post-RYGB were not statistically significant. The weight loss achieved in the calorie restriction group was not as significant as expected. There was no significant difference in the weight loss achieved between the diabetes and non-diabetes subgroups. This is shown in figure 4.3.

Serial weight and BMI changes in all groups

	Baseline visit	**Visit 2** **2-3 weeks post-surgery or 4 weeks calorie restriction**	**Visit 3** **4 months post-surgery**	**Visit 4** **12 months post-surgery**
Calorie restriction group (n = 8)				
Weight (kg)	124.4 (24.5)	120.6 (23.5) p = 0.57	-	-
BMI	45.1 (5.1)	43.8 (5)	-	-
RYGB – No diabetes group (n = 11)				
Weight (kg)	148.5 (22.4)	134 (21) p = 0.08	108.3 (19.7) p = 0.0002	94.6 (18.6) p < 0.0001
BMI	54.8 (9.5)	49.5 (9.2)	40 (8.1)	35.4 (7.1)
RYGB – Diabetes group (n = 11)				
Weight (kg)	154.8 (25.9)	138.4 (25.3) p = 0.13	116.2 (24.5) p < 0.0001	103.1 (18) p < 0.0001
BMI	53 (6.7)	47.4 (6.4)	40.3 (7.4)	36.4 (5.8)
RYGB group (n = 22)				
Weight (kg)	151.6 (23.9)	136.2 (22.8) p = 0.06	112.1 (21.9) p < 0.0001	98.4 (18.4) p < 0.0001
BMI	53.9 (8.1)	48.5 (7.8)	40.2 (7.6)	35.9 (6.4)

Values are expressed as mean (± SD) unless specified

P value not significant when comparing diabetes and non-diabetes groups at any time point

4.1.2 Changes in Body composition

The changes in waist circumference, fat mass, fat-free mass and fat percentage is shown in the table below. The reduction in fat mass and waist circumference correlated with weight loss. There was modest reduction in fat free mass and therefore a gradual reduction in fat percentage noted during the 4 month and 12 month visits.

Group	**Measure**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Calorie (n=8)	**WC**	125.8 (14.1)	121.5 (13.8)	-	-
	Fat Mass (kg)	64.8 (14.2)	62.4 (14.5)	-	-
	FFM (kg)	62.5 (12.8)	60.6 (10.8)	-	-
	Fat %	51.4 (2.2)	50.4 (2.8)	-	-
Diabetes (n=11)	**WC**	149.6 (15.4)	141.6 (16.9)	**122.4 (18.3)**	**117.2 (16)**
	Fat Mass (kg)	76.7 (19.8)	66.8 (17.5)	**44.6 (12)**	**37.5 (7.8)**
	FFM (kg)	74.5 (15.2)	71.6 (15.9)	67.9 (14.4)	**63.9 (12.4)**
	Fat %	50.5 (7.9)	48.1 (8.2)	**39.6 (7.9)**	**37.2 (7.6)**
No diabetes (n=11)	**WC**	142.2 (15.1)	131.7 (13.7)	**115.4 (24.9)**	**109 (18.3)**
	Fat Mass (kg)	72.5 (10.1)	62.6 (8.0)	**44.8 (14.6)**	**35.7 (11.9)**
	FFM (kg)	71.6 (12)	67 (12)	64.4 (11.3)	**60.4 (12.2)**
	Fat %	50.4 (4.7)	48.5 (4.5)	**40.5 (7.4)**	**36.5 (6.1)**

Values are expressed as mean (± SD)

Values in bold achieved significance ($p < 0.05$)

4.2 Blood Pressure and treatment changes

Blood pressure was significantly reduced in both RYGB diabetes and RYGB non-diabetes groups especially in the later visits after surgery. There were fewer patients needing anti-hypertensives after RYGB as detailed below.

Group		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Calorie (n=8)	**Mean BP (mm of Hg)**	148(31) / 86(14)	143(22) / 82(9)	-	-
	No on BP drugs	2/8	2/8	-	-
Diabetes (n=11)	**Mean BP (mm of Hg)**	151(24) / 88(12)	**117(17) /78(13)**	**119(21) / 74(10)**	**124(15) / 72(8)**
	No on BP drugs	8/11	4/11	2/11	1/11
No diabetes (n=11)	**Mean BP (mm of Hg)**	139(26) / 85(13)	120(15) / 74(11)	**112(13) / 72(11)**	**115(12) / 75(6)**
	No on BP drugs	7/11	2/11	0/11	1/11

Values are expressed as mean (± SD) unless specified

Values in bold achieved significance ($p < 0.05$)

4.3 HbA1c and diabetes treatment changes

As expected there was a significant drop in glycated haemoglobin (HbA1c) values after RYGB. Metformin was often continued for several months after RYGB although their glycaemia was significantly improved. None of the patients who were on insulin before RYGB needed insulin treatment after surgery. No patients reported hypoglycaemia peri-operatively (with or without diabetes treatment) but some patients were seen to have reactive hypoglycaemia (asymptomatic in this group of patients) during meal curves.

Group		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Calorie (n=8)	**Mean HbA1c (mmol/mol)**	39.4 (1.4)	38.4 (1.1)	-	-
	No on OHAS	0	-	-	-
	No on insulin	0	-	-	-
Diabetes (n=11)	**Mean HbA1c (mmol/mol)**	66.5 (17.3)	**52.1 (14)**	**38.8 (9.1)**	**36.6 (8.3)**
	No on OHAS	9/11	4/11	1/11	1/11
	No on insulin	4/11	0/11	0/11	0/11
No diabetes (n=11)	**Mean HbA1c (mmol/mol)**	38.9 (3.0)	37 (3.5)	**33.4 (2.6)**	**31.5 (3.1)**
	No on OHAS	0	-	-	-
	No on insulin	0	-	-	-

Values are expressed as mean (± SD) unless specified

Values in bold achieved significance

4.4 Lipid profile and treatment changes

There was no significant reduction in total cholesterol levels after RYGB. There was gradual increase in HDL cholesterol levels and fall in LDL cholesterol and triglycerides especially at 12 months after RYGB. Patients who were on anti-lipid treatments (mainly statins and fibrates) were usually asked to discontinue them at the time of surgery or soon after surgery. There was a slight drop in HDL cholesterol levels 2 weeks after RYGB but there was gradual increase at the subsequent visits.

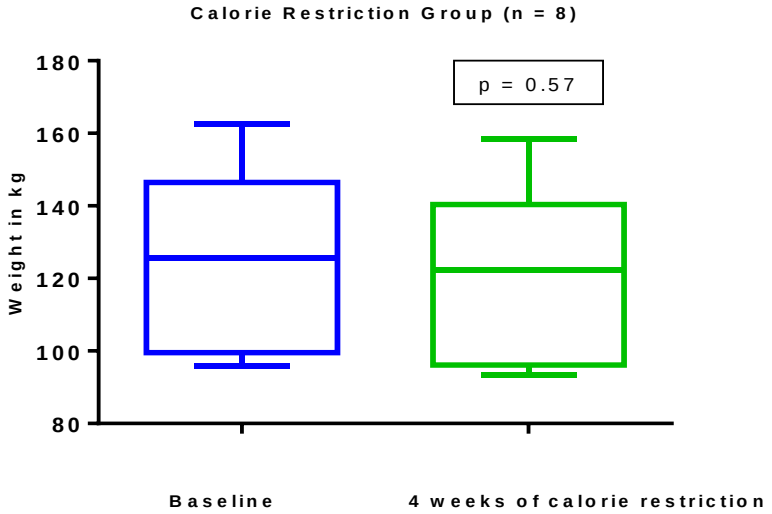
Changes in lipid profile and lipid lowering treatment

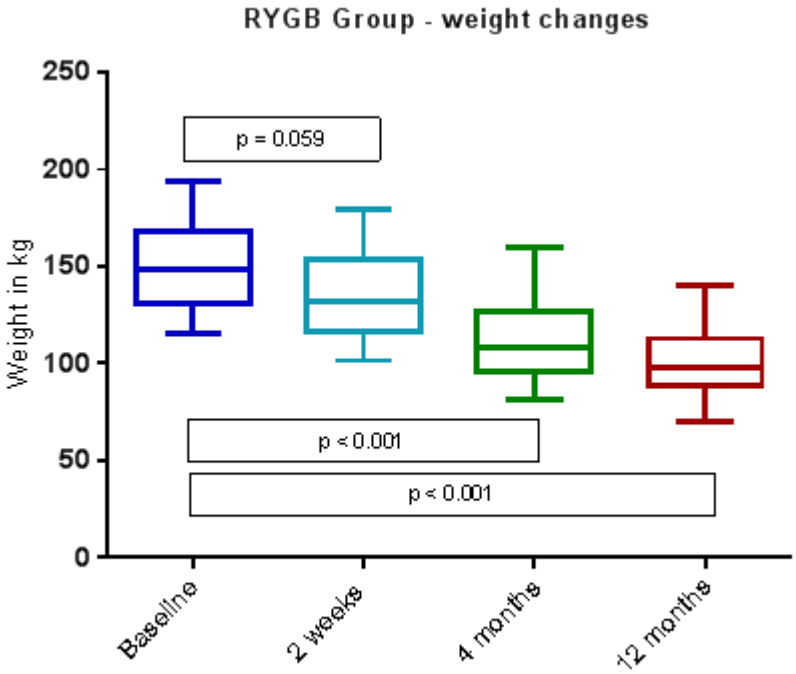
Group	**mmol/L**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Calorie (n=8)	**Total cholesterol**	4.7 (0.7)	4.5 (0.5)	-	-
	HDL	1.0 (0.2)	0.9 (0.2)	-	-
	Triglycerides	1.6 (0.6)	1.7 (0.3)	-	-
	LDL	3.0 (0.6)	2.8 (0.5)	-	-
	No on treatment	0/8	0/8	-	-
Diabetes (n=11)	**Total cholesterol**	4.2 (1.3)	4.1 (1.0)	4.4 (1.3)	3.9 (0.7)
	HDL	0.9 (0.2)	0.8 (0.2)	1.0 (0.3)	**1.2 (0.2)**
	Triglycerides	1.6 (0.4)	1.7 (0.3)	1.6 (0.6)	**1.1 (0.5)**
	LDL	2.6 (1.1)	2.5 (1.0)	2.7 (1.2)	2.0 90.4)
	No on treatment	7/11	4/11	2/11	0/11
No diabetes (n=11)	**Total cholesterol**	4.9 (0.9)	4.2 (0.7)	4.2 (0.9)	4.1 (0.9)
	HDL	1.0 (0.3)	0.8 (0.2)	1.1 (0.2)	**1.4 (0.3)**
	Triglycerides	1.6 (0.6)	1.7 (0.7)	1.2 (0.4)	**1.0 (0.3)**
	LDL	3.1 (0.7)	2.6 (0.5)	2.5 (0.7)	**2.2 (0.5)**
	No on treatment	4/11	2/11	0/11	0/11

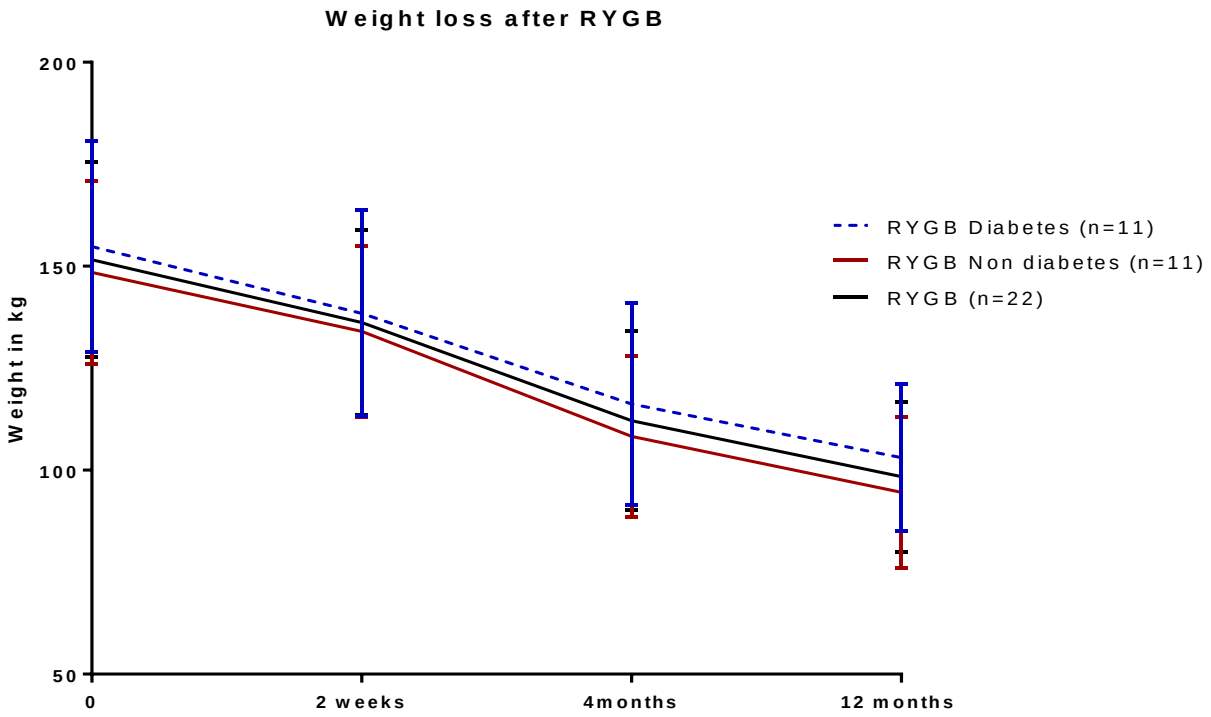
Values are expressed as mean (± SD) unless specified

Values in bold achieved significance

Weight changes







4.5 Discussion

This study has shown effective weight loss in both diabetes and non-diabetes subjects following RYGB. The weight loss achieved in keeping with the published literature [91]. This is associated with reduction in waist circumference and fat mass is reduced more than the fat free mass. There does not appear to be any significant difference in the weight loss outcome between the diabetes and non-diabetes subgroups, similar to the observation in the retrospective analysis in chapter 3.

Blood pressure, lipid profile as well as glycaemia improve significantly after weight loss surgery [92]. The weight loss at 2 weeks is not significant but there already a significant drop in HbA1c values at that time in the diabetes subgroup (66.5 vs 52.1 mmol/mol). This is likely to be related to the calorie restriction experienced in the surgery patients [117]. They are advised to follow a low calorie liquid diet for at least 2 weeks prior to weight loss surgery and their oral intake is very limited for at least 2 weeks post-RYGB. There is also an

improvement in the insulin sensitivity and peak insulin response during this time which is discussed in the next chapter. It is reassuring to note that a large majority of patients are able to safely discontinue their oral hypoglycaemic agents and insulin at the time of surgery or within days of surgery. Almost all subjects with type 2 diabetes (10/11) achieved diabetes remission by the new ADA definition at 12 months and were able to stop their diabetes treatments. One subject (was on insulin and metformin pre-RYGB) was still taking metformin at 12 months and the HbA1c was improved from pre-surgery level (74 to 57 mmol/mol).

Blood pressure and lipid profile improve within months of RYGB and many patients are able to discontinue their pre-surgery treatments. Overall the remission of type 2 diabetes along with improvements in hypertension and dyslipidaemia are likely to explain the reduced cardiovascular risk and mortality observed in the morbidly obese patients who lose weight with surgery [114]. The Qrisk 2 score (%, 10 year risk, version 2015) for the surgery patients reduced from a mean of 31.2% to 13.1% at 12 months ($p < 0.001$).

The calorie restriction group did not lose much weight probably because their calorie restriction was not adequate and some may have found it difficult to adhere to the diet prescribed to them. This highlights the difficulty in the use of lifestyle modification as a treatment option in morbidly obese subjects and the inability of some patients to stick to calorie restriction. It will however be interesting to see if there are any changes in the gut hormones, glucose homeostasis or adipokines in this group. In hindsight it may have been more appropriate to use a very low calorie diet (VLCD) to assess the effects of calorie restriction on glycaemia in comparison to changes after RYGB.

