Glucose metabolism in obese patients with type 2 diabetes mellitus undergoing Standard vs. Long Limb Roux-en-Y-gastric bypass

Anna Kamocka
MRCS, MEd.SE

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Department of Medicine
Section of Investigative Medicine
Division of Diabetes, Endocrinology and Metabolism
Imperial College London

Supervised by Professor Tricia Tan, Dr Alexander Miras and Professor Sir Stephen Bloom

"The first step in innovation is to know that a thing can be created.
After that, the rest is a matter of detail."

Brian Herbert

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Declaration of Originality

I confirm that the presented work is my own. When referring to others' data, I have referenced it in the main text, figures and tables as well as in the bibliography. All quotations have been provided in inverted comas.

I recruited the majority of the study participants, coordinated surgeries and follow up, performed clinical and mechanistic visits, conducted insulin and gut hormone assays, and collected and analysed the presented data.

Copyright Declaration

Abstract

Background

Obesity surgery has been shown to be the most effective and durable treatment for type 2 diabetes mellitus (T2DM) and obesity. The optimal length of the small bowel limbs in Roux-en-Y Gastric Bypass (RYGB), the most commonly performed obesity operation in the UK, is debated and variation in practice exists. In this study, called the LONG LIMB Trial, it was hypothesised that a longer biliopancreatic limb length of 150 cm ('Long Limb') is superior to a standard biliopancreatic limb length of 50 cm ('Standard Limb') in RYGB for the treatment of T2DM.

Methods

This was a two-centre double-blinded randomised controlled clinical trial. Fifty participants with T2DM and obesity were randomised in 1:1 ratio to either a Long Limb or a Standard Limb RYGB. Mixed meal tolerance tests were performed to measure postprandial secretion of active GLP-1 (primary outcome) and other gut hormones, insulin, and glucose excursions and hyperinsulinaemic-euglycaemic clamps to measure insulin sensitivity pre-operatively, within 2 weeks after the surgery and at matched 20% total body weight loss (TBWL). Clinical follow up took place at 3, 6 and 12 months after the surgery.

Results

Within each study group, a significant increase in insulin sensitivity, insulin and active GLP-1 secretion, and reduction in glucose concentrations were observed at 2 weeks post-operatively and 20% TBWL. HbA1c and weight were significantly reduced at all post-operative clinical visits (Standard Limb: HbA1c of 73 ± 17 pre-operatively to 43 ± 10 mmol/mol at one year, $p<0.001$, with 30 ± 8% TBWL; Long Limb: HbA1c of 76 ± 16 to 41 ± 5 mmol/mol, $p<0.001$, with 29 ± 8% TBWL), However, no difference between the groups was demonstrated in any of these outcomes nor in the percentage of patients achieving T2DM (Standard Limb 62% vs. Long Limb 77%, $p=0.23$).

Conclusion

Elongation of the biliopancreatic limb of the RYGB to 150 cm does not result in superior metabolic or clinical outcomes in terms of glucose excursions, insulin and incretin hormones secretion nor insulin sensitivity, T2DM remission or weight loss within 12 months after surgery.

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1.1. Obesity

The global pandemic of overweight and obesity represents a major issue from the healthcare and socioeconomic points of view. Obesity is characterised by an abnormal or excessive accumulation of fat (adipose tissue) that can impair health [1]. According to the World Health Organisation (WHO), obesity prevalence almost tripled since 1975. Globally, over 1.9 billion adults (39%) were overweight in 2016, with over 650 million (13%) being obese. This includes 41 million obese or overweight children under the age of five and 340 million children over the age of five [1]. Since it has been shown that the number of adipocytes, a major factor determining the fat mass in adults, is established in childhood and adolescence and remains unchanged during the adulthood regardless of weight loss [2], the prognosis for this disease prevalence in the future generation of adults is very worrying. The United Kingdom is "the fat man of Europe" [3] with the highest prevalence of overweight or obesity (64% in total, including 29% obesity) [4] amongst the Western European countries.

A balance between food intake and energy expenditure is fundamental to regulating body weight. These processes are coordinated by the central nervous system, predominantly by the arcuate nucleus in the mediobasal hypothalamus and area postrema in the brainstem. The arcuate nucleus comprises of two populations of neurons. Laterally localised anorexigenic ones (inhibiting appetite) express a neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) derived from pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Medially located orexigenic neurons (stimulating appetite) release neuropeptide Y (NPY) and Agouti-related protein (AgRP) [5]. Vagus nerve-mediated signals from the mechanoreceptors and chemoreceptors in the gut stimulate the nucleus of the solitary tract in brainstem first which then informs the hypothalamus. Various hormones, such as glucagon-like peptide 1 (GLP-1), peptide YY, pancreatic polypeptide, insulin,

cholecystokinin and leptin can act as modulators of appetite and suppress the centre of hunger in the hypothalamus and the brainstem after crossing the blood-brain barrier [6]. However, food intake homeostasis is regulated by more than just physiological needs. Taste, smell and visual perception of food all form strong signals which can override satiety and promote further consumption of highly palatable nutrients. Therefore, humans are prone to overeating sweet and salty foods and minimising intake of those that are bitter and sour [7]. Hedonic, or the "reward" system, that coordinates these processes and is capable of overruling the homeostatic appetite centres from the hypothalamus and the brainstem, is localised in limbic and cortical areas, namely in the orbitofrontal lobe, insula, amygdala, dorsal and ventral striatum, nucleus accumbens and others [8].

Obesity is a complex multifactorial disease with a major impact on an individual's wellbeing and performance status. Adipose tissue can be perceived as an endocrine organ that releases a number of signalling proteins (cytokines), known as adipokines. The increase in the adipose cell size and overall fat mass alters their secretion which can explain several pathophysiological changes in metabolism observed in obesity. With over 600 secretory proteins described, the most studied ones include leptin, adiponectin, tumour necrosis factor-α and interleukin-6 [9]. Most of the adipokines' concentrations increase with enlargement of adipocytes, whereas adiponectin levels are inversely related to the visceral fat mass as well as the adipocyte size. Adiponectin has been shown to improve insulin sensitivity and vascular function [10] and its plasma levels decrease in obesity [11, 12]. Leptin, predominantly released from the subcutaneous fat, has been shown to suppress appetite by acting on the hypothalamus in humans and rodents and to stimulate energy expenditure. A clinical syndrome arising from the rarely occurring congenital deficiency of this adipokine results in severe obesity from childhood [13]. Paradoxically, even though in ordinary obesity the abundance of adipose tissue leads to hyperleptinaemia, this does not result in a feedback suppression of appetite and restoration of weight, therefore a degree of insensitivity to it is suspected here [14]. It has been shown that leptin crosses the blood-brain barrier by a saturable transport system. Therefore, despite its serum levels being over 300% higher in

obese individuals, its concentration in the cerebrospinal fluid is only 30% higher when compared to lean individuals [15].

Other adipokines secreted by the adipocytes include interleukin-6. Together with increased blood concentration of another inflammatory marker, c-reactive protein, it increases the risk of congestive heart failure in obesity [16]. Angiotensinogen, a precursor of angiotensin, also released by the adipose tissue, can contribute to the development of hypertension. Furthermore, aromatase enzymes produced by the adipose tissue are able to convert androstenedione to oestrogen. Raised oestrogen levels can change male phenotype in obesity and also increase risk of developing breast and endometrial cancer in females, in particular after the menopause when adipose tissue is the principal source of this hormone [17]. Excess of the fat tissue leads to increased lipolysis, with decreased triglyceride storage and increased circulating free fatty acids which form ectopic deposits in multiple organs, with liver, muscle and pancreas being affected the most [18].

Obesity with excessive central adiposity has been defined as a crucial factor for the development of metabolic syndrome, defined by a combination of increased waist circumference, hyperglycaemia, hypertension and dyslipidaemia [19]. Obesity has been proven to increase the risk for the development of cardiovascular disease (including stroke and heart disease) due to the promotion of atherogenic dyslipidaemia (raised triglycerides and LDL, decreased HDL), type 2 diabetes mellitus (T2DM) and triggering proinflammatory and prothrombotic processes, which relate to the impact of excess of adipose tissue discussed above.

According to the Framingham Heart Study, the risk of death increases by 1% for each excess pound in weight (0.45 kg) in 30- to 42-year-olds and by 2% at the age of 50 and above [20]. Obesity has also been defined as a risk factor in the development of morbidities such as breast, endometrial, ovarian, colon, hepatocellular, gallbladder, colon and kidney cancer, musculoskeletal problems resulting in osteoarthritis and cholelithiasis, and has been

associated with obstructive sleep apnoea, hypoventilation syndrome, non-alcoholic fatty liver disease (steatosis, steatohepatitis and cirrhosis), depression, menstrual disturbances, infertility, polycystic ovarian syndrome, gout, phlebitis and benign intracranial hypertension [1, 18, 21]. Obesity in pregnancy predisposes to gestational diabetes, preeclampsia, hypertension and complicated delivery. It also results in higher rates of large for gestational age babies, congenital anomalies, preterm birth and perinatal death [22].

In 2017/2018 there were 711,000 hospital admissions with obesity recorded as a primary or secondary diagnosis in the UK [23]. Unsurprisingly, these alarming findings and epidemics of obesity have triggered huge interest in the subject from researchers, medical professionals, the pharmaceutical industry, the general public, and the governments. Tackling obesity through both prevention as well as treatment is high on the political agenda in the UK and the All-Party Parliamentary Group on Obesity has been formed specifically for that purpose [24]. Through collaborating with experts in the field, it aims to raise awareness of the problem, tackle stigmatisation and develop effective pathways in the prevention and treatment of this complex disease.

1.1.1. Aetiology of obesity

Obesity has been traditionally perceived as an imbalance between energy input and energy expenditure with the excess of consumed calories converted to adipose tissue. Furthermore, poor dietary choices and easy access to carbohydrate-rich and fat-saturated food as well as sedentary lifestyle induced by a change in work patterns, modes of transport and urbanisation [1] have been linked to rapidly increasing prevalence of obesity. However, reducing this disease to a simple “calories in – calories out” imbalance is misleading. It is known that obesity is a complex multifactorial disease and genetics plays a crucial role in its development. Stunkard et al. proved this over 30 years ago in their renowned study on adopted children [25]. Having investigated BMI of 540 Danish adoptees and their biological and adoptive parents, they have shown a clear correlation between biological parents’ and their children’s BMI. This correlation did not exist between children and their adoptive parents. The strong

impact of genetic factors was later confirmed again by Stunkard et al. in the study of almost 700 identical and fraternal twins from the Swedish Twin Registry who were either raised together or separately. The BMI-intrapair correlation coefficients in monozygotic twins reared apart was 0.66 for women and 0.70 for men whilst if they were reared together it was either identical in females (0.66) and only slightly higher in males (0.74). The same analysis conducted in dizygotic twins showed much lower BMI-intrapair correlation coefficients of 0.25 in women and 0.15 in men reared apart and 0.27 in women and 0.33 in men reared together [26]. Another study on monozygotic twins firmly confirming the significant contribution of genetic factors to the development of obesity has been reported by Bouchard et al [27]. In this trial, identical adult male twins were overfed by 1000 kcal/day for 6 out of 7 days over a period of 100 days. The differences in weight and adipose tissue gain and fat distribution were much greater between the pairs than within the monozygotic twin pairs. In fact, it has been estimated that the heritability of obesity is comparable to that of height [28] and it can be as high as 70% [29].

Increased body weight and fat tissue mass are believed to be linked to so-called susceptible genes [30]. It means that the influence of genotype on obese phenotype is reduced or augmented by non-genetic factors, e.g. environmental or psychosocial ones. Therefore, genes predisposing to obesity increase the risk of its development but at the same time, they are not essential for the obese phenotype to occur. Bouchard's study described above confirms this theory, where much greater similarity was shown within monozygotic twin pairs than between them [27]. Thus, in the general population, individuals with susceptible genes will most likely suffer from obesity in a favourable environment. It may seem surprising that susceptible genes evolved so rapidly in recent decades to lead to the outburst of obesity worldwide. These changes can be explained by the epigenetics. It is a study of heritable changes in gene expression that do not alter the DNA sequence but – through altered DNA methylation – deliver an additional level of transcriptional control that regulates genes' expression [31]. It results in changes at the chromosomal level through chromatin remodelling by histone deacetylases, enzymes that allow removal of the acetyl groups. DNA methylation can be

modified by factors such as diet and environment and such alterations, unlike DNA evolutionary changes, can occur over short periods.