Chapter 5

Changes in dynamic responses of insulin-glucose homeostasis, incretins and gut hormones in response to standard meal after Roux-en-Y Gastric Bypass: Relationship to changes in eating behaviour and energy homeostasis

5.1 Introduction

Weight loss outcomes after bariatric surgery have been reported widely in several observational and a few RCT and meta-analyses as discussed earlier. Diabetes remission rates, as discussed in chapter 1, were initially thought to be higher but applying stricter criteria has led to more modest yet significant reporting of outcomes. The mechanisms underlying diabetes remission need further evaluation and understanding. Several theories have been proposed to explain the mechanisms underlying diabetes remission noted after weight loss surgery.

Foregut theory

The exclusion of nutrients from the duodenum and jejunum appears to have a positive effect on incretin pathway [163]. It is proposed that when nutrients come into contact with proximal small bowel mucosa they stimulate release of unidentified anti-incretin factor/s, which decreases incretin and thereby insulin secretion. Surgical procedures that bypass the duodenum and jejunum have been shown to restore balance between anti-incretin and incretin secretion and improve glucose control [164]. The duodeno-jejunal bypass sleeve (DJBS) is currently being studied as an effective method of improving glucose homeostasis with modest weight loss [165]. Initial results are quite encouraging but complications relating to the procedure are limiting the initial enthusiasm. Following DJBS insertion, increase in postprandial release of GLP-1 and lowered secretion of gastric inhibitory polypeptide (GIP) is observed within 1 week before any significant weight loss occurred [166]. In a study evaluating DJBS in the treatment of T2DM patients, out of the 22 subjects, 16 had an HbA1c <7% at the end of the study, compared with only 1 of 22 at baseline [167, 168]. However, the hypothesis of foregut has been questioned during recent years as it has been shown that similar changes in GLP-1 and glucose metabolism occurs after SG, the operation where nutrients are in direct contact with proximal small bowel.

Hindgut theory

GLP1 is released from enteral L-cells lining the distal gastrointestinal tract when it comes into contact with nutrients. The rapid delivery of nutrients to the distal small intestine appears to be responsible for improved glucose metabolism by increasing GLP-1 and PYY release [169]. This has been confirmed by the finding that improved GLP-1 response was

found after RYGB, SG and BPD but not after LAGB [170]. Based on these observations, Edward Mason [171] proposed ileal interposition as an operation for the treatment of T2DM. Experimental studies in rats have found that ileal interposition improves glucose and lipid metabolism and delays diabetes onset in UC Davis-T2DM rats, the rat model that is most similar to clinical T2DM in humans [172]. The initial results from human studies appear to confirm that hypothesis [173].

Midgut or intestinal/hepatic regulation hypothesis

Recently it has been proposed that derivation of food into the distal small intestine after gastric bypass activates gluconeogenic enzymes and increases glucose concentrations in the portal vein, which is sensed and transmitted to the brain by vagal afferents. This results in increased suppression of hepatic glucose production by insulin and improves glucose homeostasis [174]. This has been proposed based on animal studies and replication of these changes in humans is yet to be confirmed.

Bile acids (BA)

Based on animal studies and initial observational studies, increases in fasting and post-prandial BA components post RYGB were thought to have positive metabolic effects leading to improved glycaemic control. In one RCT where both RYGB and SG were compared, similar increases in total basal plasma bile acids after both procedures were noted, with higher increase in postprandial basal acids after RYGB. As expected, improved glycaemic control and increased incretin secretion were observed after 1 week whereas basal and postprandial BA significantly increased only 1 year after surgery [175]. This does not appear to support the hypothesis that BA is responsible for early increase in incretin secretion after bariatric surgery. It is proposed that BA may inhibit gluconeogenesis, facilitate insulin-dependent control of glucose metabolism in liver, and through increase in FGF19 levels improve insulin resistance at later stages after weight loss has occurred [176].

Gut microbiota

The large intestine has a variety of microbes responsible for different metabolic pathways, mainly comprising of the bacteroidetes and the firmicutes. Obese people have reduced proportions of bacteroides, while individuals with diabetes have reduced proportions of firmicutes and clostridia [44, 177]. Recent studies show that changes in human intestinal

flora could have an impact on weight loss and glycaemic control after bariatric surgery. RYGB has been shown to alter the intestinal flora by decrease in firmicutes and increase in Gamma-proteobacteria [178]. Transfer of gut microbiota from gastric bypass mice to non-operated germ-free mice resulted in improved insulin sensitivity and reduced fasting triglyceride levels [179]. However, the impact of microbiota on metabolic changes observed after RYGB is yet to be fully understood in humans.

Changes in incretins and gut hormones after weight loss surgery

A variety of gut hormones are known to play a role in glucose homeostasis and regulation of appetite. Weight loss surgery has shown to improve post-prandial insulin response, hepatic glucose regulation and insulin sensitivity within days of malabsorptive surgery. Apart from calorie restriction, improved incretin response and favourable changes in gut hormones are thought to be the prime drivers of this change. Regulation of appetite soon after surgery is also attributed to the changes in gut and pancreatic polypeptides.

Gastrin

Gastrin is produced in G cells in the gastric antrum and duodenum and is released in response to food and gastric distension. It increases the secretion of hydrochloric acid, pepsinogen and pancreatic juices and reduces appetite. As RYGB excludes the gastric antrum or duodenum, resulting in reduced contact between the nutrients and the majority of the G cells, a fall in gastrin secretion is noted in studies [180]. Gastrin levels have been shown to be increased after both SG and LAGB [181]. Treatment with proton pump inhibitors following weight loss surgery can also increase gastrin levels.

Ghrelin

Ghrelin is mainly produced in the stomach and pancreas in response to fasting and is associated with hunger. Ghrelin is a 28 amino acid gut peptide derived from pre-proghrelin. Ghrelin levels rise with prolonged fasting and fall after a meal. Carbohydrates and proteins exert a stronger action on ghrelin suppression than fat. Ghrelin has been shown to bind with the growth hormone secretagogue receptor (GHS-R), but ghrelin is not essential for growth hormone secretion, but may increase GH pulsatility. Acylated ghrelin

is orexigenic and initially it was thought that only the acylated form was chemically active. Both total ghrelin and its acylated form (with an octanoyl group) have been studied extensively. In obesity, both fasting and postprandial total ghrelin concentrations are lower but the proportion of acylated ghrelin is higher in the obese [182]. Weight loss via calorie restriction and LAGB has been shown to increase ghrelin levels. Ghrelin levels fall after RYGB probably due to limited contact between ghrelin-producing mucosal cells in the stomach and ingested nutrients [127]. Sleeve gastrectomy has shown to decrease circulating acylated ghrelin concentrations, possibly due to the removal of ghrelin-producing cells in the stomach [183]. Dirksen et al. showed that, after RYGB, a higher degree of ghrelin suppression was seen in those who had a good weight loss response when compared to poor responders. Many studies however have shown no change or increase in postprandial ghrelin response especially in the longer term [184]. Differences in the methodology used to measure ghrelin, diverse commercial assays, type of meal used during studies and variations in surgical techniques (e.g. involvement of vagal nerve) have all been reported as possible reasons for inconclusive results .

Glucose-dependent insulinotropic polypeptide (GIP)

GIP is secreted from K cells in the small intestine but mostly from the duodenum. Like GLP-1, GIP is an incretin associated with an improved insulin response following ingestion of oral glucose. Also GIP causes a postprandial rise of glucagon and promotes lipoprotein lipase activity. Its secretion is associated with the induction of β-cell proliferation and the enhanced resistance to apoptosis. GIP was originally called gastric inhibitory polypeptide and was thought to inhibit gastric acid secretion in dogs. However, this effect was found to be negligible in humans and the hormone was later renamed. The role of GIP in the development of diabetes and obesity is unclear, but hyperglycaemia may act to directly down regulate GIP receptors in pancreatic beta cells. After RYGB, due to reduced nutrient exposure to the duodenum and jejunum, some studies have shown a reduction in fasting and postprandial GIP secretion [185]. This has not been confirmed in other studies [186]. Altered fasting and postprandial GIP responses to RYGB may be affected by the presence or absence of diabetes [187].

Glucagon-like peptide 1 (GLP-1)

GLP-1 is secreted from L cells, predominantly in the distal ileum and colon. GLP-1 is responsible for stimulation of insulin release in response to nutrient ingestion and inhibition of gastric emptying which blunts postprandial glycaemia, restoration of insulin sensitivity and inhibition of glucagon secretion. Additionally, GLP-1 acts on the central nervous system to induce satiety and decrease food intake. Rapid delivery of nutrients to the distal intestine is thought to be the cause of increased postprandial response after RYGB [8]. Levels are low in obesity and improve with weight loss. Exogenous GLP-1 administration reduces appetite and energy consumption both in normal and obese individuals.

Fasting concentrations of GLP-1 do not appear to change markedly after bariatric surgery [122, 186]. The postprandial GLP-1 levels gradually increase during the first two years after RYGB [188, 189]. These changes appear to be independent of weight loss and the caloric reduction during the early postoperative period. The rapid changes noticed in appetite regulation and glucose homeostasis within days have been attributed, at least in part, to the increased secretion of GLP-1.

Binding of GLP-1 to the GLP-1 receptor on pancreatic beta cells activates adenylate cyclase and increases cAMP concentrations which augment insulin secretion. The ability of GLP-1 to promote insulin secretion is dependent upon the glucose concentration, with reduced or absent effect at low glucose concentrations. This has made GLP-1 agonists an attractive target for pharmacological intervention to reduce hyperglycaemia with no undue tendency for hypoglycaemia. GLP-1 agonists such as Liraglutide have been approved for pharmacological use in the treatment of diabetes and more recently for obesity. The beneficial metabolic effects of RYGB upon beta-cell sensitivity is obliterated by infusion of exendin-9, a GLP-1 receptor antagonist (GLP1R) confirming that GLP-1 action is largely responsible in causing improved glucose tolerance after surgery [190]. However in animal studies RYGB achieved similar responses in glucose and weight control in GLP-1 knockout mice suggesting other mechanisms also play a part.

GLP-1 is also thought to have centrally-mediated effects upon appetite by interacting with vagal afferent nerve fibres. In rodents, GLP-1 administration activates neurones in the

arcuate nucleus and paraventricular nucleus to promote satiety. When vagotomy is performed with bariatric surgery these effects are attenuated [191].

PYY

PYY is released postprandially in proportion to the calories ingested and has an inhibitory effect on gastrointestinal mobility. Although PYY3–36 has many effects upon the body including delaying gastric emptying, inhibition of gallbladder contraction, reducing postprandial insulin production and altering colonic motility, its main role appears to involve the central regulation of appetite. It increases satiety and reduces food intake. Like GLP-1, PYY 1–36 is produced by L cells in the distal small intestine and colon. Following cleavage in the circulation by the enzyme di-peptidyl-peptidase-IV (DPP-IV), PYY 1–36 is converted to PYY 3–36 which is considered to promote satiety. The central actions of PYY are mediated through the Y2 neurons, since Y2 knock-out mice do not show any appetite inhibition after PYY administration. Fasting levels remain unchanged after RYGB. Postprandial PYY 3–36 levels are reduced in obese patients compared to healthy volunteers [192] and PYY 3–36 infusion reduces caloric intake [130]. This has led to suggestions that obesity is a state of PYY 3–36 deficiency. Following bariatric surgery, levels of PYY increase postprandially, an effect which is evident two weeks post-surgery and still present after a year [128]. This increase in postprandial PYY appears to occur following many different types of bariatric procedure including gastric banding, sleeve gastrectomy and RYGB. Animal students using rodent PYY knockout models suggest that PYY is an important contributor to weight loss following bypass surgery and has a distinct role in glucose homeostasis.

Oxyntomodulin

Oxyntomodulin also produced from L cells, like GLP-1, originates from the proglucagon gene by alternative post-translational processing pathways. Oxyntomodulin is a 37-amino acid peptide hormone which is structurally similar to glucagon with an additional C-terminal octapeptide. Postprandial levels have been shown to increase after RYGB [188]. Oxyntomodulin appears to be an agonist of both GLP-1 and glucagon receptors and is considered to be a promising pharmacological agent in the treatment of obesity. Its role in glucose metabolism however remains unclear.

Patients and methods

This is discussed in chapter 2. Glucose levels were measured at baseline ('0' fasting) and every 30 minutes after the fixed energy intake of 330 kcals until 180 minutes. Insulin and GLP1 levels were measured at fasting - 0, 30, 60, 90, 120 and 180 minutes. PYY levels were measured at baseline only. Subjects were asked to complete VAS scores for hunger and satiety (appendix 2) at each time point. They were also asked to complete the Three Factor Eating Questionnaire (TFEQ) during each visit reflecting their eating behaviour since the last visit.

HOMA2 IR and HOMA2 %B values were calculated from the fasting glucose and insulin values using the HOMA2 calculator (version 2.2.1). AUC for insulin/ glucose (pmol/mmol) was used as a marker of insulin secretion. Other measures of changes in insulin sensitivity and secretion based on oral meal curve responses are discussed in chapter 7.

Statistical analysis

Data was checked for normality using the Shapiro-Wilk test. Comparison between groups were analysed using the Student's t test or Wilcoxon signed rank test as appropriate. The trapezoid method was used to measure 'Area under the curve' (AUC) for glucose, insulin and GLP1 values. Statistical calculations were made by analysis of variance (ANOVA) for repeated measures. Data is presented as mean (+/- 1 SD) unless specified. Two sided P value of less than 0.05 was considered significant. Fisher's exact test and the Freeman–Halton extension of Fisher's exact test were used for analysis of categorical data. Data were analysed using SPSS version 22 (SPSS, Chicago, Illinois, USA) and GraphPad Prism version 6.07 (GraphPad Software Inc., CA, USA).

5.2 Results

Changes in weight, BMI, waist circumference and body composition after calorie restriction and RYGB is discussed in chapter 4. The following table details the changes in fasting and AUC values for glucose, insulin and GLP1. Changes to fasting PYY values and markers of insulin sensitivity and secretion are also detailed below.

Group	Mean value (± 1 SD)	Visit 1 Baseline	Visit 2 2 weeks after surgery (or) after 4 weeks calorie restriction	Visit 3 4 months after RYGB	Visit 4 12 months after RYGB
Calorie restriction (n=8)	Fasting Glucose (mmol/L)	5.1 (0.5)	5.1 (0.5)	-	-
	AUC Glucose (mmol/L 180 min)	1089.8	1069.3	-	-
	Fasting Insulin (pmol/L)	158.3 (79.2)	131.3 (40.7)	-	-
	AUC Insulin (pmol/L 180min) $x10^3$	80.1	102.8	-	-
	Fasting GLP1 (pmol/L)	35.4 (21.4)	25.7 (13.6)	-	-
	AUC GLP1 (pmol/L 180 min)	3988.6	5700.6	-	-
	Fasting PYY (pg/ml)	67.4 (32.9)	59 (38.1)	-	-
	HOMA2 IR	2.9 (1.3)	2.4 (0.7)	-	-
	HOMA2 %B	190.9 (82.2)	167 (38.6)	-	-
	AUC Insulin/Glucose (pmol/mmol)	80.5 (51.2)	100.6 (64.9)	-	-
RYGB Diabetes (n=11)	Fasting Glucose (mmol/L)	8.9 (2.5)	6.7 (2.3)	5.9 (2.6)	5.1 (1.3)
	AUC Glucose (mmol/L 180 min)	2005.8	1460.4	1265	1098.8
	Fasting Insulin (pmol/L)	175.9 (61.1)	111.7 (35.5)	92.7 (38.2)	91.8 (41.6)
	AUC Insulin (pmol/L 180min) $x10^3$	72.1	54.6	59	67.6
	Fasting GLP1 (pmol/L)	25.7 (12.4)	22.1 (14)	15.5 (12.2)	10.1 (11.7)
	AUC GLP1 (pmol/L 180 min)	6099.6	12237.1	6022.2	4478
	Fasting PYY (pg/ml)	71.4 (67.1)	71.4 (67.1)	87.2 (101.7)	76.8 (89)
	HOMA2 IR	3.6 (1.3)	2.2 (0.8)	1.8 (0.7)	1.7 (0.8)
	HOMA2 %B	88.1 (43.7)	100.8 (31.3)	121.3 (52.4)	140.5 (46)
	AUC Insulin/Glucose (pmol/mmol)	40.2 (21.8)	30.7 (16.4)	50.3 (22.3)	66.9 (28.7)
RYGB non diabetes (n=11)	Fasting Glucose (mmol/L)	5.9 (0.6)	5.2 (0.8)	4.9 (0.6)	4.7 (0.8)
	AUC Glucose (mmol/L 180 min)	1227.8	1071.7	1037	887.8
	Fasting Insulin (pmol/L)	131.6 (62)	72.1 (42)	44.7 (22.6)	54.5 (30.2)
	AUC Insulin (pmol/L 180min) $x10^3$	72.5	71.2	57.2	70.2
	Fasting GLP1 (pmol/L)	30.8 (21.7)	30.2 (19.4)	12.8 (12.4)	15.8 (17.9)
	AUC GLP1 (pmol/L 180 min)	6008.4	12580.3	7277.7	8282.2
	Fasting PYY (pg/ml)	66.9 (58)	36.4 (33.8)	58.5 (57.4)	54.4 (37)
	HOMA2 IR	2.5 (1.2)	1.4 (0.8)	0.8 (0.4)	1.0 (0.6)
	HOMA2 %B	125.5 (43.7)	105.1 (42.1)	86.7 (26)	110.9 (50.2)
	AUC Insulin/Glucose (pmol/mmol)	60.9 (24.7)	56.3 (25.1)	56.5 (26.4)	81.8 (69.3)
Total RYGB (n = 22)	Fasting Glucose (mmol/L)	7.4 (2.4)	6.0 (1.9)	5.3 (1.8)	4.9 (1.0)
	AUC Glucose (mmol/L 180 min)	1616.8	1275.3	1145.5	988.2
	Fasting Insulin (pmol/L)	153.8 (64.2)	91.9 (43)	67.6 (38.9)	72.3 (40)
	AUC Insulin (pmol/L 180min) $x10^3$	72.3	62.9	58.1	68.9
	Fasting GLP1 (pmol/L)	28.1 (17.2)	25.7 (16.7)	14.2 (12)	13.1 (15.2)
	AUC GLP1 (pmol/L 180 min)	6056.2	12391.6	6616.9	6480.2
	Fasting PYY (pg/ml)	69.3 (61.4)	50.8 (51.5)	72.8 (81.7)	63.8 (63)
	HOMA2 IR	3.1 (1.3)	1.8 (0.9)	1.3 (0.7)	1.3 (0.8)
	HOMA2 %B	106.8 (46.8)	102.8 (36)	103.2 (43.4)	125 (49.5)
	AUC Insulin/Glucose (pmol/mmol)	50.5 (25.1)	43.5 (24.5)	53.5 (24.1)	74.7 (53.2)