Rare exceptions are single-gene obesity-associated disorders such as Prader-Willi syndrome (chromosome 15 abnormalities), Bardet-Biedl syndrome (genetically heterogeneous) or congenital leptin deficiency (*ob* gene mutation) where the phenotype is consistently determined by the genotype. The most commonly identified gene associated with obesity is the FTO gene, fat mass and obesity-associated gene located on chromosome 16. Homozygous FTO risk allele increases the risk of obesity by 1.67-fold [32]. It is believed to predispose to increased body mass by stimulating hyperphagia and influencing food choices with the preference of energy-dense nutrients [33].

Environmental and behavioural factors have been shown to increase the incidence of obesity. Chronic stress is certainly one of them. It results in increased secretion of the corticotropin-releasing factor from the paraventricular nucleus in the hypothalamus which then stimulates adrenocorticotropic hormone (ACTH) release from the pituitary. ACTH acts on the cortex of the adrenal glands by stimulating glucocorticoids secretion, namely cortisol. Its persistently elevated levels, in turn, affect the hypothalamo-pituitary-adrenal axis, increase levels of corticotropin-releasing factor and as a result sustain high levels of circulating cortisol. Glucocorticoids can increase pleasurability of compulsive activities such as ingesting fat and carbohydrate-dense food, i.e. increase reward signals from so-called ”comfort eating”. Furthermore, glucocorticoids promote central fat deposition [34]. Cumulation of these events can promote both obesity and T2DM in chronic stress.

Those two diseases’ onset can also be influenced by sleep deprivation. Altered circadian rhythm with shortened periods of sleep is not only detrimental to glucose homeostasis but also leads to upregulation of appetite with a simultaneous decrease in energy expenditure [35].

The gut microbiome and its impact on weight regulation have generated a lot of interest over recent years. It is estimated that 3 trillion microorganisms inhabiting human intestinal lumen play a role in metabolism regulation, including weight and glycaemic control [36, 37]. It is believed that it is not just the types of microorganisms colonising the intestine that matter but also the metabolites produced by them. Individuals with obesity repeatedly showed lower Bacteroidetes to Firmicutes ratio than the lean subjects [38] [39]. However, using the example of all the twin studies discussed above, one can appreciate that its influence must be rather limited. Adoptive parents should be having similar gut flora to their adopted children (through having a similar diet and sharing their living space), yet despite that, it was the biological heritability that determined subjects' body weight. Therefore, whilst gut microbiota can influence the weight, they are likely to contribute to weight gain only in the presence of a favourable genotype.

Certain medical conditions have a strong impact on weight gain and adipose tissue distribution. The most commonly seen ones are:

1. Hypothyroidism – characterised by low concertation of plasma thyroid hormones.
2. Cushing's syndrome – caused by a high concentration of glucocorticoids, either endogenous cortisol or exogenous medications such as prednisolone.
3. Hypothalamic disorders – including craniopharyngioma, traumatic injury, surgery, Fröhlich syndrome.
4. Genetic disorders – such as already mentioned above Bardet-Biedl and Prader-Willi syndromes.

Furthermore, certain medications, including corticosteroids, anti-epileptics (e.g. valproic acid, carbamazepine), antipsychotics (olanzapine), some glucose-lowering medications (e.g. insulin, sulfonylureas) and antidepressants (e.g. amitriptyline, mirtazapine) promote weight gain.

With clearly complex and multifactorial development of obesity, where multiple mechanisms interplay on genetic level, influenced by the environment, toxins, diet, energy expenditure, psychosocial factors such as stress, sleep deprivation, medical conditions, medications, gut microbiota and others, a traditional “eat less, exercise more” approach is clearly not going to be neither effective nor sustainable for most people with obesity.

1.1.2. Assessment of obesity

A simple measure of quantifying obesity is a Body Mass Index (BMI). In adults, it divides obesity into classes [40, 41]:

1. BMI ≥ 25 kg/m^2 – overweight
2. BMI ≥ 30 kg/m^2 – grade I obesity
3. BMI ≥ 35 kg/m^2 – grade II obesity
4. BMI ≥ 40 kg/m^2 – grade III obesity

BMI in the range of 18.5-24.9 kg/m^2 is defined as normal. A large meta-analysis, The Global Burden of Disease Project, analysed the correlation between the BMI and all-cause mortality in over 10 million people from four continents: Europe, North America, Asia and Australia and New Zealand [42]. It reported the lowest mortality rates in the BMI 20-25 kg/m^2 range. After crossing the 25 kg/m^2 BMI threshold, all-cause mortality risk in overweight increased by 7% and by 20% for BMI 27.5-30 kg/m^2. In grade I obesity that risk was raised by 45%, by 94% in grade II and by 176% in grade III obesity. Furthermore, every increase in BMI by 5 units was proved to result in a 60% increase in mortality from chronic kidney disease and a 120% increase in mortality related to T2DM [43].

However, BMI is not an accurate reflection of obesity status in relation to the adipose tissue content [44]. It is not possible to assess whether measured BMI is related to the fat or the muscle mass, therefore athletes or bodybuilders may have high BMIs despite not being obese. On the other hand, certain individuals may present with relatively low BMIs whilst in fact being metabolically obese. This can be the case in the elderly with sarcopenia and excess of adipose tissue [18]. Also, BMI does not allow to assess fat tissue distribution, which is an important

aspect from the metabolic syndrome point of view and cardiovascular risk prediction, all associated with a high content of the visceral fat [45, 46]. On the other hand, a phenotype of "metabolically healthy obesity" can be seen in BMI > 30 and with no indication of insulin resistance or metabolic syndrome. The quoted prevalence of this phenomenon varies between 6 and 75% [47]. These individuals have less intraabdominal fat content, liver steatosis and lower markers of systemic inflammation than expected for their BMI [48]. However, this is usually perceived as a transient state that can progress to the unhealthy obesity phenotype with time. Despite all of its shortcomings, BMI is the most practical, easy and therefore the most common globally used tool utilised in the clinical practice and research [18].

Another measurement that is easy to perform in clinical practice and is applicable in large cohort studies is waist circumference and waist to hip circumference ratio (WHR). When compared to BMI, it is superior in estimating cardiovascular risk with cut off indicators for the substantial increase being [40]:

1. Males: >102 cm waist circumference or WHR of 0.90
2. Females: > 88 cm waist circumference or WHR of 0.85.

Polygenic risk score for increased WHR is associated with 77% higher risk for T2DM (OR 1.77, 95% CI 1.57-2.00) and 46% higher risk of developing cardiovascular disease (OR 1.46, 95% CI 1.32-16.2) [49]. However, WHR on its own is mostly useful in the lower range of BMI, <35 [50].

Body composition is believed to be a more accurate tool in the assessment of obesity as it quantifies it in relation to the fat mass instead of the total body mass. It is most commonly assessed with a dual-energy x-ray absorptiometry (DEXA) or bioelectrical impedance machines (e.g. Tanita®), which allow quantifying a subject's adipose tissue mass and fat-free mass with their distribution, alongside with basic metabolic rate. Whilst the norms for body composition have not been as firmly established as in the BMI classification, some authors use a cut-off of body fat content of 33% and above in women and 25% and above in men [44].

BMI and body fat mass exhibit different predictive values for cardiovascular risk with the latter being a more reliable predictor [51].

Assessment of cardiovascular fitness can be a meaningful additional tool in estimating all-cause mortality risks in addition to the BMI, assessment of body fat mass and WHR [52]. High levels of cardiovascular fitness have been shown to lower mortality risk in both “unhealthy” and the “metabolically healthy obesity”. In the FIT Project, where over 29,000 men and women with no evidence of cardiovascular disease or T2DM underwent a symptom-limited maximal treadmill stress test and were then followed up for an average of 11 years, a strong inverse association between the exercise capacity and all-cause mortality was noted whereas BMI on its own was a poor predictor of mortality [53].

In the process of assessing a patient with obesity, one should also consider ruling out other medical diseases that can manifest themselves as obesity (hypothyroidism, Cushing syndrome). Furthermore, screening for obesity-associated diseases should be performed, therefore baseline blood tests should also include lipid profile, liver function tests, fasting plasma glucose and glycated haemoglobin levels (HbA1c).

1.1.3. Assessment of weight loss intervention outcomes

When reporting weight loss outcomes, the most commonly used methods include:

1. Total body weight loss (TBWL) – expressed in percentage and calculated as: TBWL = (baseline weight – post-intervention weight) / baseline weight x 100%
   Many authors argue that this is the optimal tool in weight loss reporting [54]. Its main advantages include being least associated with the pre-operative BMI when compared to the excess weight loss or number of BMI units lost (as described below), thus enabling a reliable comparison between various cohorts with differing pre-intervention weight characteristics. Moreover, it is a metric that requires only a straightforward standardised calculation and is easy to comprehend by patients and the public which

can facilitate communication when discussing the outcomes of weight loss interventions [54].

2. Excess weight loss (EWL) – stands for a percentage of weight loss relative to achieving an "ideal weight". It is calculated as:

   EWL = (Weight loss/Baseline excess weight) x 100%

   Baseline excess weight is calculated as a difference between the baseline weight and the 'ideal weight'. This 'ideal weight' is usually based on an individual's weight at a BMI of 25 kg/m^2 however, different reference values have also been used [55]. EWL, even though widely used in reports on weight loss outcomes, has been criticised not only for using the non-standardised references for the 'ideal weight' but also for making a non-evidence-based assumption of that 'ideal weight' value, which in fact may not be the optimal one for individuals who have suffered from morbid obesity. Furthermore, pre-intervention BMI is a confounding factor here, which makes reliable comparisons between studies with various baseline characteristics more complicated [54].
3. The number of Body Mass Index units lost (ΔBMI) – difference between the pre- and post-intervention BMI units expressed in kg/m^2. As discussed above, the main confounding factor in this metric is its dependence on the pre-operative BMI which makes comparisons between different studies challenging.
4. Absolute weight loss (WL) – the difference between the pre- and post-intervention weight expressed for example in kilograms. Again, this metric is dependent on the pre-intervention weight which can vary vastly across the studied cohorts and make meaningful in-between studies comparisons difficult.

One should remember that, in order to fully assess the impact of the weight loss intervention, other factors than just the weight-related ones should be assessed, such as obesity-related comorbidities' remission, reduction in medications intake and changes in the functional status.

1.2. Type 2 Diabetes Mellitus

Expansion of type 2 diabetes mellitus (T2DM) and obesity are closely connected and the risk of T2DM development increases with rising weight, BMI and central adiposity [56] [57] [58]. Criteria for diagnosis of T2DM, as defined by the American Diabetes Association (ADA) [59] are:

1. Fasting plasma glucose of > 7 mmol/L or
2. Plasma glucose of > 11.1 mmol/L at 2-hours in the oral glucose tolerance test or
3. Glycated haemoglobin (HbA1c) > 48 mmol/mol (> 6.5%) or
4. Random plasma glucose of >11.1 mmol/L in patient with classic symptoms of hyperglycaemia or hyperglycaemia crisis.

It is estimated that 3.8 million people in the UK suffer from T2DM (6% of the general population) and its treatment consumes 10% of the annual NHS budget [60] [61]. T2DM has been proven to decrease life expectancy by 10 years and to carry a heavy burden of increased risk of complications affecting multiple organs, including the peripheral nervous system and micro- and macrovascular system. As a consequence, it is the leading cause of de novo blindness due to diabetic retinopathy, renal failure due to nephropathy as well as a cardiovascular disease resulting in non-traumatic lower limb amputations, acute coronary events and stroke. Reduction of hyperglycaemia significantly reduces these risks, with each 1% reduction in the glycated haemoglobin (when HbA1c is expressed in %) resulting in 21% decrease in risk of death, 14% decrease of risk in myocardial infarction and 37% decrease in risk of microvascular complications, with the lowest risks being reported when normal HbA1c is achieved [62].

1.2.1. Glucose homeostasis

To appreciate the pathophysiology of T2DM, one should first scrutinize several mechanisms involved in maintaining normal glucose homeostasis. In physiological conditions, endogenous glucose production is supplied in 85% by the liver through glycogenolysis and gluconeogenesis and in 15% by the kidneys. The rate of endogenous glucose production is set to match the baseline glucose utilisation of approximately 2 mg/kg/min [63]. During a period

of fasting, the majority of the total body glucose utilisation is undertaken by insulin-independent tissues. The main organ here is the brain, which uses 50% of all glucose and it becomes saturated at a plasma glucose concentration of 2.2 mmol/L. Further 25% of the uptake takes place in the gastrointestinal tract, including the liver. The residual 25% of glucose disposal occurs in the insulin-dependent tissues, of which muscle is the major component, with adipose tissue contributing to a much lesser degree.