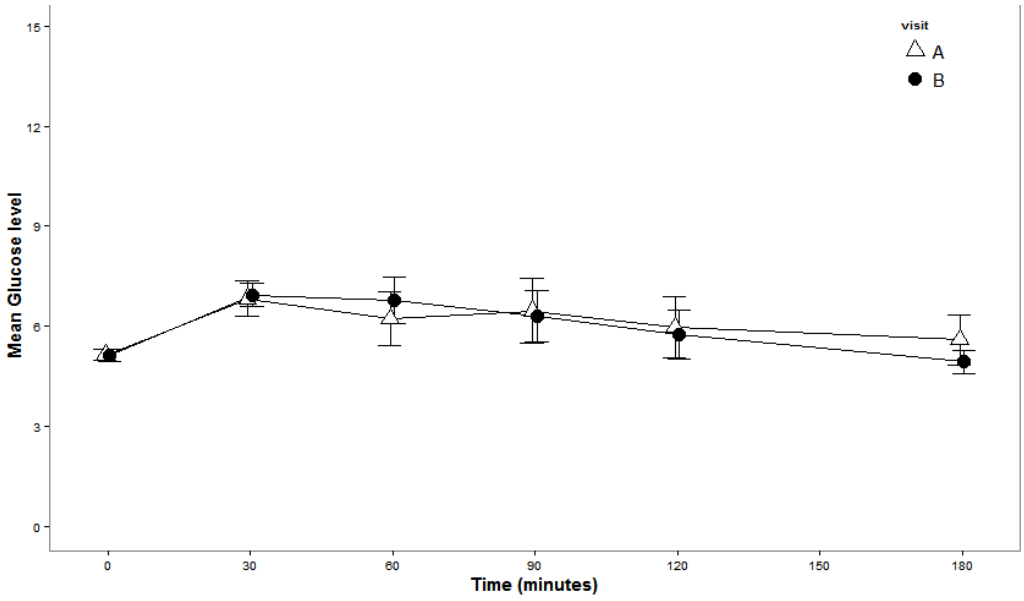
5.2.1 Changes in Glucose tolerance

Fasting Glucose levels did not change in the calorie restricted group (n = 8). The AUC for glucose also did not change significantly (1089.8 vs 1069.3, p = 0.89).

In the RYGB group (n=22), fasting glucose values fell to 6.0 ± 1.9, p = 0.037 at 2 weeks; 5.3 ± 1.8, p = 0.002 at 4 months and 4.9 ± 1, p < 0.001 at 12 months. As expected the diabetes group had more significant fall in fasting glucose values. In the RYGB group the AUC for glucose fell significantly from 1616.8 to 1275.3 at 2 weeks (p = 0.04); 1145.5 at 4 months (p = 0.004) and 988.2 at 12 months (p = 0.0001). In the RYGB non-diabetes subgroup the fall in AUC glucose was from 1227.8 to 1071.7 (p = 0.009) at 2 weeks, 1037 (p = 0.002) at 4 months and 988.2 (p < 0.0001). In the diabetes subgroup, these changes were again significant: baseline 2005.8; at 2 weeks 1460.4 (p = 0.004); at 4 months 1265 (p = 0.0002) and 12 months 1098.8 (p < 0.0001).

Change in mean glucose in response to 330 kcal liquid meal

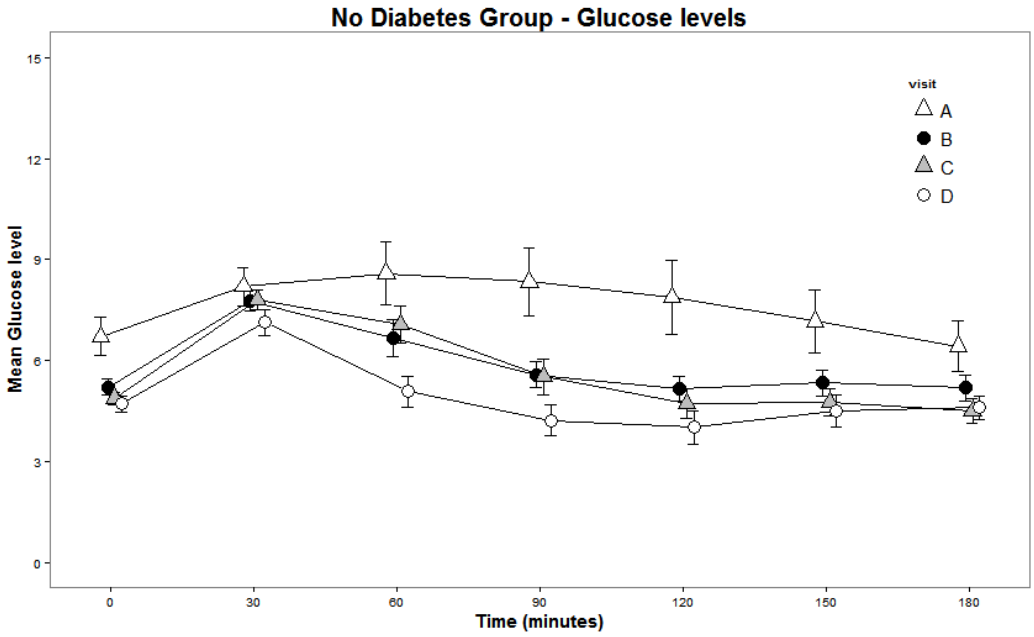
Calorie restriction group (n = 8)



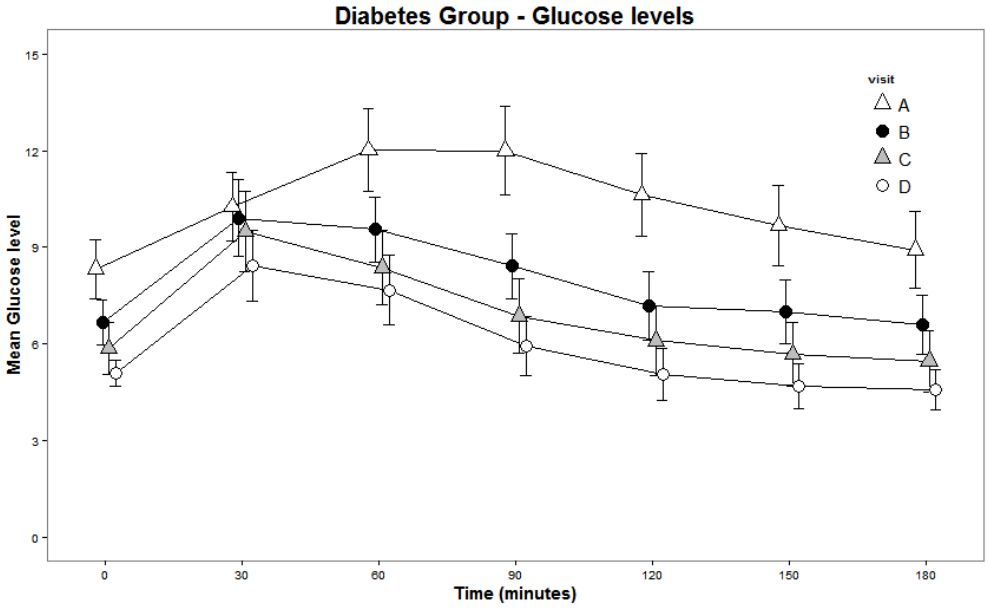
A = Baseline visit

B = after 4 weeks of calorie restriction

RYGB non-diabetes group (n=11)



RYGB Diabetes group (n – 11)



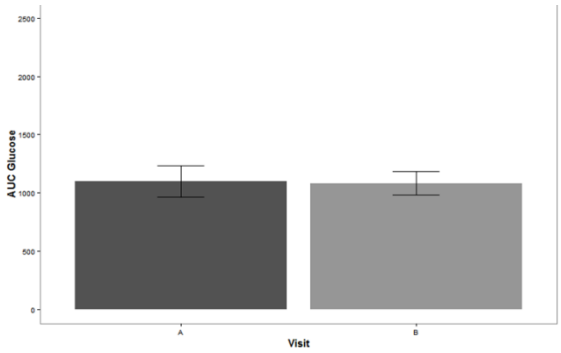
Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery

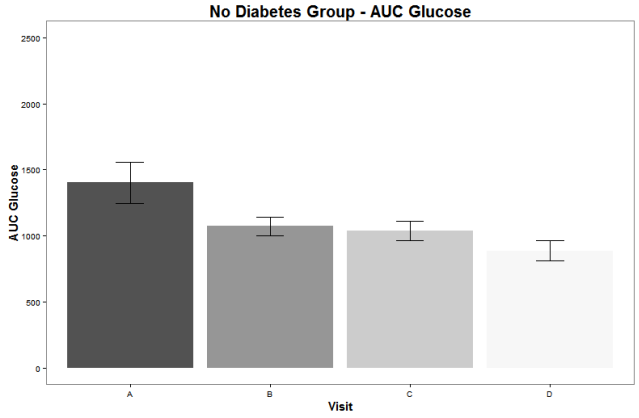
AUC Glucose – Calorie restriction group (n = 8)

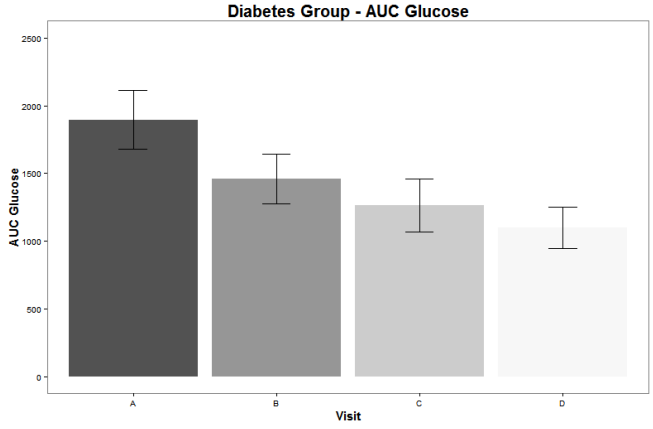


A = Baseline visit

B = after 4 weeks of calorie restriction

AUC Glucose after RYGB





Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery

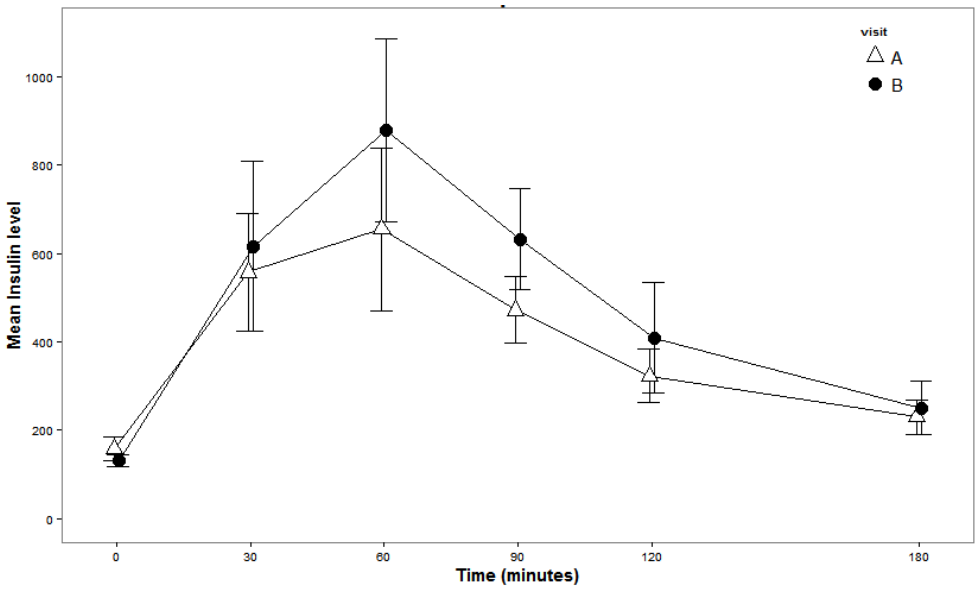
5.2.2 Changes in Insulin response

Fasting insulin levels did not change significantly in the calorie restricted group (p = 0.41). The AUC for insulin (pmol/L 180 min x 10^3) also did not change significantly (80.1 vs 102.8, p = 0.39). There was however a significant improvement in peak insulin response which is discussed in chapter 7.

In the RYGB group (n=22), fasting insulin values fell from 153.8 ± 64.2 to 91.9 ± 43, p = 0.0005 at 2 weeks; 67.6 ± 38.9, p < 0.0001 at 4 months and 72.3 ± 40, p < 0.0001 at 12 months. Both the non-diabetes and diabetes subgroups had significant fall in fasting insulin values: at 2 weeks p 0.016 vs 0.007; 4 months p 0.0003 vs 0.001 and 12 months p 0.001 vs 0.001. In the RYGB group the AUC for insulin (x 10^3) fell insignificantly from 72.3 to 62.9 at 2 weeks (p = 0.31); 58.1 at 4 months (p = 0.07) and 68.9 at 12 months (p = 0.75). In the diabetes (p = 0.12, 0.29, 0.73) and non-diabetes subgroups (p = 0.93, 0.16, 0.90) the fall in AUC insulin was not significant at any of the time points. Changes to peak insulin response, insulinogenic index and disposition index are discussed in chapter 7.