In the postprandial state, two factors play a role in triggering insulin release from the pancreatic β-cells: a rise in blood glycaemia and incretins, namely glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). Incretins are released from the enteroendocrine L-cells (GLP-1), localised predominantly in the terminal ileum and colon, and K-cells (GIP), localised in the duodenum and jejunum, following a direct stimulation by the endoluminal nutrients [64]. Their potent capability of enhancing β-cell response to blood glycaemia results in approximately 70% higher insulin secretion after oral nutrients' administration than after the intravenous glucose stimulation, which is known as the incretin effect [65]. The first, acute and early, phase of insulin secretion resulting in the early peak of postprandial insulin concentration, is released from approximately 5% of the insulin secretory granules. The second phase is a slower release from the insulin "reserve pool". The first phase is decreased when impaired fasting glucose exists, whereas both phases are reduced in glucose intolerance or T2DM [65]. A combination of hyperglycaemia and hyperinsulinaemia suppresses endogenous glucose production and prompts splanchnic and peripheral glucose uptake. Whilst liver and gut are the organs responsible for the splanchnic glucose uptake, the tissue which metabolises over 80% of the peripheral glucose is muscle, with an only small contribution from the adipose tissue (5%) [63]. Even though the involvement of the fat tissue in peripheral glucose uptake may seem small quantity-wise, it plays a crucial role in glucose homeostasis through regulating the levels of free fatty acids, released from the triglycerides, as well as producing adipokines which in turn influence hepatic and peripheral insulin sensitivity (as discussed in section 1.1).

Insulin resistance occurs when physiological concentrations of insulin induce a diminished response in insulin-dependent tissues. The impact of insulin can be compromised at a number of levels including perfusion of the insulin-dependent tissues, binding to the insulin receptor and post-receptor signalling activating glucose transporters.

Insulin sensitivity, reverse of insulin resistance, is affected by multiple factors, including: genetics (with some genes possibly being the ones that also predispose to weight gain), age (insulin sensitivity declines with age), diet (decreases with fat-saturated food), medications (reduced by corticosteroids, growth hormone and nicotinic acid), weight (decreased in obesity) and adipose tissue distribution (with central fat distribution having much higher impact on its reduction that the peripheral distribution) as well as by the physical fitness and exercise (with both improving insulin sensitivity in acute and chronic setting) [66]. Two main adipokines derived from the adipose tissue, leptin and adiponectin, have the opposite impact on the insulin sensitivity. When comparing men of the same body composition with good and with decreased insulin sensitivity, leptin levels are elevated in subjects with the latter, which suggests that insulin resistance is associated with elevated leptin levels but does not correlate with the adipose tissue mass [67]. Adiponectin, on the other hand, improves insulin sensitivity [10].

1.2.2. Aetiology of type 2 diabetes mellitus

In the process of T2DM development, two phenomena take place. Usually, resistance to circulating insulin develops first. To maintain normoglycemia, β-cells increase insulin secretion which leads to hyperinsulinaemia. As long as the normal β-cell function is maintained, T2DM should not manifest clinically as normoglycaemia will be maintained by the hyperinsulinaemia. With time, a progressive failure of the β-cell function takes place, which results in hyperglycaemia and leads to the development of T2DM [68]. Furthermore, glucagon and insulin secretion balance is disrupted with basal hyperglucagonaemia and lack of suppression of glucagon secretion in response to glucose [69]. As shown in the United Kingdom Prospective Diabetes Study, T2DM is a progressive disease with worsening and more difficult

to treat hyperglycaemia over time. Here, despite intensive medical treatment, only 1 in 4 patients met the ADA targets of HbA1c <7% (53 mmol/mol) after 9 years of the optimal medical treatment [70].

Multiple factors have been shown to contribute to the development of T2DM. Firstly, the disease has a strong genetic component and in most cases, it was shown to be polygenic [71]. Certain variants of the FTO gene (fat mass and obesity-associated gene) have been repeatedly shown to predispose to T2DM via its impact on the BMI [32]. Other genetic factors related to its development include genes for calpain 10, potassium inward-rectifier 6.2, peroxisome proliferator-activated receptor γ and insulin receptor substrate-1 [68]. It has been shown that the risk of T2DM development rises to 15% if one of the parents has the disease, 75% with both parents affected, 10% in case of fraternal twins and 90% in case of monozygotic twins [72].

On a pathophysiological level, a cascade of various events contributes to the development of T2DM. The majority of the subjects suffering from T2DM also present with obesity. Excess of adipose tissue results in chronic inflammation, both due to fat infiltration with the macrophages as well as due to the release of elevated levels of inflammatory cytokines and adipokines by the adipocytes. Inflammatory cytokines, such as tumour necrosis factor-α, impair triglyceride synthesis and storage within the adipose tissue and simultaneously stimulate their hydrolysis into the free fatty acids. Exposure of the skeletal muscle to high concentrations of fatty acids disrupts insulin signalling pathway and as a result, impairs peripheral glucose utilisation [73]. Glucose uptake into the muscle cells occurs via facilitated diffusion with glucose transporter carrier proteins, predominantly GLUT4. It is controlled by insulin and its expression is reduced in insulin resistance, which further contributes to hyperglycaemia and development of skeletal muscle resistance to insulin [74]. Furthermore, free fatty acids promote hepatic glucose production [75]. Hyperglycaemia triggers increased stimulation of pancreatic β-cells and hyperinsulinaemia. Progressive insulin resistance together with glucotoxicity, lipotoxicity and amyloid formation all lead to β-cell dysfunction [68]. β-cell impairment can also be the primary

cause of T2DM, and this can be observed in the lean individuals, however, insulin resistance will also develop with time.

1.2.3. Assessment of glucose homeostasis

Assessment of glucose homeostasis evaluates two separate phenomena: insulin sensitivity and β-cell function. Methods utilised in the assessment of both can be classified into those that:

1. Provide a static assessment of physiology in fasting steady state.
2. Assess dynamic physiological response to an oral stimulus in the Oral Glucose Tolerance Test or in the Mixed Meal Tolerance Test.
3. Evaluate 'artificial' steady-state created by the assessment tool, such as the Frequently Sampled Intravenous Glucose Tolerance Test, the Insulin Suppression Test and the Hyperinsulinaemic-Euglycaemic Clamp.

1.2.3.1. Assessments in the fasting steady state

In the clinical setting, both insulin sensitivity and the β-cell function can be assessed with the Homeostasis Model Assessment (HOMA) by using measurements of fasting glucose and insulin or C-peptide concentrations. HOMA-IR and HOMA-B were first developed by Matthews and colleagues in 1985 to assess insulin resistance and β-cell function respectively [76]. It requires only straightforward calculations:

HOMA-IR = (fasting insulin concentration [mU/mL] * fasting glucose concentration [mmol/L]) / 22.5

HOMA-B = (20 * fasting insulin concentration [mU/mL]) / (fasting glucose concentration [mmol/L] - 3.5)

HOMA model is based on the assumption that fasting glucose concentration is regulated by insulin-dependent endogenous glucose output and plasma insulin concentration is dependent on the β-cell reaction (insulin secretion) to plasma glucose concentration. Therefore, the degree of hyperglycaemia in a fasting state is determined by a combination of impaired insulin sensitivity and β-cell function. HOMA-IR <1.6 has been defined as normal and HOMA-IR >2.5

indicates insulin resistance. However, low precision and poor correlation of the HOMA model with β-cell function and insulin sensitivity derived from the hyperinsulinaemic euglycaemic clamps limited its use and the model is now thought to be out of date. Therefore, it was updated to a HOMA2 version in 1996 [77] and can be calculated through the Oxford University online software (http://www.dtu.ox.ac.uk./homa). It provides three measures: HOMA2-%B (estimated steady-state beta cell function), HOMA2-%S (insulin sensitivity) and HOMA2-IR (insulin resistance). These models have been validated and shown to correlate with clamp-derived studies [77].

Other methods of assessing insulin sensitivity or its opposite, insulin resistance, include Quantitative Insulin Sensitivity Check Index (QUICKI) [78], MacAuley index [79] and glucose/insulin ratio [80]. They are all relatively simple and low-cost however, reported results vary between laboratories and they only assess a snapshot from the fasting state.

1.2.3.2. Assessments in the postprandial state

Theoretically, measurement of circulating insulin should be the easiest way to assess β-cell secretory function both in the fasting and in the postprandial state. However, insulin concentrations in the portal vein, hepatic veins and peripheral circulation vary significantly due to inconstant hepatic and peripheral clearance, hence approximately only half of the secreted glucose reaches the peripheral circulation. Insulin clearance rates change under varying circumstances. Furthermore, insulin disposal is considerably reduced in states of insulin resistance, such as obesity and T2DM, therefore its plasma concentration is not a valid measurement of β-cell function. C-peptide is co-secreted with insulin from proinsulin in an equimolar ratio. Only a small fraction is extracted by the liver and it has a stable peripheral clearance, with a constant rate of secretion by the kidneys. Circulating levels of c-peptide in fasting and postprandial states can be used as a rough guide of the β-cell function assessment whereas a mathematically derived c-peptide deconvolution model is a reliable way of measuring insulin secretion independently of the insulin clearance [81].

Assessments in the postprandial state, Oral Glucose Tolerance Test (OGTT) and a Mixed Meal Tolerance Test (MMTT), are believed to represent a more physiological response. This is due to the incretin effect, where GLP-1 and GIP release stimulated by intraluminal nutrients significantly increases insulin secretion [65]. Postprandial incretin response is blunted or even lost in T2DM hence assessment with these tests is paramount to investigating insulin secretory capacity [82].

The insulinogenic index was designed to measure the first phase of insulin secretion in response to glucose stimulus in the OGTT [83]. After consumption of 75 g of glucose dissolved in 300 ml water, the ratio between the increments of insulin and glucose concentration at 30 minutes is calculated:
Insulinogenic index = (Δ Insulin 30 min – Insulin basal [μU/ml]) / (Δ Glucose 30 min – Glucose basal [mmol/L])
A result of 0.4 or below implies pathologically decreased insulin secretion [84].

The Matsuda Index, also called an Insulin Sensitivity Index, has also been derived from an OGTT [84]. Result of 2.5 or below suggests insulin resistance.

$$\mathrm{ISI}_{\mathrm{comp}} = \frac{10000}{\sqrt{G_b \cdot I_b \cdot Gm \cdot Im}}$$

Here, Gb and Ib are glucose and insulin concentrations in the fasting state, and Gm and Im are the mean concentrations during the oral glucose tolerance test (with sampling taken at: 0, 30, 60, 90, 120 min).

The Disposition Index is derived from the insulin sensitivity and amount of insulin secreted in response to plasma glucose. A progressive loss of the β-cell function results in a reduced capacity to compensate for insulin resistance, hence it will be represented by a lower disposition index [85]. It is calculated as:
Disposition index = Matsuda index x (Δ Insulin 120 min – Insulin basal [μU/ml]) / (Δ Glucose 120 min – Glucose basal [mmol/L])

A disposition Index of over 1 indicates normal β-cell function.

Other indices of insulin sensitivity that can be calculated from the oral glucose tolerance test include Stumvoll's Method, Oral Glucose Insulin Sensitivity Model, Avignon Index and Glucose Insulin Product. Their main limitation is that, just as in the case of the fasting indices, they are based on the assumption that a normal insulin secretion exists. Therefore, when β-cell function is impaired, they overestimate insulin sensitivity. Also, a large variation of glucose and insulin values obtained from OGTTs exists which is a result of the variability in gastric emptying rate as well as glucose absorption from the gastrointestinal tract even within the same individuals. All these indices are markers of total body insulin sensitivity and do not distinguish between hepatic and peripheral insulin sensitivity.

MMTT, which can be delivered as a liquid or solid meal combined of carbohydrates, proteins and lipids, aims to simulate more physiological response to nutrients than the OGTT, as proteins also promote glucagon release. Just like the OGTT, it is simple, minimally invasive, relatively low-cost and allows assessment of both insulin sensitivity and β-cell function [86]. Indices derived from the Oral Glucose Tolerance Test can be also applied here [87]. The main disadvantage of the MMTT is that it is poorly standardised, with different stimuli utilised by various research centres. Our research group uses a single bottle of 125 ml Ensure Compact® in all studies in obesity surgery.

1.2.3.3. Assessments in response to intravenous stimuli

The Insulin Suppression Test assesses the rate of glucose disposal in response to exogenously administered insulin after suppressing endogenous glucose production (with somatostatin or adrenaline combined with propranolol) [88] [89] [90]. With simultaneous glucose infusion, one should achieve steady-state plasma glucose and insulin concentrations after 3 hours. Plasma glucose concentrations over the normal range of 5-8 mmol/L indicate insulin resistance. However, there are quite a few shortcomings of this assessment limiting its use in the assessment of insulin sensitivity. The main shortcoming is that the exogenous

insulin infusion may not completely suppress endogenous glucose production in the liver, especially in subjects with insulin resistance or T2DM. Furthermore, simultaneous urinary glucose testing is required to correct for the glucose secretion by the kidney. Also, adrenaline (even with an attempt to block its side effects with propranolol) and somatostatin have other multiple effects on various organs, with main concerns being cardiac rhythm disturbances in the former and impact on glucose clearance and numerous gastro-intestinal and pituitary hormones in the later [91].