Change in mean insulin in response to 330 kcal liquid meal

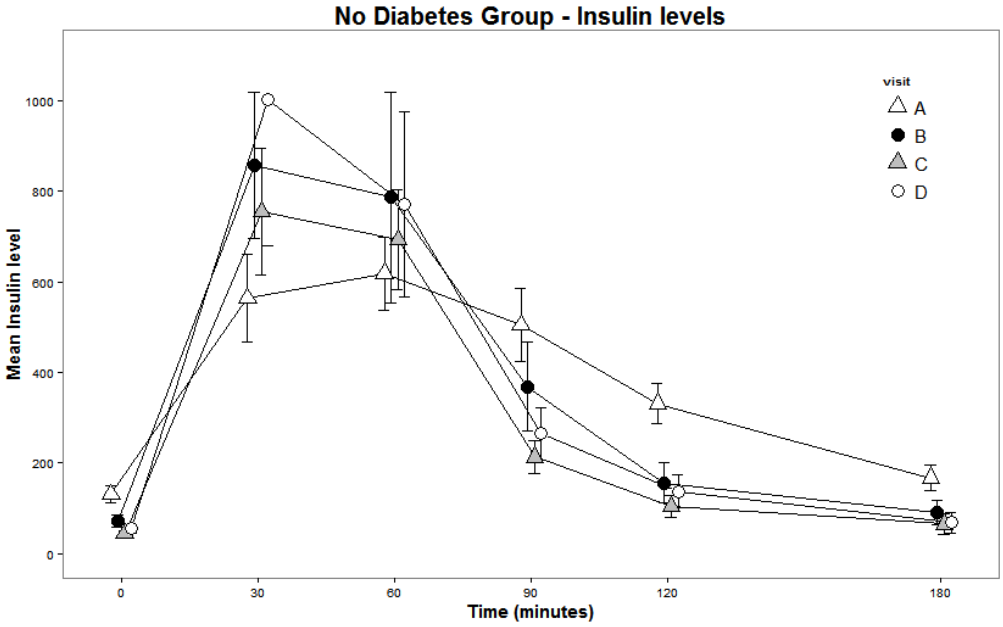
Calorie restriction group (n = 8)



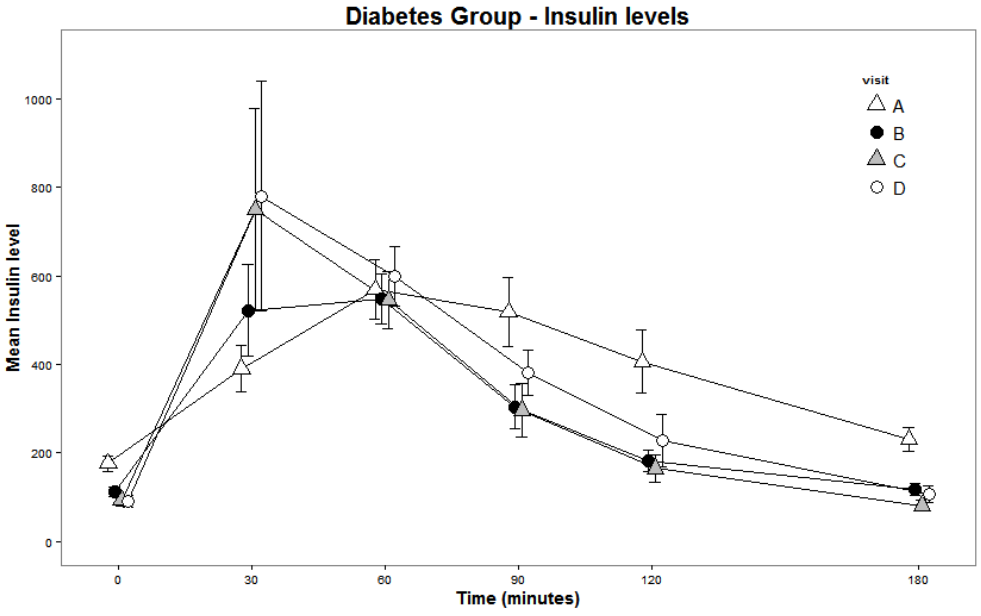
A = Baseline visit

B = after 4 weeks of calorie restriction

RYGB non-diabetes group (n = 11)



RYGB Diabetes group (n = 11)



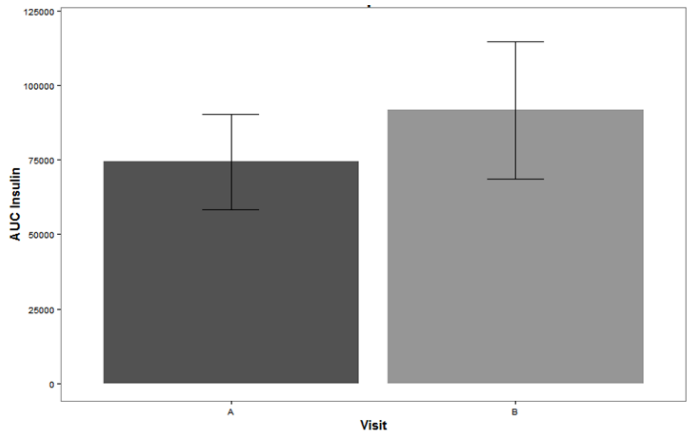
Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

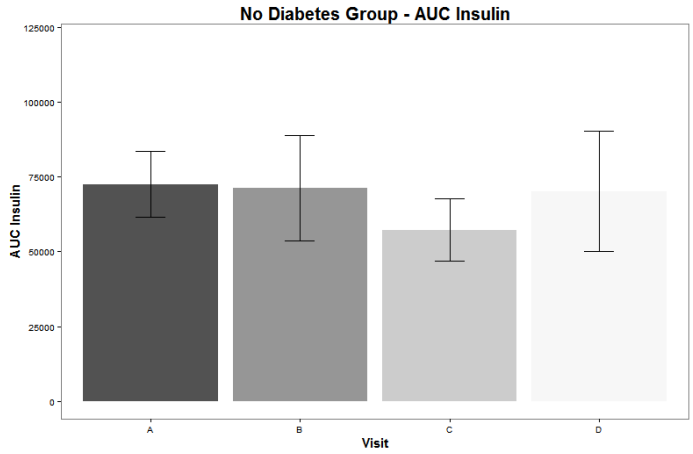
Visit C = 4 months after surgery

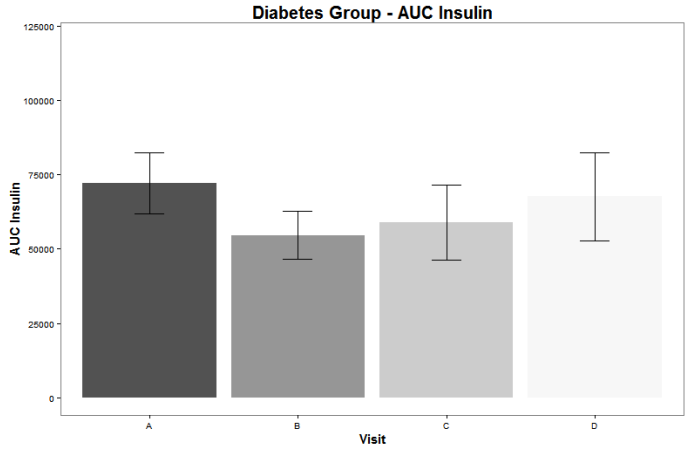
Visit D = 12 months after surgery

AUC Insulin – Calorie restriction group (n = 8)



AUC Insulin after RYGB





Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery

5.2.3 Changes in GLP1 response

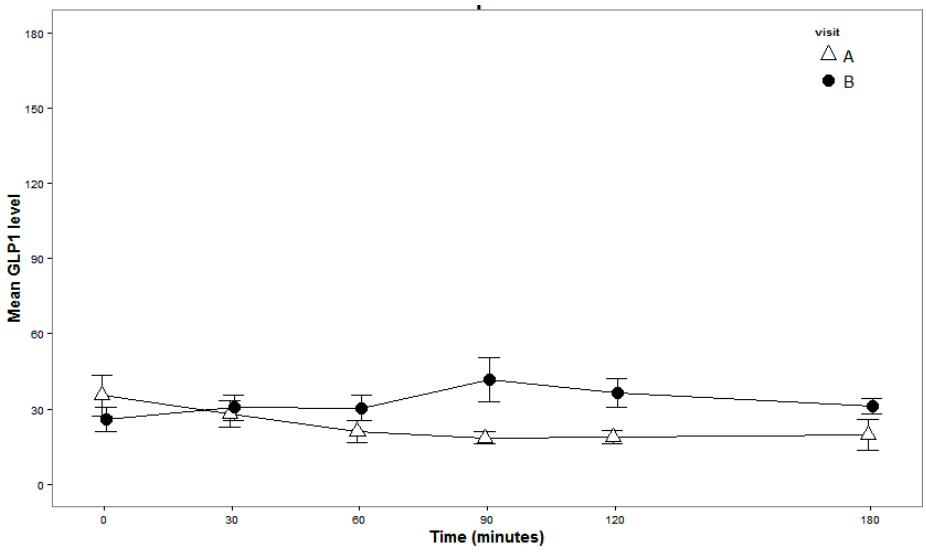
Fasting GLP1 levels did not change significantly in the calorie restricted group (p = 0.30). The AUC for GLP1 also did not change significantly (3988.6 vs 5700.6, p = 0.13). The GLP1 meal curve response was surprisingly flat in the calorie restriction group.

In the RYGB group (n=22), fasting GLP1 values fell from 28.1 ± 17.2 to 25.7 ± 16.7, p = 0. 24 at 2 weeks; 14.2 ± 12, p = 0.009 at 4 months and 13.1 ± 15.2, p = 0.01 at 12 months. In the diabetes subgroup the fasting GLP1 dropped from 25.7 ± 12.4 to 22.1 ± 14, p = 0.53 at 2 weeks; 15.5 ±12.2, p = 0.07 at 4 months and 10.1 ± 11.7, p = 0.007 at 12 months. In the non-diabetes group fasting GLP1 fell from 30.8 ± 21.7 to 30.2 ± 19.4, p = 0.95 at 2 weeks, 12.8 ± 12.4, p = 0.03 at 4 months and 15.8 ±17.9, p = 0.09 at 12 months.

In the RYGB group the AUC for GLP1 changed from 6056.2 to 12391.6 at 2 weeks (p = 0.0004); 6616.9 at 4 months (p = 0.64) and 6480.2 at 12 months (p = 0.70). In the diabetes and non-diabetes subgroup the AUC for GLP1 similarly rose significantly in the 2 week visit but there was no significant change at 4 and 12 months.

Change in mean GLP1 levels in response to 330 kcal liquid meal

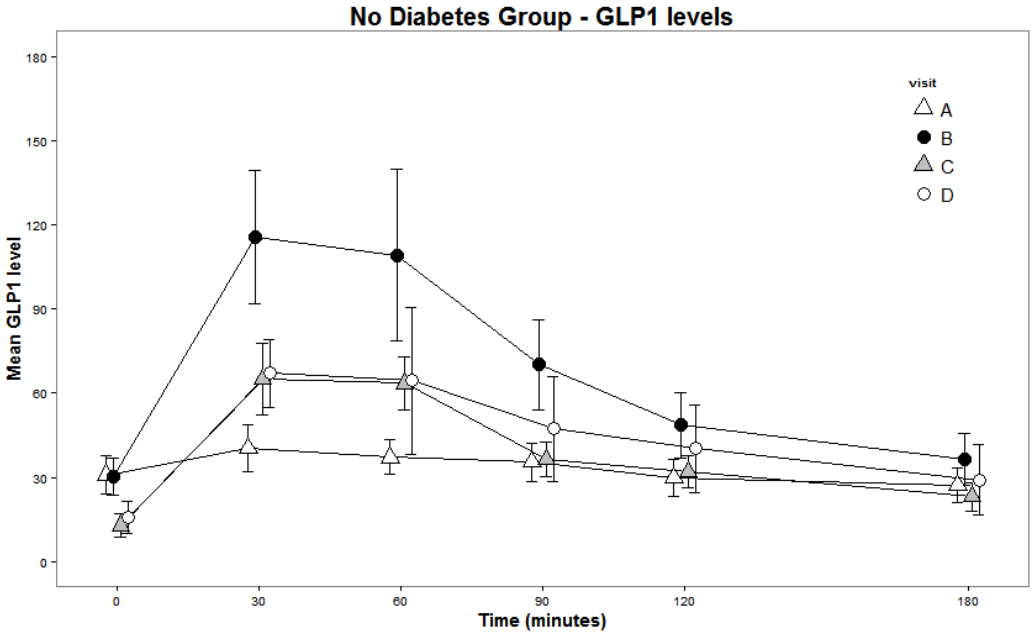
Calorie restriction group (n = 8)



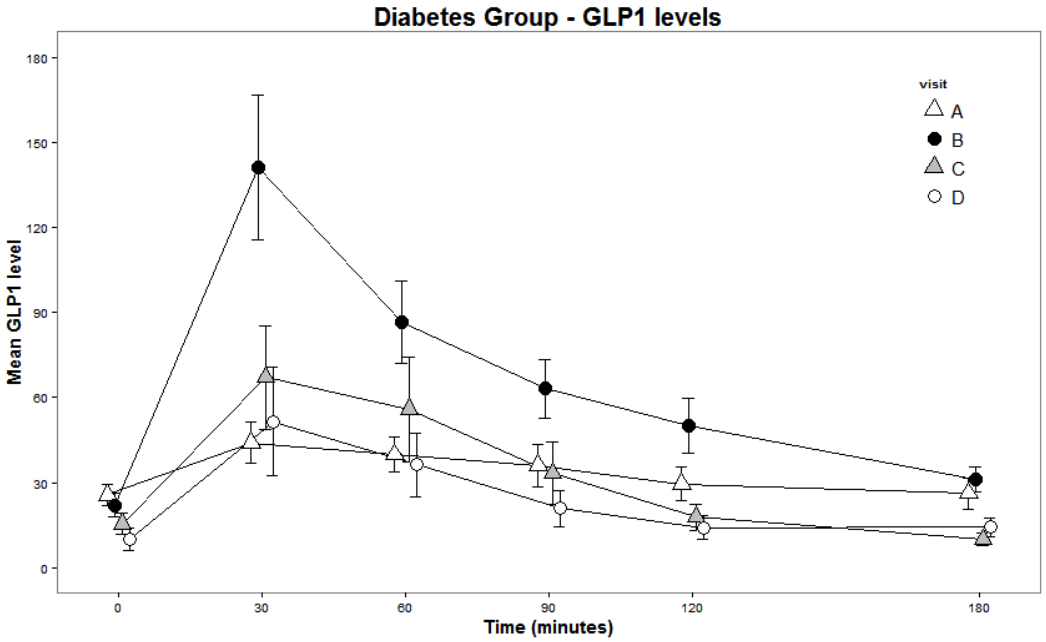
A = Baseline visit

B = after 4 weeks of calorie restriction

GLP1 response after RYGB – Non-diabetes group (n = 11)



Diabetes group (n = 11)



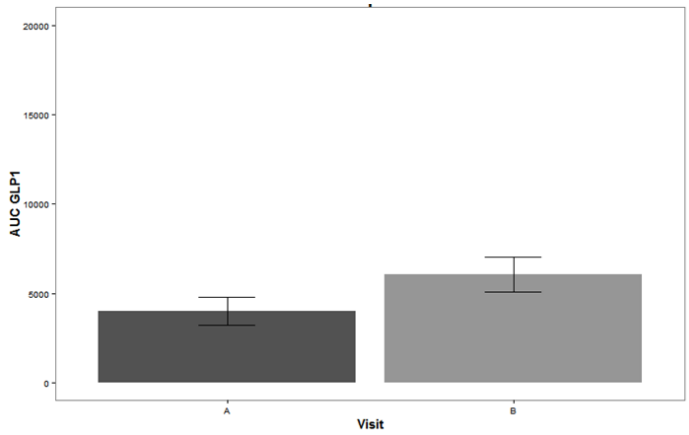
Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