The Insulin Tolerance Test is used for assessment of insulin sensitivity following an exogenous insulin injection administration in a dose of 0.1 U/kg of body weight [92] [93]. It is a simple and inexpensive method that can be easily applied in large epidemiological studies. Nevertheless, risk of inducing hypoglycaemia in insulin-sensitive subjects exists and the studied glucose fall results in a counter-regulatory response including glucagon, catecholamines and cortisol secretion which will slow down the plasma glucose disappearance rate [93] [94].

The Frequently Sampled Intravenous Glucose Tolerance Test can be used to assess both insulin sensitivity and β-cell function following an exogenous bolus of glucose. In its modified version, an insulin infusion is added after 20 minutes [95] [96]. This test calculates the insulin sensitivity index, measuring the ability of insulin to enhance glucose uptake and to inhibit glucose production as well as glucose effectiveness index, assessing the ability of glucose to disappear from plasma at constant basal insulin level. Since muscle and adipose tissues are the primary organs responsible for returning glucose to pre-test values, the insulin sensitivity index predominantly represents peripheral insulin resistance. Direct stimulation of insulin secretion by the glucose load quantifies β-cell function. The main limitations of the Frequently Sampled Intravenous Glucose Tolerance Test are that it will not work in severe insulin deficiency as minimal insulin secretion is required to assess its effect on glucose disposal. It is a work-intensive procedure that requires frequent blood sampling and the data analysis is complex.

Currently, the gold-standard tool used to assess insulin sensitivity is a hyperinsulinaemic-euglycaemic clamp (which will be called a clamp here). First described by Andres et al. and further developed by DeFronzo in 1979 [97], it serves as a measure of hepatic and peripheral insulin sensitivity. The aim of this technique is to "clamp" glucose, i.e. maintain it at a certain level and avoid hyper- and hypoglycaemia to prevent triggering of the counter-regulation mechanisms. The administration of a predetermined fixed dose of insulin inhibits hepatic glucose production and increases the glucose uptake from skeletal muscle, triggering a decline of blood glucose concentration. This decline is prevented by administering a variable infusion of glucose where the rate is determined by a negative feedback principle in order to achieve the euglycaemic plateau. Thus, the rate of glucose infusion required to maintain euglycaemia in a state of hyperinsulinaemia provides a measure for the net effect of insulin on whole-body glucose metabolised (M value) [98].

Over the years various protocols have been developed. It requires the use of two intravenous lines, one for the infusion of 20% glucose and insulin, and one in the contralateral arm for frequent blood sampling. Ideally, sampling should be performed from an arterial line, but this is rarely practical. Instead, the patient's blood sampling hand can be kept within a Plexiglas heating box which "arterialises" venous blood, i.e. makes glucose measurements more similar to those obtained from an arterial rather than venous line. Blood glucose sampling takes place every 5-10 minutes and should be analysed rapidly using with a bedside glucose analyser to enable accurate adjustment of the glucose infusion rate. Even though computerised automated methods have been developed to predict the appropriate rate of glucose infusion, they have still not managed to replace an experienced operator using empirical adjustments. The target plasma glucose is 4-6 mmol/L.

The dose of the insulin infusion which is most commonly used is 40mU per square metre of the body surface area or 1mU per kg of body weight per minute. Plateau levels of insulin are achieved at approximately 120-180 minutes. The use of a priming dose of insulin at 10 minutes after the start of the clamp can reduce this interval to approximately 120 minutes which is the

duration of most glucose clamps. The insulin syringe should contain either 2 ml of the patient's blood or albumin solution to reduce the absorption of the hormone on plastic surfaces of the giving set. In the context of euglycaemia, urinary glucose losses are considered to be negligible but if necessary, they can be measured by collecting the subject's urine during the clamp.

The M value is usually the mean of the glucose infusion rates during the final 40-60 minutes of a 120-minute clamp. It is expressed as mg of glucose infused per minute and is a reflection of the amount of metabolised glucose. M can be normalised for total body weight, fat-free mass, resting energy expenditure or steady-state plasma insulin concentration. As a rough guide, an M value of <5 mg per kg body weight per minute indicates insulin resistance.

It is important for the insulin infusion to completely suppress the hepatic endogenous glucose production. In insulin-resistant subjects, this may not be the case and the M value could be underestimated. In order to avoid errors in calculations of the hepatic insulin sensitivity, a two-stage clamp with an isotope has been designed. During the first stage, insulin is infused at a low dose for 120 minutes (usually 0.3-0.5 mU / kg body weight / minute) followed by a high dose (usually 0.9-1.5 mU / kg body weight / minute). The mean of the M value during the last 30 minutes of each stage is, therefore, a marker of hepatic and peripheral insulin sensitivity respectively. The accuracy of the hepatic insulin sensitivity measurement is further enhanced by the use of stable isotopes, i.e. glucose labelled with deuterium: [6,6-$^{2}H_{2}$]-glucose. This stable, non-radioactive metabolite of glucose which naturally exists in the environment does not enter the liver pathway and is not metabolised [99]. It is infused for 120 minutes before the clamp in order to achieve a steady concentration in blood. Its infusion continues throughout the clamp at a stable rate and all exogenously administered glucose is also 'marked' with it. Any endogenously produced glucose during the clamp is not marked with the isotope and it will dilute the previously established stable concentration of an isotope. Hence this approach, called an isotope dilution technique, allows to distinguish endogenous from exogenous glucose and to calculate the rate of endogenous glucose appearance in the circulation (Ra)

[100]. Ra enables a very reliable estimation of endogenous glucose output, which establishes hepatic glucose sensitivity. Rate of glucose disappearance (Rd), refers to the rate of glucose uptake, i.e. peripheral insulin sensitivity. The enrichment of plasma samples obtained is determined with mass spectrometry.

The hyperinsulinaemic-euglycaemic clamp has an excellent reproducibility with a coefficient of variation of 6-15% [101] [102]. It became a gold-standard method of assessment of insulin sensitivity both in diagnostic research as well as in the development and assessment of glucose-lowering medications. All other insulin sensitivity assessment methods have been validated against the clamp. In terms of disadvantages, a small risk of hypoglycaemia exists, nonetheless, it can be minimised when the investigation is performed by an experienced operator. Clamps are also labour-intensive, time-consuming and very costly which makes their use unsuitable for large studies.

1.3. Management of Obesity and Type 2 Diabetes Mellitus

Since the aetiology and progression of obesity and obesity-related T2DM are interdependent in the majority of patients, many of their management strategies are similar.

The aim of “curing” obesity is often defined as achieving BMI of a normal range of 18-25 kg/m^2. It is a rather simplistic approach that does not take a number of other factors into account. Ideally, not just the absolute weight loss but also minimizing abdominal fat content and reducing total body adipose tissue to <33% in women and <25% in men should be aimed for [44]. Furthermore, remission of obesity-associated diseases such as hypertension, hypercholesterolaemia and fatty liver disease, reduction in pharmacotherapy and improvement in physical function should be accounted for when assessing the success of the obesity treatment.

When it comes to the definition of T2DM remission, various approaches have been reported, which makes the comparison of outcomes from various research groups somewhat difficult [103]. Currently, the most thorough and commonly applied criteria worldwide are the ones described by the American Diabetes Association (ADA) [104]. They define two thresholds of T2DM remission:

1. Partial T2DM remission – an achievement of HbA1c <6.5% (<48 mmol/mol) with fasting plasma glucose of 5.6-5.9 mmol/L in the absence of glucose-lowering medications for 1 year.
2. Complete T2DM remission – an achievement of a 'normal range' of HbA1c <6% (<42 mmol/mol) with fasting plasma glucose of <5.6 mmol/L in absence of glucose-lowering medications for 1 year.

With those targets in mind, several approaches to obesity and T2DM management have been developed. T2DM turns out to be a very heterogeneous disease, with recently reported 5 subtypes, therefore it is expected that response to treatment can vary vastly and needs to be adjusted on an individual basis [105].

1.3.1. Lifestyle interventions

Lifestyle management, including dietary modifications and increasing physical exercise levels, have been advocated as the first-line intervention by the ADA in order to reduce hyperglycaemia and cardiovascular risk [106]. It has been shown that a sustained loss of 5-10% of total body weight loss (TBWL) or 10kg can improve fasting glucose by 30-50% and generate a relative HbA1c reduction of 15%, simultaneously reducing the pharmacotherapy requirements and overall morbidity and T2DM-related mortality by 30-40% [107] [108] [109]. Therefore, maximising weight loss and improving exercise tolerance are the main targets of lifestyle interventions.

Dietary interventions aim not only to decrease the number of consumed calories but also to improve the quality of food. If introducing a deficit in calorie intake would translate directly into weight loss, daily reduction by 500 kcal would accumulate to 3500 kcal deficit per week,

equivalent to the amount of energy in 0.45 kg (1 pound) of adipose tissue [110]. However, this dietary restriction does not lead to a linear decline in body weight loss. It has been well established that the most rapid weight loss takes place in the initial stages of caloric restriction, followed by slowing down to plateau despite ongoing low-calorie diets [110] [111]. This can be explained by the fact that in response to a diminished calorie load, compensatory mechanisms are activated in order to prevent weight loss. They stimulate hunger, adaptive reduction in energy expenditure and as a result, reduce the extent of weight loss [112]. Such mechanisms can be evolutionarily explained, when historically the human race was more likely to suffer from hunger (with limited access to food) than from the abundance of nutrients, therefore developing physiological mechanisms which maintain body weight seems perfectly reasonable. Another theory explaining this phenomenon is the “set point” of one’s body weight [113]. This means that an individual’s metabolism is set to maintain a certain weight, therefore compensatory mechanisms will be triggered in periods of both over- and underfeeding, in order to maintain that weight.

We now know that it is not just the calorie count, but the macronutrient composition and the quality of the consumed product that matters. Hall et al. have shown that reduction in carbohydrate intake results in decreased insulin secretion, increased fat oxidation and more adipose tissue loss when compared to a eucaloric baseline diet. On the other hand, selective reduction in dietary fat intake leads to even higher loss of adipose tissue mass than the low-carbohydrate diet, despite no differences in insulin secretion or fat oxidation when compared to the eucaloric baseline diet [114]. Low-carbohydrate and high-protein diets have been shown to have a positive impact on improving glycaemic control, with the Mediterranean diet having the strongest impact. It was shown to decrease the need for the introduction of pharmacotherapy in newly diagnosed T2DM over 4 years when compared to the low-fat diet (HR 0.63, 95% CI 0.51, 0.86) [115].

Caloric restriction is relatively cheap and easy to implement in the primary care and its potential benefits have been shown in the DiRECT Trial. Here, 300 participants were

randomised in 1:1 ratio to either best-practice care or a total diet replacement with a very low calorie diet (of approximately 800 kcal/day) for 3-5 months, followed by gradual food re-introduction to a eucaloric diet with an intense follow up by the multidisciplinary team. In their intention-to-treat population, 24% participants achieved at least 15kg total body weight loss at 1 year in the intervention arm versus none in the control arm, with overall mean weight loss of 10 ± 8 kg in the first and 1 ± 3·7 kg in the latter group (adjusted difference -8.8 kg, 95% CI -10.3 to -7.3; p<0·0001). Diabetes remission was reported in 46% participants on the total diet replacement and only in 4% in the control group (OR 19.7, 95% CI 7.8-49.8; p<0·0001) [116]. However, the enthusiasm that these impressive results have triggered may be somewhat reduced by the fact that the long-term benefits after the intervention was ceased have not yet been proved. In a follow-up paper with 2 years follow up, the proportion of patients maintaining 15 kg weight loss dropped to 11% in the intervention arm versus 2% in the control arm and T2DM remission rates were reduced to 36% and 3% respectively [117]. It has to be noted that T2DM remission criteria applied here were less strict than the commonly used ADA criteria. Moreover, total diet replacement with a very low calorie diet is an intervention that is difficult to maintain. Even in the DiRECT trial settings, with an intense multidisciplinary team input, a 23%, high drop-out rate from the intervention group was reported, which makes it difficult to apply as an intervention in a population-level setting.

The Look AHEAD Trial investigated the impact of an intensive lifestyle intervention including diet modification and physical exercise versus diabetes support and education in over 5000 participants assigned to either of those two interventions. Initial promising weight loss of 8.6% at one year gradually decreased to 4.7% in a follow-up of the intensive lifestyle intervention at 4 years. Meeting the HbA1c targets of <7% (<53 mmol/mol) increased from 47% to 72% in the intervention arm versus 45% to 50% in the control arm at 1 year. However, with progressive weight regain, these rates decreased to 57% in the intervention arm and were maintained at 51% in the control arm [118]. Lifestyle interventions in the Look AHEAD Trial have not been shown to bring any cardiovascular benefits. Furthermore, this trial illustrates limited sustainability and efficacy of conservative management of both obesity and T2DM.

1.3.2. Pharmacotherapy for Obesity

Clinical guidelines state that pharmacological interventions should be only used in combination with other interventions such as exercise and diet.

Since 2010, Orlistat (Xenical®) is the only drug in the UK funded by the NHS that is recommended specifically for the management of obesity. Orlistat acts by reducing the absorption of dietary fat through selectively inhibiting pancreatic lipase. It is quoted to induce a weight loss of 2.9 kg (95% CI 2.5 to 3.2kg) [119] with reports of a maximum of 6.65 kg TBWL. Its side effects, predominantly flatulence and steatorrhea, are the main limitations to its use and lead to up to 29% cessation of treatment [120].

GLP-1 agonists, primarily prescribed for treatment of T2DM, also have a significant impact on weight loss. The most commonly used, liraglutide, comes in a dose of 3 mg daily injection (Saxenda) when prescribed specifically for obesity. A meta-analysis of 3 trials of liraglutide showed a maximum weight loss of 7.7 kg, with half of its impact on weight loss occurring within 3 months of treatment [120]. Another GLP-1 agonist, semaglutide, has been proven to facilitate TBWL of 13.8% at one year (when at a maximum dose of 0.4mg/day) versus 7.8% in liraglutide and 2.3% in placebo groups [121].

1.3.3. Pharmacotherapy for Type 2 Diabetes Mellitus

The aim of pharmacotherapy for T2DM is to minimise the degree of hyperglycaemia and by this to reduce the occurrence of disease complications. Several classes of glucose-lowering medications are currently recommended for use, either in mono- or in polytherapy with the aim of improving insulin sensitivity, insulin secretion or both [106]:

1. Biguanides

   The only available drug from this group is metformin. This most commonly used first-line glucose-lowering medication decreases endogenous hepatic glucose production, decreases intestinal glucose absorption and improves insulin sensitivity through

increasing glucose uptake and utilisation by the peripheral tissues [122]. It can lower HbA1c% levels by 1.4% and assist in weight reduction [123]. Its main side effects are gastrointestinal ones such as nausea, diarrhoea and abdominal pain and it should be used in caution in patients with increased risk of lactic acidosis (i.e. renal insufficiency, alcoholism) [124].

2. Sodium-glucose transport protein-2 (SGLT-2) inhibitors

   The most commonly used drugs from this group are empagliflozin, dapagliflozin and canagliflozin. By inhibiting sodium-glucose co-transporters in the kidney, they minimise glucose reabsorption in the renal system and increase its excretion with urine. Furthermore, they have been shown to reduce plasma glucose levels and also to have a positive impact on weight reduction. Canagliflozin, assessed in the CANVAS trial, has been shown to not only reduce HbA1c% by 0.6% and weight by 1.6kg but also to have a positive impact on blood pressure control and minimizing cardiovascular risk. However, it did increase the risk of amputation at the toe or metatarsal level [125]. Other risks of their use include renal dysfunction, urinary tract infections and genitourinary fungal infections [126] which can limit their use in a proportion of patients.

3. Sulfonylureas

   They act by binding to subunits of the potassium channel receptors and triggering their closure. By this, they stimulate membrane depolarization and insulin secretion. They can be specific to the receptors in the β-cell (gliclazide, tolbutamide) or can act both in the β-cells and on the receptors in cardiac, skeletal and smooth muscle (glibenclamide, glimepiride) [127]. They can induce HbA1c% reduction of 1-2% [128]. The large drawbacks of this group are the promotion of weight gain and risk of hypoglycaemia [129].

4. Thiazolidinediones

   They are peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists. They have a dual mechanism of action – they improve skeletal muscle insulin sensitivity and suppress endogenous glucose production in the liver, thus their additional benefit in the treatment of the fatty liver disease. Of the two most commonly used

thiazolidinediones, rosiglitazone has been withdrawn from use due to the increased risk of myocardial infarction. Pioglitazone can potentially increase the risk of bladder cancer. Furthermore, their well-documented side effects include weight gain, heart failure and increased risk of bone fractures. All of these factors contribute to their rare use nowadays [124].

5. Dipeptidyl peptidase-4 (DPP-4) inhibitors

   Dipeptidyl peptidase-4 is an enzyme which is responsible for the degradation of incretins, therefore its inhibitors enable to potentiate the effect of endogenous GLP-1 and GIP. Apart from linagliptin and vildagliptin, the most commonly used drug from this group is sitagliptin. They reduce HbA1c% by a mean of 0.7%. DPP-4 inhibitors are weight-neutral and possess a minimal risk of hypoglycaemia [130] however, they have not been shown to reduce mortality in treated subjects [131].

6. Glucagon-like peptide-1 (GLP-1) agonists

   GLP-1, an incretin hormone released from the gut neuroendocrine L-cells, stimulates insulin and suppresses glucagon secretion, slows down gastric emptying and increases satiety [132]. Synthetically produced agonists of its receptor, such as liraglutide, characterised by longer half-life due to resistance to DPP-4. have an analogous therapeutic profile and induce 1% to 1.5% reduction in HbA1c [133]. Furthermore, as shown in the SCALE Trial, liraglutide in a dose of 3mg/day, combined with lifestyle interventions, can reduce the risk of T2DM onset by 80% in patients with pre-diabetes over 3 years [134]. An additional benefit includes significant weight loss of up to 8% TBWL, as discussed above [121]. Furthermore, GLP-1 agonists have been shown to reduce cardiovascular (predominantly atherosclerotic events) and all-cause mortality [131]. Their main side effects are gastrointestinal ones, such as nausea and vomiting, but they can potentially also increase the risk of acute pancreatitis, cholangiocarcinoma and medullary thyroid cancer hence they are contraindicated when a family history of medullary thyroid cancer, multiple endocrine neoplasia syndrome type 2 or history of pancreatitis is present [18].

7. Insulin

Insulin is usually added to the T2DM pharmacotherapy at more advanced stages of the disease, where endogenous insulin secretion is markedly reduced, and its replacement is required. Intermediate- and long-acting insulins are most commonly used. The main risk of insulin use is hypoglycaemia, hence regular glucose monitoring is required. It is also known to cause weight gain [106].

Pharmacotherapy in obesity and T2DM includes not only the medications described above but also a range of medications used to treat associated comorbidities, such as antihypertensives, lipid-lowering and antiplatelet therapy, as frequently a polytherapy is required to minimise the long-term impact of these diseases and the cardiovascular risks.

1.4. Interventional Management of Obesity and Type 2 Diabetes Mellitus

With multifactorial aetiology of obesity and type 2 diabetes, effective medical treatment for both is yet to be found. As supported by a large body of evidence, the most effective and durable treatment for both of these diseases is obesity surgery [135-146]. Initially labelled as bariatric surgery (*báros* meaning weight in Greek), nowadays it is more often referred to as metabolic surgery, since it does not only promote weight loss, but it also produces powerful changes in the whole-body metabolism with a potential of inducing remission of T2DM, improving multisystem function and treating the end-organ damage. At least 11 RCTs and large prospective cohort studies with long-term follow up have shown its superiority to the optimal medical management of obesity and/or T2DM [135-145]. Metabolic surgery decreases morbidity and/or mortality from diseases such as T2DM and its microvascular complications, namely retinopathy, neuropathy and nephropathy [147], cardiovascular disease [148], obstructive sleep apnoea [149], non-alcoholic fatty liver disease [150], functional impairment [151, 152], infertility [153] and cancer [154].

1.4.1. Development of metabolic surgery

Attempts to introduce metabolic surgery to a wider clinical practice started over half a century ago. Kremen and Linner performed the first successful weight-loss procedure, a jejunoileal bypass, at the Varco University of Minnesota in 1953 [155]. This procedure was later abandoned due to significant morbidity and mortality associated with malabsorption. The first bariatric procedure in the UK, a jejunoileal bypass, was performed in Birmingham by Michael Baddeley in 1970 [156].

The first gastric bypass for weight loss, then believed to be a combined restrictive and malabsorptive procedure, was performed in 1966 by Mason and Ito [157]. Following that, experiments with various procedures and devices took place in order to optimise outcomes and minimise surgical risks. In 1980, Mason introduced a vertical banded gastroplasty, also known as the stomach stapling. It involved the formation of a small gastric pouch by vertical stapling of the upper part of the stomach and restricting its outlet with a non-adjustable gastric band. However, it soon lost its popularity due to poor outcomes and significant complication rates [158].

Biliopancreatic Diversion (BPD) is a complex surgery which was introduced by Nicola Scopinaro in 1976 [159]. It involves a horizontal resection of the stomach with a formation of a large (200-500 ml in capacity) gastric pouch and bypassing most of the small bowel, with alimentary limb of up to 250 cm in length and a long biliopancreatic limb anastomosed to the distal ileum 50-150 cm from the ileocaecal valve in order to form a very short common channel. To minimise its morbidity resulting from a significant degree of malabsorption, BPD was modified by introducing a Duodenal Switch (DS) in the 1990s [160, 161]. Here, gastric resection is performed vertically, enabling pyloric preservation (Figure 1). Despite initial enthusiasm with regards to outcomes of these procedures, such as over 90% rates of T2DM remission [162], nowadays they are not as commonly performed due to significant complications such as malnutrition and very high reoperation rates [163].

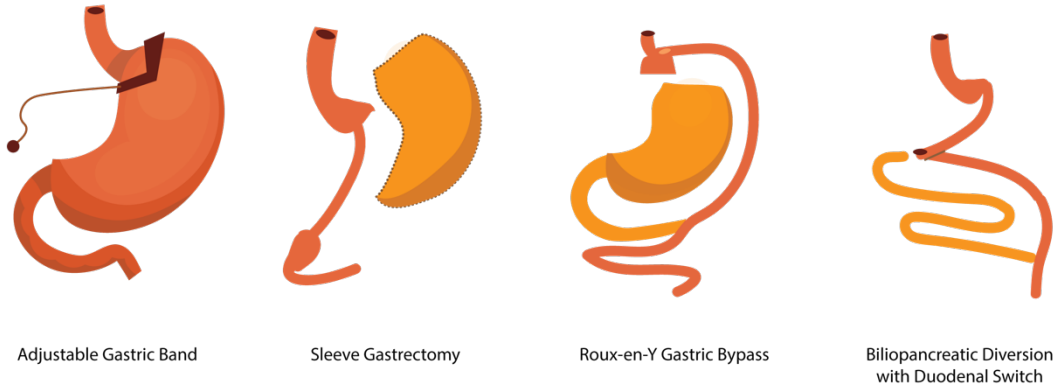


Figure 1: Schematic drawing of the most commonly performed metabolic surgical procedures. Courtesy of Mr Paul Cowley.

An adjustable gastric banding, where an inflatable band is secured around the upper part of the stomach to restrict its capacity (Figure 1), was first used by Kuzmak in 1983 [164]. It rapidly gained popularity across the globe, as this relatively easy to perform procedure provided reasonably good results of 18% TBWL and approximately 30% T2DM remission rate [165]. However, in the long-term, it proved to be often problematic due to device-associated complications such as slippage or erosion, which resulted in high reoperation and explantation rates [166]. Furthermore, a review of over 7000 patients with an adjustable gastric band in the PCORnet Cohort study demonstrated that at 5 years TBWL declines to 11.7% [167]. Due to the factors listed above, its utilisation has been on the decline over the recent years [168, 169].

The development of minimally invasive surgical techniques has revolutionised the surgical world and stimulated the rapid development of laparoscopic bariatric surgical techniques over the past three decades. A wide range of laparoscopic equipment, including a variety of stapling

devices, allowed a prompt progression from open to laparoscopic bariatric surgery with improved outcomes, including a reduction in morbidity and mortality [170, 171].

The first documented laparoscopic Roux-en-Y Gastric Bypass (RYGB) for treatment of obesity was performed by Wittgrove and Clarke in 1993 [172]. A quarter of a century later, it sustains its position as the gold-standard metabolic procedure in the UK and many other countries [168]. It involves the formation of an approximately 4cm long narrow gastric pouch (30-50 ml volume) formed over the lesser curve of the stomach which is anastomosed to a 100-150 cm long alimentary (Roux) small bowel limb. The alimentary limb is then anastomosed to a 50-100 cm long biliopancreatic limb (Figure 1). A segment of the small bowel distal to the jejuno-jejunal anastomosis of the alimentary and biliopancreatic limb is called the common channel and it varies in length depending on the total small bowel length (TSBL) on an individual level. Expected TBWL at 1 year is around 30% and settles at the level of approximately 25% at 5 years after the RYGB [167].