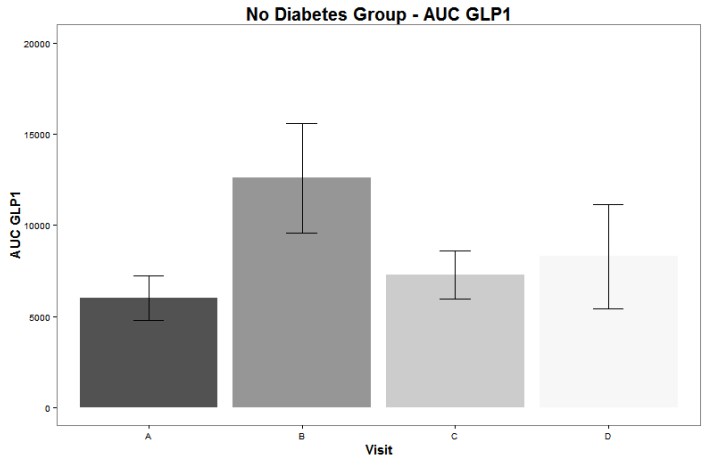
Visit C = 4 months after surgery

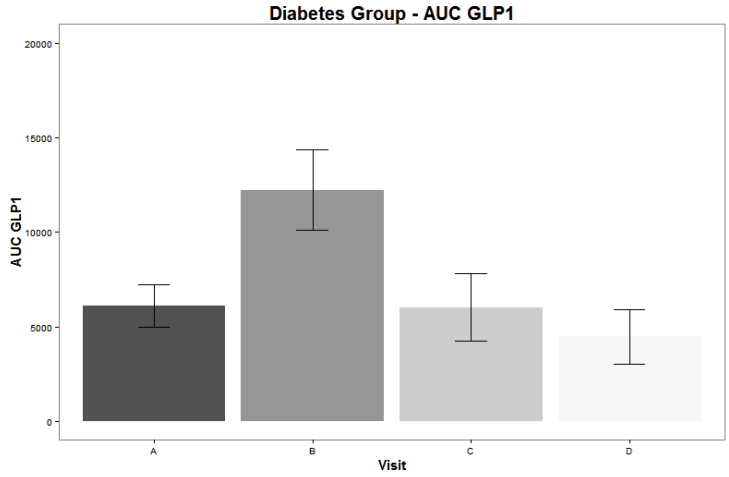
Visit D = 12 months after surgery

AUC GLP1 – calorie restriction group



AUC GLP1 after RYGB





Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

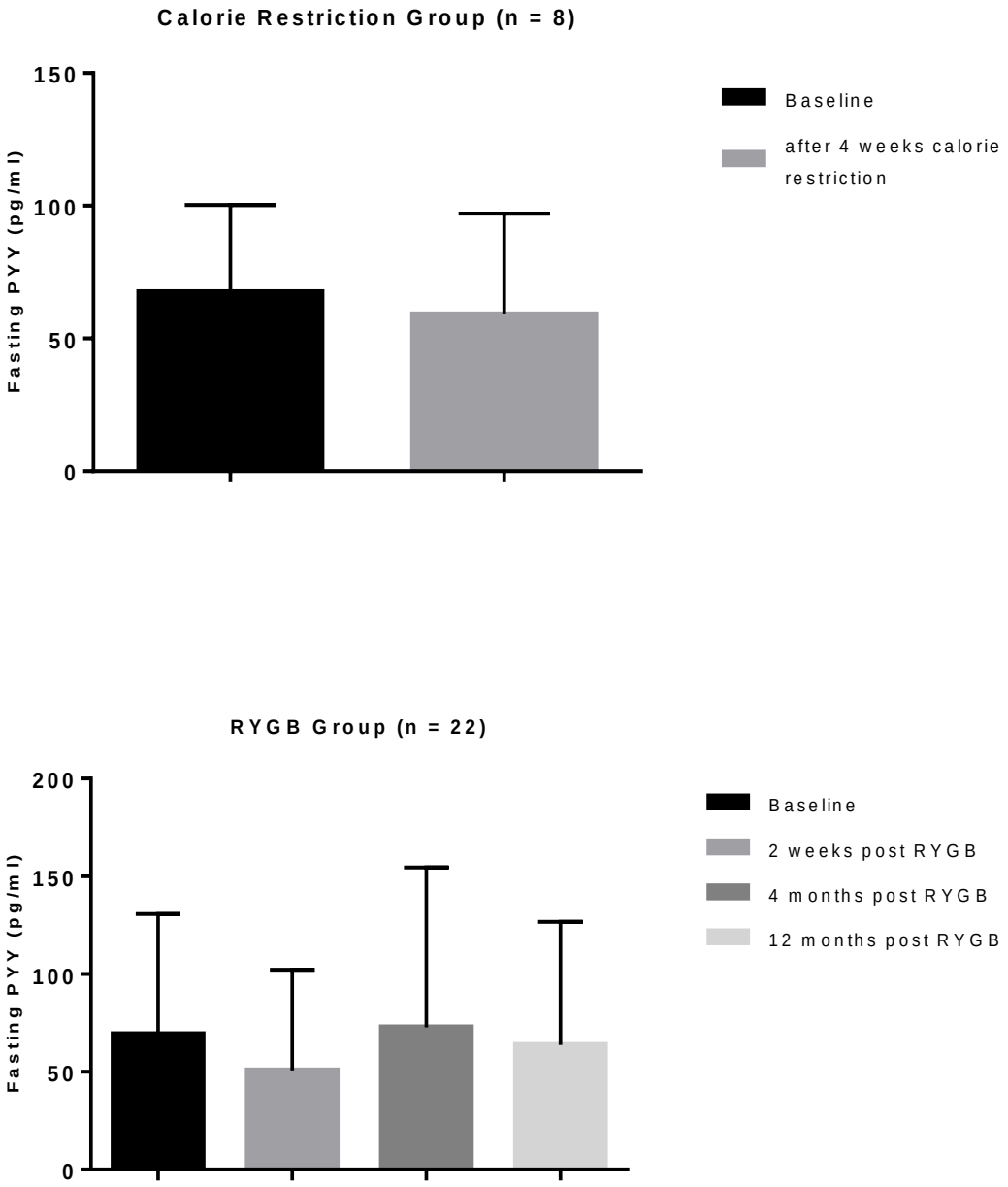
Visit C = 4 months after surgery

Visit D = 12 months after surgery

5.2.4 Changes in fasting PYY levels

Fasting PYY levels were not different between the groups and did not change significantly with calorie restriction (67.4 ± 32.9 to 59 ± 38.1, p = 0.64). In the RYGB group the changes in fasting PYY levels (69.3 ± 61.4) did not achieve significance at 2 weeks (50.8 ± 51.5, p = 0.29), 4 months (72.8 ± 81.7, p = 0.87) or at 12 months (63.8 ± 63, p = 0.77).

Changes in Fasting PYY



5.2.5 Changes in markers of insulin sensitivity and secretion

In the calorie restriction group the changes were as follows: HOMA2 IR 5.0 ± 2.3 to 4.2 ± 1.6, p = 0.43; HOMA2 %B 190.0 ± 82.2 to 167 ± 38.6, p = 0.46 and AUC Insulin/Glucose (pmol/mmol 180 minutes) 80.5 ± 51.2 to 100.6 ± 64.9, p = 0.20.

Changes in HOMA2 IR, HOMA %B and AUC Insulin/Glucose in the RYGB group and the diabetes & non-diabetes subgroups are shown in the table below. P values reflecting change from baseline are included in the table. The changes in other indices of insulin sensitivity and secretion are discussed in chapter 7.

HOMA2 IR

	Baseline	2 weeks after RYGB	4 months after RYGB	12 months after RYGB
RYGB Diabetes n= 11	9.7 (4.9)	4.8 (3) p = 0.01	3.4 (2.1) p = 0.0008	3.0 (1.6) p = 0.0003
RYGB non-diabetes n = 11	4.9 (2.5)	2.4 (1.7) p = 0.01	1.4 (0.9) p = 0.0003	1.7 (1.3) p = 0.001
RYGB n = 22	7.3 (4.5)	3.7 (2.7) p = 0.003	2.4 (1.9) p < 0.0001	2.3 (1.5) p < 0.0001

HOMA2 %B

	Baseline	2 weeks after RYGB	4 months after RYGB	12 months after RYGB
RYGB Diabetes n= 11	88.1 (43.7)	100.8 (31.3) p = 0.46	121.3 (52.4) p = 0.10	140.5 (46) p = 0.04
RYGB non-diabetes n = 11	125.5 (43.7)	105.1 (42.1) p = 0.10	86.7 (26) p = 0.01	110.9 (50.2) p = 0.21
RYGB n = 22	106.8 (46.8)	102.8 (36) p = 0.68	103.2 (43.4) p = 0.77	125 (49.5) p = 0.39

Values are expressed as mean (± SD) unless specified

AUC Insulin/Glucose (pmol/mmol 180 minutes)

	Baseline	2 weeks after RYGB	4 months after RYGB	12 months after RYGB
RYGB Diabetes n= 11	40.2 (21.8)	30.7 (16.4) p = 0.16	50.3 (22.3) p = 0.28	66.9 (28.7) p = 0.04
RYGB non-diabetes n = 11	60.9 (24.7)	56.3 (25.1) p = 0.46	56.5 (26.4) p = 0.58	81.8 (69.3) p = 0.41
RYGB n = 22	50.5 (25.1)	43.5 (24.5) p = 0.11	53.5 (24.1) p = 0.75	74.7 (53.2) p = 0.05

Values are expressed as mean (± SD) unless specified

5.2.6 Three Factor Eating Questionnaire (TFEQ) scores before and after RYGB

Group (n)	**TFEQ Domains**	**Visit A**	**Visit B**	**Visit C**	**Visit D**
Calorie restriction n = 8	Restraint	7.18 ± 3.9	7.42 ± 4.1 p = 0.90	-	-
	Disinhibition	9.32 ± 2.9	9.61 ± 3.3 p = 0.85	-	-
	Hunger	8.59 ± 2.6	8.71 ± 1.8 p = 0.91	-	-
Surgery n = 22	Restraint	7.52 ± 3.7	11.53 ± 3.8 p = 0.001	11.12 ± 4.4 p = 0.005	10.58 ± 4.2 p = 0.01
	Disinhibition	9.84 ± 5.1	5.23 ± 3.6 p = 0.001	5.19 ± 3.8 p = 0.003	6.1 ± 2.1 p = 0.003
	Hunger	8.32 ± 4.7	3.36 ± 3.7 p = 0.0004	3.72 ± 4.2 p = 0.001	4.4 ± 3.9 p = 0.004

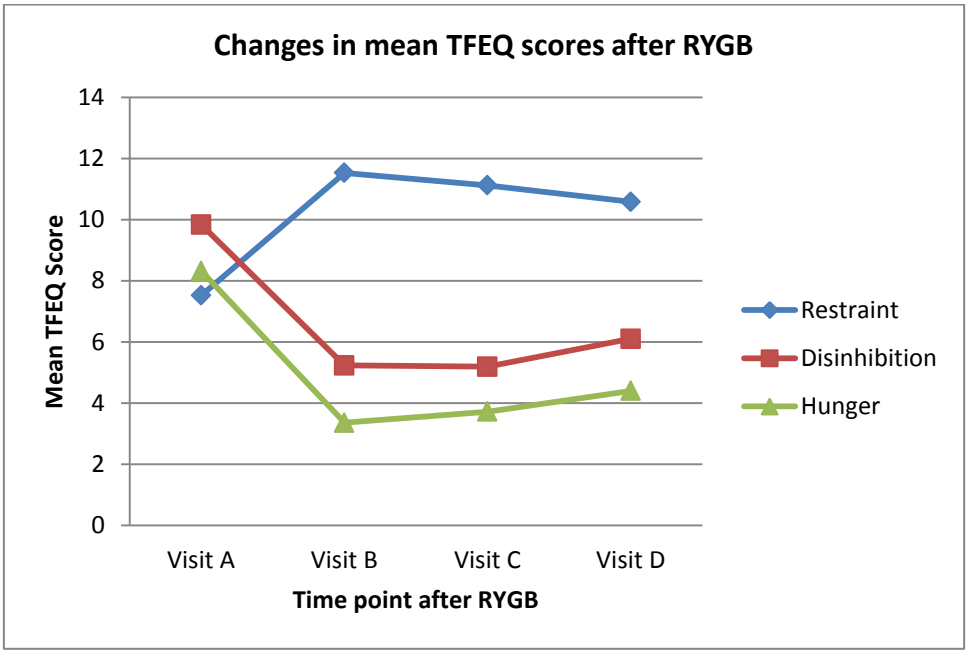
Data expressed as mean score ± standard deviation

Visit A = pre-surgery or pre-dietary intervention

Visit B = after 4 weeks of calorie restriction (or) 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery



Visit A = pre-surgery

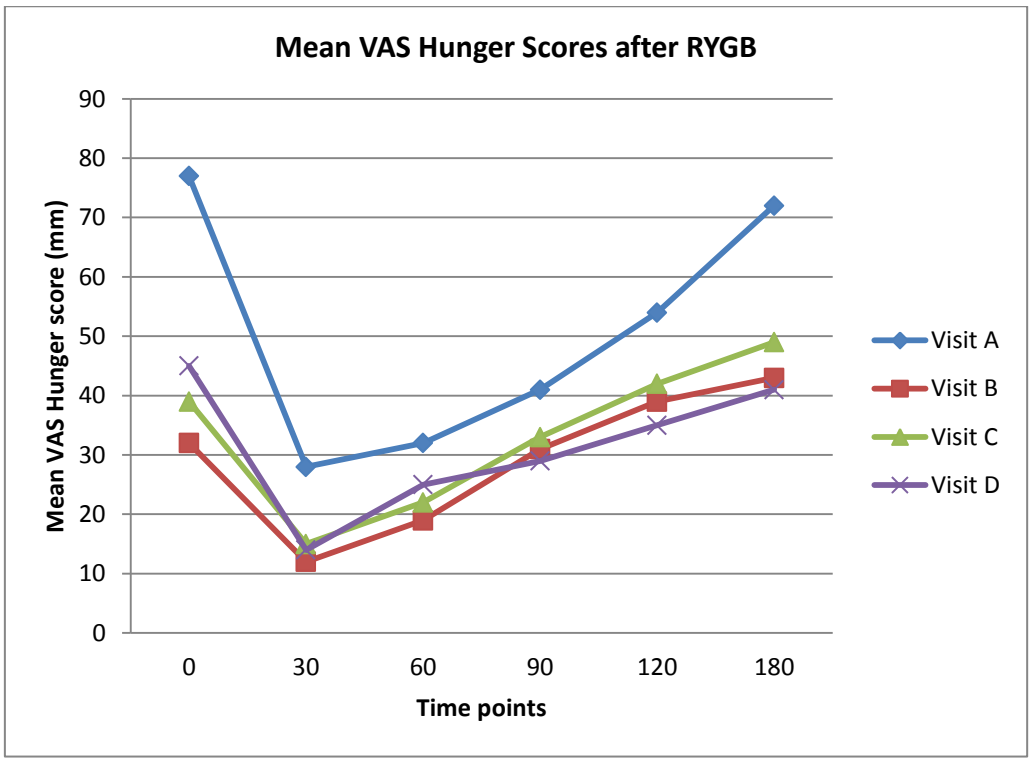
Visit B = 2 to 3 weeks after RYGB

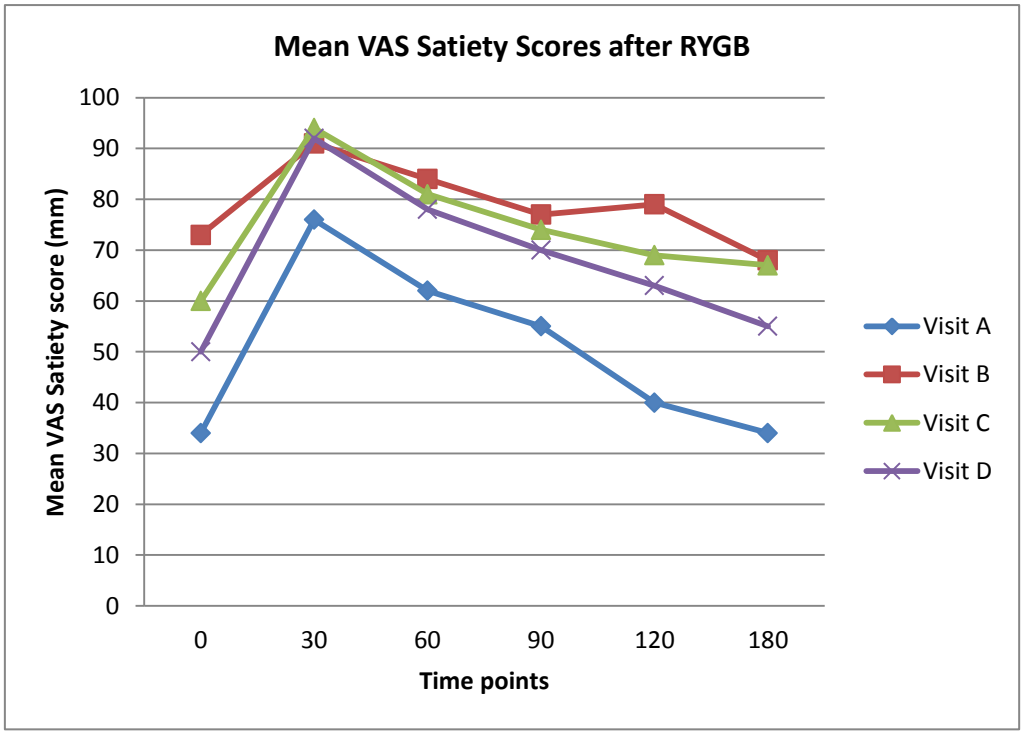
Visit C = 4 months after surgery

Visit D = 12 months after surgery

5.2.7 Changes in mean Hunger and Satiety VAS scores after RYGB (mm)

VAS score	Visit	0	30	60	90	120	180
Mean Hunger	**A**	77.2	28.5	32.3	41.8	54.3	72.7
	B	32.4	12.1	19.5	31.8	39.3	43.6
	C	39.3	15.4	22.2	33.1	42.2	49.4
	D	45.1	14.7	25.3	29.7	35.6	41.5
Mean Satiety	**A**	34.4	76.2	62.8	55.3	40.4	34.2
	B	73.2	91.5	84.3	77.8	79.3	68.2
	C	60.1	94.6	81.2	74.4	69.8	67.4
	D	50.3	92.3	78.7	70.2	63.6	55.5





Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery

5.3 Discussion

The main findings of this study was that there was a significant fall in the fasting glucose and fasting insulin values in both the diabetes and non-diabetes subjects who had RYGB. As expected the mean fasting glucose, fasting insulin and arear under the curve for glucose were significantly reduced within 2 weeks of RYGB when the weight loss was not significant. There was a progressive fall in the fasting glucose and insulin levels at 2 weeks, 4 months and 12 months. The area under the curve for glucose reduced significantly and progressively in both diabetes and non-diabetes groups. These results are consistent with the results from other similar studies.

Although the fasting insulin values fell significantly there was no reduction in the AUC for insulin in both the surgery subgroups. This is not surprising as the insulin response required in relation to the fixed calorie intake is lower due to the rapidly improving insulin sensitivity and insulin clearance. This has also been shown in other studies. The peak insulin response (I^{30} & I^{60}) values are significantly better in both the RYGB subgroups but the overall AUC did not change. The GLP1 response to meal closely mimicked the insulin response which confirms that opinion that the better insulin response to calorie intake is driven by improved incretin response. This has been shown in several studies as described before. Some studies have also shown these changes in GIP responses confirming that the overall incretin response to calorie intake improves dramatically after RYGB which is independent of weight loss.

We did not find any significant change in fasting PYY levels with calorie restriction or RYGB. This has been seen in other studies as discussed earlier. Korner et al showed that fasting PYY values do not change after RYGB but there is an early exaggerated PYY response to meal [128] mimicking the changes in GLP1 responses which may have an effect on insulin response and suppression of appetite post RYGB [146] leading to sustained weight loss in the long-term.

HOMA2 IR scores fell significantly 2 weeks after RYGB in both the diabetes and non-diabetes subjects. This may be driven by calorie restriction, changes in adipokines and gut hormones. There was a fall in HOMA2 IR scores in the calorie restriction group but the

changes did not reach significance. We may have noticed the true changes in insulin sensitivity in this group with more number of subjects or stricter calorie restriction. Likewise the changes in fasting glucose, fasting insulin and AUC glucose were not significant in the calorie restriction group but there was an overall trend towards improved glycaemia in this group.

There is very limited published data about the changes in eating behaviour after weight loss surgery. The SOS study showed significant improvement in mean scores of restraint and fall in disinhibition and hunger [146]. This was also seen in our surgery group. There was a dramatic fall in hunger as expected after RYGB which was maintained at 4 months and 12 months. There was also fall in disinhibition and improvement in restraint which were statistically significant. This shows that the reduction in hunger and disinhibition with improved restraint plays a significant part in long term weight loss and maintenance in the surgery group. This is likely to be due to the impact of surgery itself (restrictive effect) and the net effect of changes in appetite regulating hormones. VAS scores done serially during meal tests also confirm a progressive improvement in hunger and satiety scores as shown before.

Although HOMA2 IR is a reliable marker of insulin sensitivity and the changes in the study reflect the rapid improvement after RYGB there has been a lot of debate about the usefulness of the various markers of insulin sensitivity and secretion. Matsuda index (Composite index ISI) is an alternate marker of insulin sensitivity which is widely used in many studies [151]. It shows better correlation with insulin clamp studies ($r > 0.73$) when compared to HOMA alone. Whilst HOMA-IR score is measured using fasting glucose and insulin, the Matsuda index uses the insulin and glucose values following an oral glucose tolerance test (OGTT 0, 30 and 120 minute values).

It is much more difficult to find a marker of insulin secretion which correlated with insulin response to a test meal. We initially measured HOMA %B and AUC insulin/glucose values but these did not appear to reflect the improved insulin response seen after RYGB. As there is a rapid fall in insulin sensitivity after RYGB the insulin response to a fixed calorie intake is likely to be less and not reflective of the improved insulin response driven by better incretin response. Review of literature concurred with the notion that there is no

proven measure of insulin secretory capacity after weight loss surgery except an insulin clamp study which is not possible in large numbers of patients [153]. We therefore measured a variety of indicators to try and measure the improved insulin response to a meal in the background of falling insulin resistance. This is discussed in chapter 7.

Chapter 6

Changes in Adipokines and Inflammatory cytokines following Roux-en-Y Gastric Bypass for Morbid obesity

6.1 Role of Adipokines and Inflammatory cytokines in Obesity

The main role of adipose tissue, which is commonly called as 'body fat', was thought to be to store energy in the form of fat and to cushion and insulate the body. It constitutes between 10-30% of total body weight and is proportionately higher in overweight and obese individuals. In the last 2 decades, adipose tissue has been widely recognised as an important and active endocrine organ [132]. There are two types of adipose tissue: Brown Adipose tissue (BAT) and White adipose tissue (WAT). BAT, whose main role is to produce body heat, is thought to have negligible contribution to energy balance in adults.

WAT is thought to be metabolically active endocrine organ producing a number of biologically active products collectively called the adipokines (adipocytokines). The known adipokines secreted by WAT are listed below. WAT undergoes expansion with weight gain and develops throughout life in response to demands fasting, exercise and nutrient delivery. It is also richly innervated by autonomic nervous system which influences lipolysis. Adipokines can also send signals back to the central nervous system which are known to modulate thermogenesis and appetite regulation.

The location and distribution of adipose tissue also appears to have physiological and prognostic significance. Degree of intra-abdominal and visceral fat rather than subcutaneous fat deposits is thought to have a greater correlation with components of metabolic syndrome as described in chapter 1. Fain JN in a recent review described that nearly 37 circulating adipokines are known to be clinically significant [46]. The table below shows the adipokines and the changes in obesity. The roles of few of the adipokines have been widely reported in obesity and their changes after weight loss surgery are discussed in detail.

Major Adipokines secreted by WAT and their relationship to obesity [46]

	Adipokines
Elevated in obesity	FABP-4, IL-8, PAI-1, MCP-1, IL-6, Adipsin, Leptin, Amyloid A, Migration inhibitor factor, Cathepsin S, IL-1Ra, HGF, Haptoglobin, ICAM-1, ACE, IL-10, VEGF, VCAM-1, TNFα, TGF-β1, sTNF RII, NGF, CRP, IL-18
Decreased in obesity	Adiponectin, glutathione peroxidase 3 (GPX-3)
No change	Visfatin/PBEF/Nampt, CD14, ZAG, Lipocalin-2, RANTES, Osteoprotegerin, LPL, VEGFR/sFLT1, Resistin,
No data	PGE2, IL-1β

Adiponectin

Adiponectin is a 30-kDa polypeptide plasma protein exclusively expressed in WAT and found in higher expression in subcutaneous adipose tissue than visceral fat. It has been shown to lower insulin resistance and inflammation [193]. Adiponectin levels and its mRNA are decreased in obesity and insulin resistance and these improve with weight loss. Adiponectin levels have been reported to correlate inversely with BMI, percentage body fat and fasting insulin levels. It enhances the action of insulin in the liver and reduces hepatic glucose production [193]. It also induces the oxidation of fats, reducing TAG levels in liver and muscle. Its anti-inflammatory action is a result of decreased synthesis and action of TNF-α and IL-6, and induction of IL-10 production. Macrophage secretion of TNF-α and its metabolic effects are attenuated by adiponectin. Due to its anti-inflammatory and anti-diabetogenic effects adiponectin protects against vascular injury and progression of atherosclerosis [194]. Adiponectin exerts a protective role in the vascular wall through inhibition of monocyte adhesion and transformation of macrophages to foam cells by reducing the expression of adhesion molecules and scavenger receptors. Several studies, both in adults and children have shown significant increases in adiponectin levels proportionate to weight loss following both surgical and non-surgical treatments [133, 135]. However the increase in adiponectin levels tend to occur several weeks to months after weight loss surgery (in keeping with the degree of weight loss) much later than the rapid improvements seen in glucose and insulin homeostasis.

Leptin

Leptin is encoded from the 'Ob' gene which is expressed mostly in adipose tissue (mainly in WAT). Leptin is a non-glycosylated 16-kDa peptide with 146 amino acids. Lower levels are also thought to be expressed in the hypothalamus, pituitary, gastric epithelium, placenta, mammary glands and gonads. When body weight is steady reflecting a balance between expenditure and intake, leptin levels are an indicator of body fat mass. Leptin has been studied extensively and has been shown to be involved in the regulation of food intake and energy expenditure, storage of fat and insulin signalling. Leptin primarily regulates energy homeostasis by controlling satiety and body weight through three leptin-sensitive neurons found within the arcuate nucleus of the hypothalamus: neuropeptide Y (NPY), γ-aminobutyric acid (GABA), and proopiomelanocortin (POMC) neurons. Leptin crosses the blood-brain-barrier (BBB) and inhibits the orexigenic NPY and GABA neurons, whilst simultaneously stimulating the anorexigenic POMC neurons, thereby promoting the sensation of satiety and increasing energy expenditure [195, 196].

Weight loss, starvation and food deprivation reduce leptin levels and increase appetite and parasympathetic tone, preserving energy expenditure at the same time. Leptin has been related to appetite as well with studies showing that decreased leptin levels induced hunger and exogenous leptin reduced desire for food. For those with congenital leptin deficiency, a condition associated with extremely low levels of circulating leptin, extreme obesity, and severe hyperphagia, daily subcutaneous injections of leptin completely reversed the above phenotype [197]. This lead to the belief initially that leptin was likely to be an effective treatment of obesity by promoting satiety and energy expenditure, but most obese humans were found to be leptin resistant with very little weight loss response [198]. Nevertheless, a tendency to develop leptin resistance through chronic overfeeding is demonstrated by high leptin levels in obese humans and animals.

In addition to its role in energy homeostasis, leptin is able to modulate the immune system due to its structural similarity to cytokines and its class I cytokine receptors being found on immune cells such as monocytes, lymphocytes, and neutrophils. The chronic inflammatory state observed in obesity is attributed to the elevated leptin levels through up-regulation of phagocytosis by macrophages, promotion of T-helper 1 cell responses, and mediating the release of further pro-inflammatory cytokines such as TNF-α and IL-6.

Weight loss surgery has been shown to decrease leptin levels in many studies [125, 135] but patients do not display many of the characteristic responses associated with low levels of leptin. For example, patients receiving weight loss surgery actually have decreased hunger and increased satiety. However, most studies find a correlation with body fat, body weight or body mass index (BMI) after surgery [133]. Thus, it has been hypothesised that leptin sensitivity improves after weight loss surgery. The direct effect of leptin decrease immediately after WLS on glucose homeostasis is not fully explained. In the long term improved leptin sensitivity related to weight loss might have a significant role.

Tumour Necrosis Factor-alpha and TNF receptors

Tumour necrosis factor-alpha (TNF-α) is a 26-kDa transmembrane protein with an active 17-kDa polypeptide form. It is predominantly known for its role in inflammation [199]. Its possible involvement in energy homeostasis was suspected after identifying its ability to induce cachexia in vivo. Although TNF-α was implicated in the pathogenesis of cachexia initially, it became apparent that TNF-α was positively correlated with obesity with expression in adipocytes. The manner in which TNF-α exerts its negative metabolic effects is not fully understood. One mechanism involves modulation of gene expression within metabolically active adipose and liver tissue [200]. Within adipose tissue, TNF-α dampens the influx and storage of glucose and NEFAs, represses genes implicated in the formation of both lipids and adipocytes, and alters the expression of adipokines such as adiponectin and IL-6. Similarly, TNF-α represses the genes regulating glucose utilization and metabolism at the liver, but also those involved in oxidation of FFAs. However, TNF-α simultaneously increases the synthesis of cholesterol and fatty acids at the liver. Another mechanism involves impairment of insulin signalling at both the skeletal tissue and the adipocyte level [201].

In addition TNF-α is central to the chronic inflammatory state present in obesity. TNF-α activates NF-κB which mediates transcription of numerous genes linked to the inflammatory process. TNF-α has also been shown to increase the rate of atherosclerosis by inducing the expression of adhesion molecules in both endothelial cells and smooth muscle cells in the vascular wall. With elevated levels of TNF-α being shown to cause a spectrum of negative effects, it was proposed that neutralization or deletion of genes

coding for TNF-α receptors could possibly improve both adiposity and insulin resistance. Although such effects have been demonstrated in obese rodents, clinical trials in obese and/or insulin resistant humans have proven inconclusive.

TNF-receptors (TNF-R) were originally designated as TNF-binding proteins. These TNF-binding proteins present in urine and plasma were later found to be the extra-cellular part of the membrane bound cell signalling receptor for TNF-alpha and TNF-beta. Two types of TNF-receptors (molecules belonging to the family of nerve growth receptor CD27, CD40 etc.) have been identified: TNF-RI and TNF-RII. The molecules share substantial identity in their extracellular part although the intra-cellular parts are structurally unrelated. This difference is reflected by the difference in function of the two receptors. Activation of various cell types leads to proteolytic cleavage of TNF-R resulting in soluble (extracellular part) TNF-R. Soluble TNF-R (sTNF-R) is considered to play a physiological role in TNF binding and inactivation, although also other functions such as stabilization of TNF in circulation have been mentioned. Detection of sTNF-R offers a means of investigating activation of the immune system both in vitro and in vivo [202]. Recent studies have favoured measuring the sTNF-α RII in plasma as a measure of overall TNF-α activity in relation to inflammation [202, 203].

Interleukin-6

Interleukin-6 (IL-6) is a 185 amino acid protein known for its ability to potentiate and modulate the immune system. However 15 to 35% of circulating IL-6 arising from adipose tissue and with IL-6 being expressed by both adipocytes and the stromovascular matrix of visceral WAT, studies has shown that the secretion and expression of IL-6 are directly proportional to the degree of obesity, glucose intolerance, and insulin resistance [204]. With levels of circulating IL-6 increasing with weight gain and decreasing upon weight loss, assessment of genetic variations in the IL-6 gene has shown that it is linked to both indices of obesity and homeostatic regulation of glucose and insulin [205]. The reports of the effects of IL-6 on hepatic insulin resistance have also been inconsistent, with IL-6 being implicated in both improvements as well as worsening of hepatic insulin resistance. On the other hand, both in vitro studies and in vivo human studies have shown that IL-6 enhances insulin mediated glucose uptake in the skeletal muscle. Peripherally, IL-6 impairs insulin

signalling by dampening the expression of insulin receptor substrate 1 (IRS-1) and inhibits insulin signalling by inducing the expression of SOCS-3. Also there is a negative correlation between body weight and levels of central IL-6. Indeed, the central deficiency in IL-6 may result from a possible underexposure of IL-6 to regions within the brain known to regulate body weight. Chronic exposure of adipocytes to IL-6 has been shown to enhance insulin resistance. Thus the effect of IL-6 on insulin resistance is dependent on the type of target tissue and the duration of exposure. Additionally, IL-6 inhibits the expression of adiponectin which negates any beneficial effects of this adipokine. IL-6 levels are shown to fall after weight loss surgery [133].

hs-CRP

High sensitivity C-reactive protein (hs-CRP) is thought to be mainly produced by the liver but may also be synthesized by the adipose tissue. Its physiological function is to bind to the phosphatidylcholine on the surface of the dead cells and bacteria in order activate the compliment system. It is a 224 amino acid, 25 kDa protein. Both IL-6 and TNF-α can induce production of CRP in the liver, and IL-6 in particular has been shown to be an independent predictor of CRP levels. At the molecular level, IL-6 has been shown to induce CRP gene transcription. Obesity, in particular increased intra-abdominal fat, has been shown to be associated with increased CRP levels and weight loss causes reduction in levels [138]. Dietary methods of weight loss have been shown to decrease CRP levels; this has been shown to be related more to the degree of weight loss than the macronutrient composition. A prospective study involving 66 patients reported that patients with greater baseline insulin sensitivity had a greater decrease in CRP after gastric bypass, independently of the change in body weight [206]. This direct relation between CRP and insulin sensitivity is supported by several non-surgical studies [207].