Vertical sleeve gastrectomy involves removal of 80% of the stomach with its size calibrated over an orogastric bougie. Stomach resection typically starts about 4cm from the pylorus on the greater curvature and serial applications of a stapler follow from that point towards the angle of His (Figure 1). This operation emerged as the first stage procedure from the biliopancreatic diversion with duodenal switch in morbidly obese patients [173]. When a substantial amount of weight loss was noted just after the sleeve gastrectomy, prior to proceeding to the second stage procedure, sleeve gastrectomy evolved to be a stand-alone obesity operation and has been gaining popularity over the past two decades [174]. According to the 4th IFSO Global Registry Report from 2018, sleeve gastrectomy has become the most frequently performed procedure for the treatment of obesity and associated diseases worldwide [169]. Two RCTs have even suggested that its outcomes are comparable to RYGB in terms of post-operative weight loss (reported excess weight loss (EWL) of over 60% in SM-BOSS Trial) [175] and T2DM remission (37% in SLEEVEPASS Trial) [176] [175]. However, the National Patient-Centred Clinical Research Network (PCORnet), the largest cohort study on over 46,000 patients, which investigated 5-year weight loss outcomes following the RYGB,

sleeve gastrectomy and gastric band, has shown that sleeve gastrectomy is inferior to RYGB in terms of weight loss outcomes both in short- and long-term: at 1 and 5 years TBWL was 25.2% and 18.8% after sleeve and 31.2% and 25.5% after RYGB respectively [167]. RYGB though was shown to result in twice as many major adverse events within 30 days post-operatively when compared to the sleeve (5% vs 2.6% respectively) [167].

One anastomosis gastric bypass, a variation of Mason and Ito's bypass, includes the formation of a long narrow gastric pouch over a lesser curve with a loop gastrojejunostomy at the inferior aspect the pouch to a 150-200 cm long biliopancreatic limb [177]. It has been advocated as technically less challenging than the RYGB, yet still providing good weight loss of 35%TBWL and T2DM remission of 60%, rates comparable to RYGB at 2 years post-operatively as presented in the YOMEGA Trial [178]. However, longer-term follow-up outcomes are awaited in this relatively new procedure.

Single anastomosis duodenoileal bypass (SADI), based on the principles of the biliopancreatic diversion, was first described by Torres over 10 years ago [179]. It involves sleeve gastrectomy and division of the first part of the duodenum, enabling pyloric preservation, hence Roux-en-Y type of reconstruction is not required. Duodenoileal anastomosis is then performed to a segment of small bowel measured 200-250 cm proximally to the ileocaecal valve. With EWL of 95% and T2DM remission reaching 100%, it seems to be a good procedure of choice for morbid obesity. However, significant technical challenges in its performance are limiting its application in a wide clinical practice [180] and longer-term follow-up data are still required.

All of the procedures described above have their specific risks and benefits, hence it is impossible to objectively define which surgery is superior to the others in the treatment of obesity, T2DM and associated comorbidities [171]. Nevertheless, obesity surgery is considered to be overall a safe treatment for obesity, with an in-hospital mortality rate in the UK of 0.07%, comparable to many other common major gastrointestinal procedures [168]. In

a clinical setting, the choice of surgery depends on a patient's comorbidities and BMI; as well as the patient's and surgeon's preferences.

1.4.2. Endoscopic Management of Obesity

Endoscopic interventions for obesity include intragastric balloon [181], gastric plication [182][183][184], endoscopic duodeno-jejunal bypass [185], gastroduodenojejunal bypass sleeve [186], duodenal mucosa resurfacing [187], Endo-Aspire gastrostomy [188], transpyloric shuttle, endoluminal magnetic partial jejunal diversion [189],

The endoscopic duodeno-jejunal bypass, EndoBarrier (GI Dynamics), is a 60 cm-long sleeve-like device anchored endoluminally in the proximal duodenum which precludes digestion and absorption of nutrients in the duodenum and proximal jejunum. By this, it is believed to partially simulate the impact of the RYGB, where the proximal small intestine is also bypassed. Its initial results were promising, with 5 kg body weight loss (or 13% EWL) over dietary intervention and with 0.9% HbA1c reduction [185] to even 35% of EWL at 1 year [181]. However, during several trials on the device, concerns were raised with regards to its safety (its major risks include hepatic abscess formation, device migration, intestinal obstruction and need for extraction via laparotomy, bleeding) and nowadays it is not licensed for use.

The aim of the Duodenal Mucosa Resurfacing procedure with Revita (DMR; Fractyl) is to stimulate a renewal of the duodenal mucosa by performing an endoscopic hydrothermal ablation. In 2016, the first human study on DMR was published. It showed promising results in terms of its impact on glycaemic control, with 1.2 ± 0.3% reduction in HbA1c at 6 months post-procedure with no significant weight loss. The mechanisms of DMR outcomes are currently under investigation. The main potential side effects include abdominal pain and duodenal stenosis [187].

1.5. Mechanisms of action of the Roux-en-Y Gastric Bypass

Roux-en-Y gastric bypass (RYGB) is considered by many the gold-standard procedure in the field of obesity surgery. Apart from its impact on the clinical practice, RYGB can also constitute a useful tool in research to learn about the impact of the gastrointestinal signalling on the physiology of weight and glycaemic regulation as well as metabolism [190], with the aims of:

1. Improving metabolic surgery procedures.
2. Improving endoscopic obesity procedures.
3. Developing targeted drugs development.

With those targets in mind, RYGB has been thoroughly investigated to review its mechanisms of action, which will be discussed below.

The main anatomical impact of the RYGB involves excluding the majority of the stomach as well as the proximal small intestine from nutrient contact (biliopancreatic limb) and digestion (biliopancreatic and alimentary limb). Although it was initially thought that RYGB worked by nutrient malabsorption with physical restriction of calorie intake, this theory was proved to be inaccurate. Firstly, if it was indeed a 'restrictive' procedure, it would promote increased hunger as a reaction to a negative energy balance, a result of a decreased calorie intake [191] [192]. In practice, an opposite phenomenon takes place following RYGB, where patients report lower hunger levels post-operatively, despite decreased caloric intake [190]. Secondly, forming larger gastric pouches would result in inferior post-operative outcomes due to theoretically less "physical restriction" however, the size of the gastric pouch in RYGB has been shown to have no major impact on post-operative outcomes [193]. If malabsorption was the primary mechanism of action of the RYGB, patients would be losing a much larger amount of calories in stool after the surgery and a significant proportion of patients would develop an excessive weight loss with significant micro- and macronutrient deficiencies but yet again, this rarely takes place [194].

Mechanisms of the metabolic impact of obesity surgery are in fact much more complex. These mechanisms can be divided into weight loss-independent and weight loss-dependent. The

former occur early post-operatively, before a substantial weight loss taking place, and when maintained in the long-term, they are not related to the extent of the post-operative weight loss. The latter become apparent at later stages after the operation, after a considerable amount of weight has been lost.

1.5.1. Effects of RYGB in clinical studies

Bypass of the proximal small bowel is believed to play a major role in regulating both glycaemic homeostasis and weight loss, as illustrated by a number of clinical studies. Therefore, RYGB is expected to result in better metabolic outcomes than an adjustable gastric band or a vertical sleeve gastrectomy. RCTs have consistently shown superior EWL after RYGB of 51-67% at 1-3 years when compared to the adjustable gastric banding (EWL 35-38% in the same follow-up period) [195]. The reported difference in EWL between the two procedures is 19-34% [196]. The previously mentioned largest retrospective PCORnet cohort study of over 65,000 bariatric surgical patients, reports 31.2% TWBL at 1 year after the RYGB (95% CI 31.1-31.3%), 25.2% (95% CI 25.1-25.4%) after sleeve gastrectomy and 13.7% (95% CI 13.3-14%) after the adjustable gastric band [167]. Even though Schauer's STAMPEDE Trial has shown that the amount of weight loss decreases with a longer follow-up, it is still superior in RYGB when compared to sleeve gastrectomy at 5 years (absolute weight loss of -23.2 ± 9.6 kg vs 18.6 ± 7.5 kg, p=0.01) [137].

Furthermore, RYGB is more potent in introducing T2DM remission, with up to 78% achieving it versus 50% after the band [196] but since weight loss after RYGB exceeds the one achieved after a band, these results can be weight-dependent. However, the weight-independent impact of RYGB on glucose homeostasis has been presented in 2003 by Schauer et al., when 30% of 240 RYGB patients with a pre-operative diagnosis of T2DM recorded normalization of glucose excursions and were able to discontinue all glucose-lowering medications within 3 days after the surgery [197].

Biliopancreatic diversion (BPD) can serve as the basis for the theory of the importance of the proximal small intestinal bypass. The main differences between the BPD and RYGB are a longer bypass of the proximal small intestine (the length of the biliopancreatic limb in the BPD depends on the total small bowel length and it can frequently be above 200 cm with an alimentary limb of up to 250 cm) and as a result, a shorter common channel (50-150 cm in the BPD, 300-500 cm on average in the RYGB). Mingrone et al. have clearly shown that, at matched weight loss (EWL of 69.4 ± 17.6% at 2 years after BPD and 68.1 ± 12.7% after RYGB), the impact of the BPD on glycaemic control is superior to the one delivered by the RYGB with a relative reduction in HbA1c of 43 ± 9.6% versus 25.2 ±. 20.9% ($p<0.001$) [198]. This illustrates well weight loss-independent effects of metabolic surgery on glycaemic control. Higher T2DM remission rates of 63% in BPD group versus 50% in the RYGB group were maintained at 5 years post-operatively [139], which suggests that a longer proximal small bowel bypass provides superior glycaemic control outcomes both in the short- and longer-term follow-up. Superiority of the proximal intestinal bypass procedures has been confirmed by Buchwald et al. in a large meta-analysis of 136 studies involving over 22,000 patients, where RYGB and BPD or duodenal switch resulted in EWL of 61.6% (56.7%-66.5%) and 70.1% (66.3%-73.9%) respectively, and gastric band in 47.5% EWL (40.7%-54.2%) [199]. Reported T2DM remission rates were 98.9% (95% CI, 96.8%-100%), 83.7% (95% CI, 77.3%-90.1%) and 47.9% (95% CI, 29.1%-66.7%) respectively.

Another procedure involving proximal small bowel bypass, one-anastomosis gastric bypass, also proved to be an effective intervention in the treatment of obesity (35% TBWL at 2 years) and T2DM (60% remission rate). It involves a formation of a larger gastric pouch but also a longer biliopancreatic limb (150-200 cm) than the RYGB, which is believed to play a crucial role in its excellent results.

Similarly, procedures that do not involve any surgical manipulation of the gastrointestinal tract but act in the proximal small bowel have a potential of inducing changes in glucose homeostasis without (Duodenal Mucosa Resurfacing, DMR) or with (EndoBarrier) concurrent

moderate weight loss. Six months after the stimulation of duodenal resurfacing with DMR, 1.2 ± 0.3% reduction in HbA1c% has been observed. Endoscopic duodeno-jejunal bypass with an EndoBarrier intraluminal sleeve can result in 0.8% [200] to 0.9% HbA1c% reduction [185] at 12 months with a simultaneous weight loss of 5kg [185] to 15kg [200].

As illustrated above, mechanisms involved in the remission of T2DM after obesity surgery are both independent and dependent of weight loss. The former is reiterated by the fact that improvement in glycaemic control after obesity surgery occurs before any significant weight loss takes place [201] and it is superior in BPD over the RYGB at matched weight loss [198]. A well-known phenomenon of stopping all glucose-lowering medications within days after the RYGB or BPD when only 1-2% of body weight has been lost has been reported in 30-100% bariatric surgical patients with T2DM [202] [203] [197]. Moreover, metabolic surgery is also effective in treating T2DM in the absence of obesity [204]. Weight-dependent mechanisms play certainly an important role in improving glucose homeostasis by diminishing fat deposits and glucose and lipid toxicity, decreasing inflammation associated with fat tissue excess and improving insulin sensitivity. Impact of weight-dependent mechanisms is reiterated not only by the fact that the significant improvement in glycaemic control increases with decreasing weight post-operatively, but also by the fact that relapse of T2DM or worsening of glycaemic control after the surgery is associated with weight regain [65]. Sjoholm et al have also confirmed the importance of the degree of weight loss on glucose metabolism in long-term follow up. In their analysis of the SOS data, they demonstrated that it was predominantly the degree of weight loss, not the type of surgery, that had an impact of glucose homeostasis within 10 years after the RYGB, gastric banded or vertical gastroplasty [205].