Objectives

To assess longitudinal changes in serum adiponectin, leptin, IL6, TNF-α and hs-CRP levels in morbidly obese subjects who undergo weight loss surgery in relation to weight and fat loss achieved.

Patients and methods

The selection of patients who underwent RYGB for morbid obesity has been discussed in chapter 2. A total of 30 subjects were studied for changes in adipokines. 11 patients who were known to have type 2 diabetes before WLS, 11 patients who did not have diabetes and 8 patients who followed a calorie restricted diet for 4 weeks were included. As described before, the surgical group were studied at baseline (pre-surgery before pre-op diet), 2 weeks, 4 months and 12 months after RYGB. The calorie restriction group were studied before intervention and 4 weeks after following a 1200 kcal/day diet.

Study visit protocol is described in chapter 2.

Biochemical assays

Plasma glucose and insulin were measured at baseline and at 30, 60, 90, 120 and 180 minutes following a standard liquid meal of 330 kcals. Adipokines were measured from the fasting sample at each study visit.

The assays used to measure the adipokines are described in chapter 2.

Statistical analysis

Data was checked for normality using the Shapiro-Wilk test. Comparison between groups were analysed using the Student's t test or Wilcoxon signed rank test as appropriate. All results for adipokines were not normally distributed and therefore they were log transformed before analysis. The trapezoid method was used to measure 'Area under the curve' (AUC) for glucose and insulin values. Statistical calculations were made by analysis of variance (ANOVA) for repeated measures. Data is presented as mean (+/- 1 SD) unless specified. Two sided P value of less than 0.05 was considered significant.

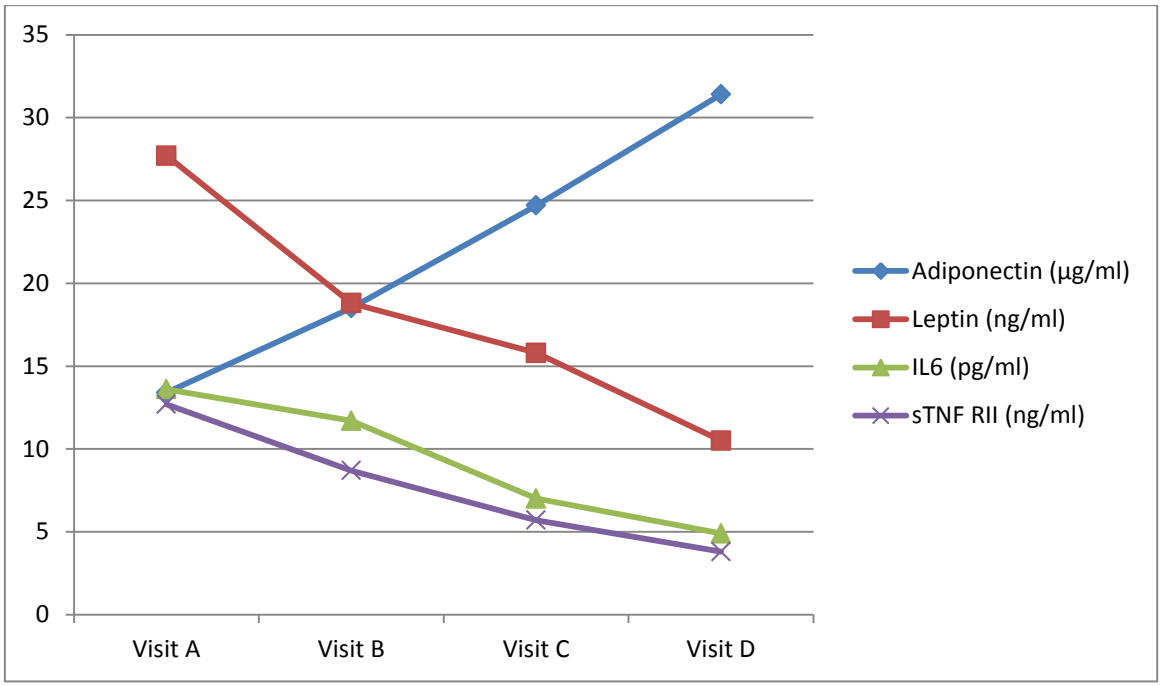
6.2 Results

The baseline characteristics of study subjects and their weight-related outcomes are discussed in chapter 4. Changes in glucose tolerance, insulin response curves to fixed calorie intake and HOMA2 scores are discussed in chapter 5.

Serial changes in fasting adiponectin, leptin, IL6, sTNF RII and hs-CRP are shown in Table 6.1 and figure 6.2. Values are expressed as mean (± SD) unless specified.

Group	Mean value (± 1 SD)	Visit 1 Baseline	Visit 2 2 weeks after surgery (or) after 4 weeks calorie restriction	Visit 3 4 months after RYGB	Visit 4 12 months after RYGB
Calorie restriction (n=8)	Adiponectin (µg/ml)	21.6 (13.3)	30 (16.7)	-	-
	Leptin (ng/ml)	28.3 (16.6)	23.7 (13.9)	-	-
	IL6 (pg/ml)	5.7 (3.6)	4.5 (3.4)	-	-
	sTNF RII (ng/ml)	3.7 (1.1)	3.3 (1.1)	-	-
	hs-CRP (µg/ml)	71.6 (89.6)	37.4 (50.2)	-	-
RYGB Diabetes (n=11)	Adiponectin (µg/ml)	13.5 (9.2)	20.1 (10.5)	26.5 (11.7)	35.1 (14.5)
	Leptin (ng/ml)	22.4 (11)	17.9 (7.6)	14.6 (6.1)	11.6 (4.2)
	IL6 (pg/ml)	11.8 (13.8)	10.5 (10.8)	7.5 (9)	5.6 (7.4)
	sTNF RII (ng/ml)	8.8 (4.3)	7.8 (2.7)	4.8 (1.1)	3.6 (1)
	hs-CRP (µg/ml)	115.9 (100)	88.7 (94.4)	13.7 (7.4)	5.6 (3.7)
RYGB Non-diabetes (n=11)	Adiponectin (µg/ml)	13.2 (7.8)	16.8 (11.7)	22.9 (13.4)	28 (13.8)
	Leptin (ng/ml)	33.6 (14.7)	19.9 (6.8)	17 (11.5)	9.7 (6.2)
	IL6 (pg/ml)	15.6 (9.8)	13 (9)	6.5 (5.8)	4.3 (5.7)
	sTNF RII (ng/ml)	16.7 (23.7)	9.6 (7.5)	6.5 (4.8)	4 (2.5)
	hs-CRP (µg/ml)	155.3 9237)	93.9 (173.2)	73 (141.7)	21.5 (47.2)
Total RYGB (n = 22)	Adiponectin (µg/ml)	13.4 (8.4)	18.5 (10.9)	24.7 (12.4)	31.4 (14.2)
	Leptin (ng/ml)	27.7 (13.8)	18.8 (7.1)	15.8 (9.1)	10.5 (5.3)
	IL6 (pg/ml)	13.6 (12)	11.7 (9.8)	7 (7.4)	4.9 (6.4)
	sTNF RII (ng/ml)	12.7 (17.1)	8.7 (5.6)	5.7 (3.6)	3.8 (2)
	hs-CRP (µg/ml)	134.7 (175.1)	91.3 (136.2)	43.3 (102.3)	14.3 (35.3)

Changes in Adipokines following RYGB (n = 22)



Visit A = pre-surgery

Visit B = 2 to 3 weeks after RYGB

Visit C = 4 months after surgery

Visit D = 12 months after surgery

6.2.1 Adiponectin (µg/ml)

Fasting adiponectin levels at baseline were lower in the surgery group when compared to the calorie restricted group (13.4 ± 8.4 vs 21.6 ±13.3, p = 0.054) in keeping with their higher BMI at baseline. There was no statistical difference in the fasting adiponectin levels between the diabetes and non-diabetes RYGB groups (13.5 ± 9.2 vs 13.2 ± 7.8. p = 0.94). There was a modest increase in the fasting adiponectin levels after 4 weeks of calorie restriction but this did not reach statistical significance (p = 0.28).

In the surgery group there was progressive increase in the adiponectin levels which correlated inversely with their weight loss: at 2 weeks post-surgery 18.5 ± 10.9, p = 0.09; at 4 months 24.7 ± 12.4, p = 0.001 and at 12 months 31.4 ± 14.2, p < 0.001. The increase in adiponectin levels after surgery reached statistical significance at 4 months and 12 months when compared to baseline values.

6.2.2 Leptin (ng/ml)

There was no significant difference in the fasting leptin levels between the calorie and surgery groups at baseline (28.3 ± 16.6 vs 27.7 ± 13.8, p = 0.92) or the diabetes and non-diabetes subgroups (22.4 ± 11 vs 33.6 ± 14.7, p = 0.06).

Leptin levels fell to 23.7 ± 13.9 after 4 weeks of calorie restriction which was not significant (p = 0.56). There was significant and gradual decline in the leptin levels with surgery when compared to baseline: at 2 weeks 18.8 ± 7.1, p = 0.01; at 4 months 15.8 ± 9.1, p = 0.002 and at 12 months 10.5 ± 5.3, p < 0.001.

6.2.3 IL-6 (pg/ml), hs-CRP (µg/ml) and sTNF RII (ng/ml)

There was a slight fall in the levels of IL-6, hs-CRP and sTNF RII levels after calorie restriction but these did not reach significance: IL-6 (5.7 ± 3.6 to 4.5 ± 3.4, p = 0.50); hs-CRP (71.6 ± 89.6 to 37.4 ± 50.2, p = 0.36) and sTNF RII (3.7 ± 1.1 to 3.3 ± 1.1, p = 0.48).

There were significant decreases in IL-6 levels post-RYGB at 4 and 12 months but not at 2 weeks: 2 weeks 11.7 ± 9.8, p = 0.57; at 4 months 7 ± 7.4, p = 0.03 and 4 months 4.9 ± 6.4, p = 0.004. Similarly hs-CRP levels fell to 91.3 ± 136.2, p = 0.36 at 2 weeks; 43.3 ± 102.3, p = 0.04 at 4 months and 14.3 ± 35.3, p = 0.003 at 12 months. Decreases in sTNF RII levels were gradually progressive but statistically significant only at 12 months: at 2 weeks 8.7 ± 5.6, p = 0.30; at 4 months 5.7 ± 3.6, p = 0.07 and at 12 months 3.8 ± 2, p = 0.02.

There were no statistically significant differences between the baseline values or the fall in the levels of IL-6, hs-CRP or sTNF RII between the diabetes and non-diabetes sub-groups (Table 6.1).

6.3 Discussion

The main findings of this study were that the adipokines levels changed favourably after calorie restriction and RYGB. The adiponectin levels increased with weight loss after RYGB and there was progressive fall in the levels of leptin, IL-6, sTNF RII and hs-CRP levels. These correlated with weight loss, reduction in fat mass and waist circumference. The changes in adipokines were not significant in the first few weeks after RYGB but significant after there is weight loss. Several studies have shown that the adiponectin levels increase after RYGB in proportion to weight loss. Fall in Leptin and makers of inflammation have also been shown after RYGB [133, 135, 138]. There is also evidence to support the view that these have a positive impact on glucose metabolism through improved insulin sensitivity but there is limited evidence to assess their effect on insulin secretion.

Fasting adiponectin levels rose at 2 weeks after RYGB but this was not significant. The rise at 4 months and 12 months after RYGB was significant. This mirrors the weight loss achieved in this group which was not significant at 2 weeks but significant at 4 months and 12 months. Fall in Leptin levels and the inflammatory markers IL6, hs-CRP and soluble TNF RII receptors also were more significant few months after surgery. All these changes reflect a gradual decline in the state of chronic inflammation which appears proportionate to the degree of weight loss. This reduction in systemic inflammation could explain many of the improvements seen in cardio-vascular outcomes after weight loss surgery. There is currently insufficient data to directly attribute these changes to the improvements seen in insulin production but there is plenty of evidence to suggest that they are the main drivers for improvements in insulin sensitivity.

A number of studies have reported significant rise in adiponectin, fall in leptin, hs-CRP and IL6 but the changes in TNF levels in adult human studies have been modest. In this study we measured sTNF RII as a marker of TNF activity. Although there was a gradual fall in the sTNF RII levels after RYGB, this only reached significance at 12 months. Presence of type 2 diabetes pre-RYGB did not appear to have any effect on these changes. Both diabetes and non-diabetes subgroups had comparable results at all the time points.

The changes in adipokines are therefore thought to be the main drivers of improved insulin sensitivity and improved appetite regulation after RYGB but their direct or indirect effect on improved insulin secretion remains difficult to measure or explain.

Chapter 7

Mechanisms underlying Diabetes remission following RYGB

7.1 Summary of results and discussion

Obesity and type 2 diabetes are closely interlinked both epidemiologically and in their patho-physiology. People with type 2 diabetes are more likely to be obese and the increased risk of type 2 diabetes in morbidly obese population is well established. Weight loss, even modest amounts, has been shown to have significant improvements in glycaemia and calorie restriction can cause dramatic changes in glucose regulation without significant weight loss. Older treatments for type 2 diabetes were prone to cause weight gain which sometimes can be considered counter-productive. Fortunately all the newer treatment options for type 2 diabetes are weight neutral (e.g. DPP IV inhibitors) or cause weight loss (e.g. GLP1 analogues, SGLT2 inhibitors). Weight loss surgery not only causes durable weight loss in the morbidly obese subjects but also leads to remission or improvement of several obesity-related comorbidities and complications. Diabetes remission and improvement in cardiovascular risk factors are some of the most beneficial results of weight loss through surgery. A number of mechanisms have been shown to contribute to diabetes remission both through weight-dependent and weight-independent changes. This study, although not exhaustive, aims to understand these mechanisms in a bit more detail to understand the complex changes that lead to diabetes remission or improvement in a majority of subjects who undergo weight loss surgery.

Diabetes remission

Diabetes remission in older studies was not clearly defined and the assumptions of the diabetes remission rate were often an over-estimate. Using the ADA consensus definition has given us a benchmark and most newer studies now provide a clearer picture about the likelihood of diabetes remission. The retrospective analysis of the Aintree database shows that the diabetes remission rates after weight loss surgery is as follows: LAGB 15%; RYGB 42.2%; SG 33.3% AND LDS 62%. The overall diabetes remission rate for all forms of weight loss surgery is 41.6%. This is similar to the rates noted in other studies.

In terms of weight loss, malabsorptive procedures cause the most weight loss followed by mixed (restrictive + malabsorptive) procedures. SG although mainly restrictive appears to

be nearly as effective as RYGB and much better than LAGB. It is reassuring to note that the patients with type 2 diabetes have similar weight loss outcomes to non-diabetes subjects in the longer term. The weight loss in patients with diabetes in the initial year or two is relatively less but there is no difference at 2 years and further. Blood pressure and lipid profile also improve after weight loss surgery and a majority of patients are able to discontinue their treatments safely. Weight loss is accompanied by favourable changes in body composition as noted by gradual reduction in waist circumference and fat mass.

Changes in glucose homeostasis

In both patients with diabetes and non-diabetes, there is a rapid and significant fall in fasting glucose and insulin after weight loss surgery. These changes are seen at 2 weeks before meaningful weight loss has occurred. The glucose tolerance improves progressively as seen by gradual fall in the AUC of glucose at 2 weeks, 4 months and 12 months. This is likely to be due to combination of improved insulin sensitivity and better early and rapid insulin response.

7.2 Markers of Insulin sensitivity

HOMA2 IR scores fall progressively after weight loss surgery both in the calorie restricted and the RYGB subjects. This change is seen at 2 weeks after RYGB before weight loss is significant. We wanted to confirm this observation by measuring the Matsuda (Composite) index of insulin sensitivity (ISI) as well. This is discussed later in this chapter. The fall in HOMA2 IR scores are significant at 2 weeks confirming the suspicion that there are factors other than weight loss and changes in adipokines that contribute to this. The exact reasons for this rapid improvement in insulin sensitivity are still unclear but it is suspected to be due to calorie restriction. Unfortunately we were unable to confirm this hypothesis in our study as the control subjects did not achieve significant changes in weight or glucose tolerance.

7.3 Markers of Insulin secretion

Fasting insulin levels fall after calorie restriction and RYGB probably due to improved insulin sensitivity. But the AUC for insulin did not show a significant change after RYGB. This has been noted in other studies. It is however noticeable that the peak insulin response improves significantly after RYGB. This is most probably due to improved incretin response as shown by the similar improvement in GLP1 response in this study. We did not measure Ghrelin, Oxyntomodulin, GIP and post-prandial PYY responses which have been shown to contribute to the observed improvement in insulin response.

We measured HOMA2 %B and AUC Insulin/Glucose to assess the changes in insulin secretion. As discussed before the HOMA %B scores and the AUC Insulin/Glucose measurements did not reflect the changes in early insulin response seen after RYGB. Moreover there is a rapid improvement in insulin sensitivity and improvement glucose clearance after RYGB which makes the validity of these measurements inaccurate. Review of literature shows that there is no agreed marker that has been shown to be reliable in measuring the insulin secretory capacity after weight loss surgery. The only reliable way of measuring insulin secretion is by performing insulin clamp studies. There are many unproven markers that have been compared to the gold standard by using oral glucose tolerance values. None of these studies are however in post-bariatric patients. We chose Insulinogenic index and Disposition index for further analysis as they had the most likelihood of measuring the changes in early insulin response after RYGB.

Matsuda index (ISI – Composite index)

	Baseline	**2 weeks after RYGB**	**4 months after RYGB**	**12 months after RYGB**
Calorie restriction n = 8	3.2 (1)	4 weeks after diet 3 (1) p = 0.69	-	-
RYGB Diabetes n= 11	1.6 (0.5)	3.1 (1.4) p = 0.003	3.9 (1.8) p = 0.0006	4.1 (1.9) p = 0.0004
RYGB non-diabetes n = 11	3.1 (1.8)	4.9 (2.7) p = 0.08	6.3 (3) p = 0.007	6.8 (3.9) p = 0.009
RYGB n = 22	2.3 (1.5)	4 (2.2) p = 0.005	5.2 (2.7) p < 0.0001	5.5 (3.3) p = 0.0002

Values are expressed as mean (± SD) unless specified

Insulinogenic index (IGI)

	Baseline	**2 weeks after RYGB**	**4 months after RYGB**	**12 months after RYGB**
Calorie restriction n = 8	2.4 (2.3)	4 weeks after diet 2.2 (1.7)	-	-
RYGB Diabetes n= 11	3.2 (6.2)	1 (0.5)	1.3 (0.7)	1.5 (0.9)
RYGB non-diabetes n = 11	2.2 (1.9)	2.7 (1.9)	2 (1.5)	3.9 (4.5)
RYGB n = 22	2.7 (4.5)	1.8 (1.6)	1.7 (1.2)	2.7 (3.4)

Disposition index (DI = ISI x IGI)

	Baseline	2 weeks after RYGB	4 months after RYGB	12 months after RYGB
Calorie restriction n = 8	6.6 (4.9)	4 weeks after diet 6.3 (5.7)	-	-
RYGB Diabetes n= 11	6.6 (15.2)	2.8 (1.6)	4.6 (2.4)	5.8 (3.6)
RYGB non-diabetes n = 11	7.6 (9.8)	13.2 (11.7)	13.3 (15.3)	36.4 (73.6)
RYGB n = 22	7.1 (12.5)	7.8 (9.5) p = 0.84	9.1 (11.8) p = 0.59	21.1 (53.1) p = 0.24

Values are expressed as mean (± SD) unless specified

Masuda index which is a reliable marker of insulin sensitivity [151] rose significantly in both the diabetes and non-diabetes subjects following RYGB. This correlated well with the fall in the HOMA2 IR score. This confirms that insulin sensitivity improves within 2 weeks of RYGB and is progressively better at 4 months and 12 months. Unfortunately neither the Insulinogenic index nor the Disposition index showed any significant changes in insulin secretion after RYGB. We therefore measured the peak insulin response which is shown to be a marker of early insulin response to a fixed oral calorie intake. The early insulin response for the calorie restriction group and the RYGB group is shown in the table below. This shows that the peak insulin response within oral consumption of a 330 kcal liquid meal improves within 2 weeks of RYGB and the response is maintained at 4 months and 12 months.

Early insulin response (ΔInsulin 30 - ΔInsulin 0)

	Baseline	2 weeks after RYGB	4 months after RYGB	12 months after RYGB
Calorie restriction n = 8	410.4 (289.9)	4 weeks after diet 489.1 (480.5) p = 0.69	-	-
RYGB n = 22	323.7 (269.9)	597.7 (463.2) p = 0.02	685.1 (581.1) p = 0.01	823.8 (935.8) p = 0.02

Values are expressed as mean (± SD) unless specified

7.4 Mechanisms underlying Diabetes remission following RYGB

2 weeks after RYGB

The weight loss after RYGB did not reach significance at 2 weeks. However there were significant changes to glycaemia. 2 weeks after RYGB the following changes occur: fasting glucose and AUC for glucose are significantly reduced; early insulin response is better following oral calorie intake; insulin sensitivity improves shown by a fall in HOMA2 IR and rise in Matsuda index; fasting GLP1 falls insignificantly but the AUC for GLP1 improves significantly. The rise in GLP1 following calorie intake mirrors the insulin response. Fasting PYY does not change significantly. Hunger and disinhibition falls and restraint improves as measured by TFEQ. Hunger scores are lower and satiety improves significantly. Leptin levels fell significantly but the rise in adiponectin or the fall in IL6, TNF-α & hs-CRP did not reach significance.

It appears that the improvement in glucose tolerance at this stage is largely weight independent. Insulin secretion defined as peak insulin response to oral calorie intake improves probably related to the improved GLP1 response. Insulin sensitivity improves soon after RYGB and this is not explained by changes in adipokines. Although leptin levels are thought to be directly proportionate to fat mass there is a relative leptin resistance in morbidly obese individuals. Post-RYGB there appears to be rapid improvement in leptin sensitivity which leads to fall in leptin levels. The exact mechanism for this change remains unclear. There is further fall in leptin levels with weight loss which also contributes to improved glucose tolerance and insulin sensitivity.

Calorie restriction has also been shown to improve glycaemia in other studies. We were unable to show the changes in insulin secretion and sensitivity secondary to calorie restriction due to small numbers in the study and inadequate calorie restriction. A very low calorie diet would be more appropriate to study these changes.

In summary the rapid improvements in glucose tolerance in the first few weeks of RYGB appear to be secondary to calorie restriction, improved incretin response and somewhat due to improved leptin sensitivity.

12 months after RYGB

Subjects who undergo RYGB lose a significant amount of weight by this time. Majority of the patients who are oral or insulin treatments for type 2 diabetes are able to stop their therapy early due to improved glycaemia. Waist circumference, fat mass, fat percentage, blood pressure and dyslipidaemia improve favourably. Glycaemic control measured by HbA1c, fasting glucose and OGTT improves significantly. Fasting insulin and AUC glucose fall significantly. Insulin sensitivity is improved shown by lower HOMA2 IR score and higher Matsuda index. AUC for insulin and AUC for GLP1 are no different from baseline at this time. Peak insulin response to oral calorie intake is however better. Hunger and disinhibition are lower and restraint is improved as measured by TFEQ. Hunger is lower and satiety is better as reported by visual scales.

The significant weight loss achieved by RYGB leads to favourable changes in body composition. Adiponectin levels are significantly improved and the levels of leptin are lower. Reduction in IL6, soluble TNF RII and hs-CRP levels confirm a significant improvement in the chronic inflammatory state that is often associated with morbid obesity.

In summary at 12 months the improvements in glycaemia are predominantly due to weight dependent mechanisms. Insulin sensitivity and secretion is improved and the improved incretin response is preserved. Favourable changes in appetite regulating hormones ensure that the hunger is lower and satiety is better. This probably leads to improved eating behaviour and better portion control. Reduction in chronic inflammatory state aided by favourable changes in adipokines and inflammatory cytokines further contributes to improved insulin sensitivity. This maintains the earlier improvements in glycaemia and it has been shown that the incidence of developing type 2 diabetes in the future is also lowered in those who did not have type 2 diabetes before surgery.

7.5 Limitations of this study

As discussed earlier the calorie restriction group did not achieve significant weight loss or changes in glycaemic indices. Bigger numbers in the group and stricter calorie control probably with the use of VLCDs may have been ideal. We did not measure all the known appetite regulating gut hormones such as ghrelin and oxyntomodulin which have been shown to influence change significantly following RYGB but their exact role in glucose

homeostasis remains unclear. We did not measure post prandial PYY levels which are shown to mirror changes in incretins and insulin responses. It would have been useful to have studied a group who undergo LAGB as the comparisons between the RYGB group and the LAGB group would have further explained the weight-dependent and weight-independent effects on glycaemia. The overall numbers that were recruited for this study were dictated by the number of patients who were able to get funding approval for weight loss surgery at that time. Bigger numbers in each group would have made it possible to correlate and analyse the 'effect size' of the various parameters in improved glucose tolerance.

Future research considerations

There does not appear to be a reliable marker to measure insulin secretion from oral glucose tolerance tests. We studied a number of indices and found most of them unreliable. After weight loss surgery, glucose clearance improves rapidly and insulin sensitivity improves continuously. We need a marker that can assess pancreatic beta cell function or insulin secretion in response to oral calorie or glucose intake which can take into account the background changes in insulin sensitivity. If there are no reliable markers then insulin clamp study appears to be the only reliable way of assessing insulin secretion.

It would be worthwhile performing the same study measurements in a group with VLCD. This would give us a better picture of the effect of calorie restriction in glucose metabolism.

There has been a lot of interest on the role of non-esterified fatty acids (NEFA) and gut microbiota on the metabolic changes after weight loss surgery. Further studies including these newer targets will shed more light on the possible mechanisms that underlie diabetes remission.

7.6 Final statement

This study presents evidence that in the initial stages after RYGB, glucose tolerance is improved by increased insulin secretion due to the effect of incretins and improved insulin sensitivity probably due to calorie restriction. In the later stages the improved glycaemia is maintained by weight loss, reduced fat mass, favourable changes in adipokines and inflammatory cytokines. Appetite regulation is improved by changes in gut hormones and adipokines which helps maintain eating behaviour and weight.

Appendix 1

APPENDIX: THREE-FACTOR EATING QUESTIONNAIRE

One point is given for each item in Part I and for each item (numbered question) in Part II. The correct answer for the true/false items is underlined and beside it is the number of the factor that it measures. The direction of the question in Part II is determined by splitting the responses at the middle. If the item is labelled '+', those responses above the middle are given a zero. Vice versa for those with a '−'. For example, anyone scoring 3 or 4 on the first item in Part II (item No. 37) would receive one point. Anyone scoring 1 or 2 would receive a zero.

Part I

				Factor Number
1.	When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	T	F	2
2.	I usually eat too much at social occasions, like parties and picnics.	T	F	2
3.	I am usually so hungry that I eat more than three times a day.	T	F	3
4.	When I have eaten my quota of calories, I am usually good about not eating any more.	T	F	1
5.	Dieting is so hard for me because I just get too hungry.	T	F	3
6.	I deliberately take small helpings as a means of controlling my weight.	T	F	1
7.	Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	T	F	2
8.	Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	T	F	3
9.	When I feel anxious, I find myself eating.	T	F	2
10.	Life is too short to worry about dieting.	T	F	1
11.	Since my weight goes up and down, I have gone on reducing diets more than once.	T	F	2
12.	I often feel so hungry that I just have to eat something.	T	F	3
13.	When I am with someone who is overeating, I usually overeat too.	T	F	2
14.	I have a pretty good idea of the number of calories in common food.	T	F	1
15.	Sometimes when I start eating, I just can't seem to stop.	T	F	2
16.	It is not difficult for me to leave something on my plate.	T	F	2
17.	At certain times of the day, I get hungry because I have gotten used to eating then.	T	F	3
18.	While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	T	F	1
19.	Being with someone who is eating often makes me hungry enough to eat also.	T	F	3
20.	When I feel blue, I often overeat.	T	F	2
21.	I enjoy eating too much to spoil it by counting calories or watching my weight.	T	F	1
22.	When I see a real delicacy, I often get so hungry that I have to eat right away.	T	F	3
23.	I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	T	F	1
24.	I get so hungry that my stomach often seems like a bottomless pit.	T	F	3
25.	My weight has hardly changed at all in the last ten years.	T	F	2
26.	I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	T	F	3
27.	When I feel lonely, I console myself by eating.	T	F	2
28.	I consciously hold back at meals in order not to gain weight.	T	F	1
29.	I sometimes get very hungry late in the evening or at night.	T	F	3

30.	I eat anything I want, any time I want.	T	F	1
31.	Without even thinking about it, I take a long time to eat.	T	F	2
32.	I count calories as a conscious means of controlling my weight.	T	F	1
33.	I do not eat some foods because they make me fat.	T	F	1
34.	I am always hungry enough to eat at any time.	T	F	3
35.	I pay a great deal of attention to changes in my figure.	T	F	1
36.	While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	T	F	2

Part II

Directions: Please answer the following questions by circling the number above the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?

1	2	3	4	
rarely	sometimes	usually	always	+ 1

38. Would a weight fluctuation of 5 lbs affect the way you live your life?

1	2	3	4	
not at all	slightly	moderately	very much	+ 1

39. How often do you feel hungry?

1	2	3	4	
only at mealtimes	sometimes between meals	often between meals	almost always	+ 3

40. Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4	
never	rarely	often	always	+ 1

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	3	4	
easy	slightly difficult	moderately difficult	very difficult	+ 3

42. How conscious are you of what you are eating?

1	2	3	4	
not at all	slightly	moderately	extremely	+ 1

43. How frequently do you avoid 'stocking up' on tempting foods?

1	2	3	4	
almost never	seldom	usually	almost always	+ 1

44. How likely are you to shop for low calorie foods?

1	2	3	4	
unlikely	slightly unlikely	moderately likely	very likely	+ 1

45. Do you eat sensibly in front of others and splurge alone?

1	2	3	4	
never	rarely	often	always	+ 2

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	2	3	4	
unlikely	slightly likely	moderately likely	very likely	+ 1

47. How frequently do you skip dessert because you are no longer hungry?

1	2	3	4	
almost never	seldom	at least once a week	almost every day	− 3

48. How likely are you to consciously eat less than you want?

1	2	3	4	
unlikely	slightly likely	moderately likely	very likely	+ 1

49. Do you go on eating binges though you are not hungry?

1	2	3	4	
never	rarely	sometimes	at least once a week	+ 2

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?

0
eat whatever you want, whenever you want it + 1

1
usually eat whatever you want, whenever you want it

2
often eat whatever you want, whenever you want it

3
often limit food intake, but often 'give in'

4
usually limit food intake, rarely 'give in'

5
constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behavior? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4	
not like me	little like me	pretty good description of me	describes me perfectly	+ 2

Appendix 2

Visual Analogue Scales for Hunger ans Satiety

I am not hungry at all	How hungry do you feel?	I have never been more hungry
I am completely empty	How satisfied do you feel?	I cannot eat another bite
Not at all full	How full do you feel?	Totally full
Nothing at all	How much do you think you can eat?	A lot
Yes, very much	Would you like to eat something sweet?	No, not at all
Yes, very much	Would you like to eat something salty?	No, not at all
Yes, very much	Would you like to eat something savoury?	No, not at all
Yes, very much	Would you like to eat something fatty?	No. not at all

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