In order to understand how such remarkable metabolic outcomes are possible, a review of the mechanistic studies in RYGB is presented below.

1.5.2. Metabolic effects of RYGB in the mechanistic studies

A number of mechanisms, which can be activated either in presence or absence of weight loss after metabolic surgery, have a major impact on the whole-body metabolism after RYGB. Metabolic post-operative changes are a result of the interplay of changes in insulin secretion and sensitivity, gut nutrient sensing and neural signalling, glucose absorption from the intestine and its intestinal utilisation, enteroplasticity, energy expenditure, hunger and fullness and food preferences. The main mediators that play a role in these processes are gut hormones, bile acids and gut microbiota. There is also a theory created by Prof Francesco Rubino about the anti-incretin factor that could be synthetized in the proximal small intestine, the effect of which is alleviated by bypassing this segment of bowel [206]. However, no such factor has yet been identified in the 15 years since this theory has been created.

1.5.2.1. Insulin secretion

Anatomical rearrangement after the RYGB leads to a rapid glucose absorption and early peak in blood glycaemia [207]. This stimulates a higher and earlier than the pre-operative peak in insulin secretion, which has been proved to be the result of the incretin effect (it will be described further below in section 1.5.3.1). Most studies report an increase in glucose- or meal-stimulated post-prandial insulin area under the curve (AUC) at 15% or more TBWL after the obesity surgery. However, early post-operatively, before 10% TBWL is achieved, an increase in insulin AUC has been reported after the BPD, but not RYGB, sleeve or gastric band [65]. Disposition index, which is a measure of the response of insulin sensitivity and insulin secretion to a glucose challenge, has been shown to increase after RYGB and gastric band after moderate to substantial weight loss takes place [65].

1.5.2.2. Insulin sensitivity

Weight loss due to caloric restriction is one of the main contributors to the improvement of glucose homeostasis after obesity surgery. Improvement in insulin sensitivity can be observed during a very low-calorie diet alone (usually up to 600-800 kcal/day) and some researchers believe that this is the main mechanism leading to improved glucose homeostasis post-

operatively, through a reduction of adipose tissue infiltration of the liver and the pancreas [208]. As little as 4-10 days of a very low-calorie diet of under 800 kcal/day can rapidly improve hepatic insulin sensitivity, demonstrated by the lower levels of fasting plasma glucose, insulin concentrations and HOMA-IR (Homeostasis Model Assessment for Insulin Resistance) [65]. There are studies demonstrating indifferent insulin sensitivity in the early days after the RYGB and a very low-calorie diet, at a matched small degree of weight loss [209] [210]. However, these studies used only crude indicators of insulin sensitivity, such as HOMA-IR. Furthermore, the stress response present after the surgery is expected to increase blood glycaemia in response to a higher concentration of circulating catecholamines [211], yet this has not been observed after obesity surgery [212]. Moreover, patients are calorie-restricted for only a relatively short period after the surgery and when their diet is reviewed beyond one year post-operatively, their caloric intake is several times higher than in the early post-operative days, yet they maintain the weight loss and improved glycaemic control.

Strong evidence of the post-RYGB glucose homeostasis changes independent of the reduced caloric intake after the surgery has been elegantly shown by Mingrone's group. After assessing hepatic and peripheral insulin sensitivity with a hyperinsulinaemic-euglycaemic clamp during a very low-calorie diet, they repeated the study one week after the surgery and found superior outcomes after RYGB when compared to the adjustable gastric band as well as the caloric restriction on its own [213]. Early post-operative improvement in insulin sensitivity independent of caloric restriction after the RYGB, but not band or sleeve, was also shown by Kashyap et al. [214].

With progressive weight loss after RYGB, even more, profound increase in insulin sensitivity can be observed, with many researchers reporting improvement in peripheral insulin sensitivity only after a minimum of 20-40% TBWL has been achieved [65]. This indicates the contribution of weight-dependent mechanisms at further post-operative stages. However, BPD has been shown to consistently improve both hepatic and peripheral insulin sensitivity early after the surgery (1-2 weeks) [202], before any substantial weight loss takes place.

A majority of the studies did not report improvement in peripheral insulin sensitivity after RYGB until several months after the surgery when weight loss of at least 15% TBWL was recorded [215] [216] [217] [217]. This indicates that a longer bypass of proximal bowel can trigger weight-independent mechanisms leading to an improvement in the peripheral insulin sensitivity.

The literature review regarding hepatic, peripheral or whole-body insulin sensitivity and all studies investigating the impact of RYGB with hyperinsulinaemic-euglycaemic clamps with isotopes (a technique used in this study, explained in detail in the Methodology), with a couple of examples of studies without an isotope or after BPD are presented in Table 1.

Authors	Sample	Technique	Post-op follow-up	Results
Hepatic & peripheral insulin sensitivity improvement early post-RYGB				
Gastadelli 2016 [213]	10 RYGB 10 LAGB + VLCD in same subjects 3months pre-op	[6,6-$^2H_2$] glucose bolus (22 mmol/kg) → constant infusion 0.22 mmol/kg/min → tracer gradually decreased after insulin infusion start by 50% and stopped at 30mins Insulin: 2h of 40 mU/min/m^2 + 20% glucose with [6,6-2H2] isotope Insulin secretion measured with a bolus of glucose (IVGTT) at the end of clamp Hep-IS = 1/(EGP · insulin)]	1week (5kg WL)	VLCD: No change in Hep-IS LAGB: ↓basal EGP and Hep.IS ↑ only **RYGB early post-op:** ↓basal EGP and Hep.IS ↑ & ↑Periph.IS
Hepatic insulin sensitivity improvement post-RYGB				
Dunn 2012 [215]	40 RYGB ± omentectomy (incl.17 DM)	(3-3H) glucose for 2.5hr (basal) – EGP calculated from last 30mins → insulin infusion 4 mU/kg/min for 8 mins → 2.4 mU/kg/min for 2hrs. – M from last 30mins	1 month (11% TBWL)	**RYGB early post-op:** ↓ EGP & ↑ Hep.IS (more in DM) No change in Periph.IS

Camastra 2011 [218]	RYGB: 13 DM + 12 non-DM Controls: 8 lean + 14 obese	[6,6-$^2H_2$] glucose infusion at 0.22 µmol/min/kg → down to 0.11 at 0 timepoint + 2[H^5] glycerol 0.01 mg/min/kg for 3hrs → insulin: 240 pmol/min/m^2 for 3hr in DM or 2hr in non-DM + 20% glucose with isotope M=GIR in last 40 mins, corrected for changes in glucose concentration in distribution of 200ml/kg for FFM or energy expenditure TGD=tracer from last 40mins corrected for FFM	2 weeks (4% TBWL) & 1 year (35% TBWL)	**RYGB early post-op:** No change in Hep.IS & Periph.IS **RYGB late post-op:** ↑ Hep.IS & Periph.IS proportional to weight loss NO evidence of weight loss-independent mechanism
Anderwald 2012 [216] and Promintzer 2011 [219]	6 RYGB non-DM, BMI > 40 Lean & obese controls	[6,6-$^2H_2$] glucose for 5 mins at 4 mg/FFM → 0.04 mg/min/FFM for 2hrs Insulin infusion: 40 mU/min/m^2 BSA for 140mins EGP calculated from timeponts-120, -5, 0, 80, 100, 120 M/I – last 40mins Basal Hep.IS = 100 / (EGP*basal insulin secretion) Hepatic IS in clamp=duration of halving EGP by insulin infusion	7 months (35kg WL)	**RYGB late post-op:** Slightly ↑ whole-body IS Basal Hep.IS ↑ post-RYGB but insulin-mediated EGP remains impaired

Bojsen-Moller 2017 [217] and Bojsen-Moller 2014 [220]	RYGB 10 DM & 10 non-DM	[6,6-$^{2}H_2$] glucose primed basal infusion for 2 hrs at 0.036 mg/kg/min → ↓ to 25% at time 0 Insulin: primed-continuous 40 mU/m_2/min + 20% glucose enriched with [6,6-2H2]-glucose Rd in the last 30 min: non-steady-state equations, mg/min/kg FFM & corrected Rd/I HISI: Ra(mg/min) in last 30mins of basal inf. HISI=10^6 / [Ra * C-peptide]	1 week (4% TBWL), 3 months (15% TBWL), 1 year (22% TBWL in DM, 28% in non-DM)	**RYGB early post-op:** ↓ EGP, ↑ basal Hep.IS (HISI ↑ by 60%) and. ↑ insulin clearance Periph.IS unchanged in DM; non-DM: ↓Rd, but Rd/I unchanged **RYGB late post-op:** 3 & 12M: IS ↑ HISI ↑ by 100% at 3M & 1Y
Fabrini 2010 [221]	22 RYGB± omentectomy DM & non-DM vs 10 omentectomy in obese DM	Primed infusion of [3-3H] glucose at 0.14 μCi/min for 2.5 hrs Insulin infusion: 2hrs of 2 mU/kg/min (with priming of 4 mU/kg /min for 8 mins) To account for surgical weight loss-induced ↓ in basal plasma insulin & ↑ in insulin clearance, the rate of insulin infusion in clamp post-op was empirically ↑: 2.75 at 6 months & 3.1 at 12 months	6 months (27% TBWL) & 12 months (34% TBWL)	**RYGB late post-op:** ↑ Hep.IS & Periph.IS HISI ↑ 4x and Periph.IS x2 at 1yr (already improved at 6M)

Weijer 2013 [222]	18 F RYGB	0.11 μmol/kg/min [6,6-$^2H_2$] glucose & glycerol isotope Insulin infusion: 20 mU/m2 BSA/min for 2hrs → 60 mU/m2 BSA/min for 4hrs 20% glucose with [6,6-$^2H_2$] Hep-IS=suppression of EPG in 1st phase Rd corrected for insulin as serum insulin levels lower in 2nd phase post-op	2 weeks (8kg WL)	**RYGB early post-op:** ↓basal EGP but no change in Hep.IS or Periph.IS
Hansen 2015 [223]	RYGB: 16 DM + 17 non-DM	[6,6-$^2H_2$] glucose bolus of 23 μmol/FFM → infusion of 0.55 μmol/FFM/min If the fasting glucose was >5 mmol/l, prime bolus was ↑ (plasma glucose (mmol/l) * 5^{-1}) Insulin infusion 80 mU/m^2 for 2.5 h with bolus of 23 μmol [6,6-$^2H_2$] glucose/ FFM, inf 1.65 μmol/FFM/min OR NO isotope (18M) – 120 mins clamp	4 months & 18 months (35-40 kg WL)	1/3 of GIR (whole-body IS) improvement after diet (6kg WL) but non-significant **RYGB late post-op:** ↑whole-body IS & Hep-IS

Tamboli 2014 [224]	RYGB: 22 DM + 23 non-DM	primed (33 μCi), continuous 0.14 μCi/min inf of [3-3H] glucose for 2.5 h (basal period) followed by primed insulin infusion for 2hrs: pre-RYGB, 2.3 1 month 2.5 6 months, 2.9 1 year 3.3 2 years 3.3 μU/ml Sampling last 30mins basal (HISI) & clamp (M/I=periph.IS)	1 month (11kg), 6 months (27kg), 1 year (33kg), 2years (33kg)	**RYGB early post-op:** 1 month: Periph-IS unchanged; HISI ↑ **RYGB late post-op:** ↑ Periph-IS from 6M onwards (stable 1-2yrs) HISI ↑ at 6M, then remained stable
Studies with no isotope				
Guidone 2006 [202]	10 BPD DM with 1800kcal TPN for 6 days post-op	Insulin infusion 6 pmol/min/kg M in last 40mns of 2hr clamp NO isotope	1 week (6 kg WL) & 4 weeks (16kg WL)	**BPD early post-op:** Normalised IS with no change in insulin clearance
Kashyap 2010 [214]	9 RYGB, 7 SG or LAGB, all DM	HYPERGLYCAEMIC clamp for 2hrs: bolus of glucose 0.3mg/kg	1 & 4 weeks	**RYGB early post-op**: ↑ IS at 1 & 4 weeks

		given over 2min; glucose maintained at125mg/100ml over basal levels NO isotope		Pre-op test during 800kcal diet-changes independent of caloric restriction
Salinari 2013 [225]	RYGB: 7 DM + 7 non-DM + 6 controls	Insulin infusion: 6 pmol/min/kg NO isotope	1 month	**RYGB early post-op**: IS improved in all subjects
Lima, 2010 [226]	RYGB in females: 6 DM, 7 IGT, 6 non-DM	Ins.40mU/m2/min for 3hrs M=GIR in last 60 mins corrected for glucose distribution space & adjusted for FFM NO isotope	1 month	**RYGB early post-op:** No change in IS (assessed only peripheral)
Campos, 2010 [227]	12 RYGB + 10 caloric restriction	Ins.40mU/m2/min for 2hrs NO isotope	2 weeks (10 & 8kg WL in RYGB & diet) 6 months (28kg=50% EWL% in RYGB)	**RYGB early post-op:** No change in Periph.IS **RYGB late:** ↑ Periph.IS (assessed only Periph.IS)

Table 1. Literature review of studies utilising hyperinsulinaemic-euglycaemic clamps in the assessment of insulin sensitivity after the Roux-en-Y gastric bypass and biliopancreatic diversion.

RYGB – Roux-en-Y gastric bypass, IS – insulin sensitivity, Periph – peripheral, GIR – glucose infusion rate (i.e. insulin-mediated whole-body glucose uptake), IGT – impaired glucose tolerance, EGP – endogenous glucose production HISI – hepatic insulin sensitivity index (inverse product of EGP and fasting serum insulin), M – insulin sensitivity (glucose disposal rate per kg of body weight), M/I – whole-body insulin sensitivity, TGD – total glucose disposal, IR – insulin resistance, WL – weight loss, EWL – excess weight loss, TBWL – total body weight loss

To summarise the literature review from Table 1, a majority of studies report an increase in the hepatic or cumulative whole-body (depending on the clamps technique used) insulin sensitivity in early stages after the RYGB, i.e. before any substantial weight loss has taken place. This phenomenon implies weight loss-independent mechanisms, especially when an improvement in insulin sensitivity after the RYGB was proved superior to a comparable very low-calorie diet on its own. Secondly, a preponderance of the studies found a rise in the peripheral or cumulative whole-body insulin sensitivity only late post-operatively, i.e. months or years after the intervention, when significant weight loss of a minimum of 15% TBWL took place.

1.5.2.3. Nutrient sensing and neural signalling

Nutrient sensing plays an important role in mediating the anorexigenic and glucose-regulating signalling to the brain, liver and pancreas [228]. In the past, taste and nutrient sensors were believed to be localised only in the oral cavity, however their distribution in the lower parts of the alimentary tract (intestine) has now also been proved [229]. G protein-coupled taste and nutrient receptors that respond to sweet and bitter stimulants as well as fatty acids, similar to those found on the tongue, have been identified in the enteroendocrine cells in the intestinal mucosa. Therefore, chemosensory mechanisms are involved in the regulation of gut hormone secretion and as a consequence, influence energy homeostasis and glucose metabolism [230]. Glucose and lipids are believed to be the main triggers in activating the gut-brain-liver/pancreas axis in this process. Glucose stimulates GLP-1 secretion from the enteroendocrine L-cells via the sodium-coupled glucose transporter-1 (SGLT-1) receptors. On

the other hand, ingestion of lipids leads to stimulation of the vagus nerve by the esterified long-chain free fatty acids converted to fatty acyl-coenzyme A molecules and cholecystokinin which in turn suppresses hepatic glucose output [231] [232]. These processes are believed to be augmented following an anatomical rearrangement of the gastrointestinal tract in RYGB [212]. The impact of gastrointestinal manipulation on nutrient sensing has been investigated in a number of studies. Wang et al. demonstrated that a direct intraduodenal infusion of lipids in rats activates a gut-brain-liver neurocircuit by the fatty acyl-coenzyme A molecules via the vagus nerve, which results in decreased hepatic glucose output and increased hepatic insulin sensitivity [233]. This phenomenon seems to fit with the hindgut theory, where enhanced stimulation takes place due to faster nutrient delivery to jejunum and ileum. The ability of jejunal nutrient sensing to improve blood glycaemia dramatically, independently of weight loss in rodents after a duodenal-jejunal bypass was presented well by Baud et al. [234]. Furthermore, vagotomy disrupts nutrient-triggered negative feedback of food intake regulation and impairs glucose homeostasis [235].

1.5.2.4. Role of the gut: enteroplasticity, intestinal glucose absorption and gluconeogenesis

Epithelial cell turnover time in the intestine is estimated to be as fast as 3-5 days [236]. Proximal segments of the small intestine (duodenum and jejunum) absorb predominantly the macronutrients, whereas distal ones (ileum) are mainly responsible for micronutrient absorption. Surgical rearrangement of the bowel segments has been shown to promote intestinal adaptation, i.e. enteroplasticity. RYGB has been shown to have a potent impact on it, mainly by stimulating cell proliferation in the alimentary and common limbs, raising the height of villae and crypt depth [237] as well as increasing biliopancreatic limb diameter [238]. GLP-2 is believed to play a major role here. By promoting epithelial cell proliferation and inhibition of apoptosis as a result, it increases the absorptive surface area of the intestine [239]. Redirection of nutrient flow is believed to be the major trigger in this process since other procedures such as EndoBarrier and ileal interposition also result in hyperplasia of certain segments of the bowel (as well as leading to improved glucose homeostasis). Especially

important in the process of post-operative intestinal remodelling are changes to gut hormone-producing enteroendocrine cells. Studies utilising intra- and post-operative intestinal biopsies have shown that the expression of gene encoding preproglucagon (GCG), which is a substrate for GLP-1 production, as well as density of the GLP-1 producing cells increases after RYGB as soon as 4 months after the surgery. Increased density of these enteroendocrine cells in the common limb has been linked to an increased flow of nutrients (including fatty acids, carbohydrates and oligopeptides) and bile through its lumen, all known to be secretagogues of GLP-1 and thus, stimulators of GCG expression. These findings have been reported in patients with and without T2DM and are consistent with elevated postprandial plasma levels of GLP-1 after RYGB [240]. However, the most recent work by Fiona Gribble's group demonstrated contradictory findings, with no post-operative changes in the enteroendocrine cells density neither in mice nor in lean humans. Here it is believed that the driver for an enhanced postprandial GLP-1 peak is solely a direct stimulation of a larger number of L cells by amplified nutrient transit to the distal gut. However, the human part of this study was performed only in lean individuals undergoing resection for cancer (including total gastrectomy) and RYGB reconstruction, not as an obesity procedure [241]. Rodent studies confirmed that exposure of the alimentary limb after the RYGB to the undigested nutrients stimulates its hyperplasia and hypertrophy through increased circulating glucose uptake via the GLUT1 receptors. Furthermore, through the process of intestinal reprogramming of glucose metabolism, increased utilisation of glucose by the hypertrophied intestine takes place and contributes to the systemic lowering of circulating plasma glucose [242].

On the other hand, Francois Pattou's studies on minipigs undergoing RYGB showed that the intestinal glucose uptake from intraluminal undigested nutrients in the alimentary limb is significantly decreased. By applying a bowel clamp to the distal part of the alimentary limb, intraluminal glucose absorption in this segment after a meal could be studied and it was negligible. However, when the clamp was taken off and the nutrients were transferred with peristalsis to the common limb, a rapid increase in blood glycaemia was observed. Post-RYGB diversion of bile flow and gastric secretions led to low intraluminal sodium in the alimentary

limb, which was shown to be responsible for the decreased glucose absorption via the glucose-sodium co-transporters. The only bowel segment where glucose absorption is possible is the common limb, thus reduction of the absorption surface and time is believed to lead to the improved glucose excursions post-operatively, independently of weight loss [243].

Intestinal gluconeogenesis has been shown to suppress appetite and promote improvement in whole-body glucose utilisation [244]. Gluconeogenesis within the intestine after RYGB has been shown to increase, and through releasing synthetised glucose into the portal circulation, suppresses hepatic glucose production via a GLUT-2 dependent pathway. This process is observed early after the surgery and is dependent on the anatomical rearrangement of the gut, not on the weight loss induced by the surgery [245].

1.5.2.5. Energy expenditure

Food restriction resulting in negative energy balance leads to a decrease in energy expenditure in order to minimise its impact on weight [190]. An excessive reduction in energy expenditure has been correlated with poor long-term weight loss results [246, 247]. However, after RYGB in rodents, in the initial 4 post-operative weeks when decreased calorie intake and rapid weight loss take place, energy expenditure remarkably increases [248, 249]. Some human studies have also shown an increase in resting energy expenditure when adjusted for the amount of lost fat and fat-free mass [250, 251] however, a consensus is that in humans RYGB tends to decrease the overall oxygen utilisation and basic metabolic rate. A balance between those changes was elegantly presented by Prof Carel le Roux's group where, apart from demonstrating reduced basic metabolic rate and unchanged non-activity related thermogenesis, an increase in postprandial energy expenditure when corrected for body composition was shown [252].

1.5.2.6. Hunger and fullness

After metabolic surgery, humans and rodents consume less due to decreased hunger (not as previously thought due to physical restriction of stomach capacity), despite losing weight. This

phenomenon is opposite to the response to a calorie-restricting diet on its own and seems to happen due to the fact that surgery might be lowering the previously mentioned 'set point'. Once the new 'set point' weight is achieved, mechanisms such as increased hunger and food intake are triggered in order to maintain it. Hence if caloric restriction is forced after the new 'set point' has been achieved, rodents will make up for the excessive weight loss as soon as ad libitum diet is reintroduced [253]. Furthermore, in pregnancy, female rodents after metabolic surgery change the 'set point' again and physiological weight gain takes place [254]. The same process has been observed in humans. Increased output of "satiety hormones", namely GLP-1, oxyntomodulin and PYY are believed to be the mediators of hunger suppression.

1.5.2.7. Food preference

Changes in food preferences have been reported both in animal and human studies. Rodents that undergo RYGB or a sleeve gastrectomy display a shift in food choices from palatable calorie-dense diets to the less caloric but carbohydrate-rich ones [255] [256]. In humans, food preference assessments are inevitably burdened with some bias introduced by the peri-operative dietary counselling with motivation to eat smaller amounts, make healthy dietary choices and lose weight. However, evaluation with functional MRI in humans still confirmed a reduction of neural activity in preference to calorie-dense food [257].

1.5.3. Mediators of the RYGB impact

1.5.3.1. Gut hormones

Pories et al. were the first researchers to link the gastric bypass to T2DM remission due to the enteroendocrine changes [57]. Enteroendocrine cells, which form approximately 1% of the intestinal epithelium [258], secrete potent signals that regulate whole-body metabolism, including glucose and insulin homeostasis and energy balance. Their apical side is exposed to the intraluminal contents of the gastrointestinal tract, whereas the basolateral side, which

is proximal to the innervation of the intestine, secretes peptides and stimulates local paracrine signalling [259]. Traditionally, enteroendocrine cells have been characterised by the peptides they secrete. L-cells, predominantly placed in the terminal ileum and colon, but also duodenum and throughout the small intestine, with their density increasing distally, produce incretin GLP-1. K-cells, which are preponderant in the duodenum, secrete the other incretin, GIP. Stimulation of these enteroendocrine cells by the intraluminal nutrients leads to a surge in their release which stimulates β-cells to secreting insulin. This incretin effect has been nicely demonstrated when comparing intravenous and oral stimulation with glucose which resulted in higher serum insulin levels in the latter [65] and it has been shown to contribute to up to 70% insulin release after the oral glucose challenge. GLP-1 has been consistently shown to increase after RYGB. Its incretin effect post-operatively has been confirmed after RYGB by blocking GLP-1 receptors with exendin (9-39) and subsequently observing decreased insulin secretion rate in a hyperglycaemic clamp [260]. A study comparing postprandial GLP-1 excursions depending on the route of administration 5 weeks after RYGB (transoral versus gastroduodenal via the gastric remnant gastrostomy) has shown a nearly fivefold surge in GLP-1 release after transoral meal administration. This is believed to be the consequence of gastrointestinal tract anatomical rearrangement and increased nutrient transit, causing a direct stimulation of L-cells in the distal segments of the small intestine and colon. As a consequence, glucose homeostasis and insulin secretion improve independently of weight loss, changes in insulin sensitivity or caloric restriction [207].

GLP-1 is believed not only to stimulate glucose-dependent insulin secretion and biosynthesis but also to suppress hunger, slow down gastric emptying, alter food preference and even support the suppression of glucagon and prevent hyperglycaemia in obesity. GIP also promotes satiety and glucose conversion to fatty acids and their storage in the adipose tissue by activation of lipoprotein lipase [261].

GLP-2, also released from the L-cells in the ileum and stimulated by nutrients in the intestinal lumen, promotes gut hypertrophy after RYGB [239] and slows down motility to facilitate

nutrient absorption. GLP-2 concentrations after RYGB in humans and rodents seem to be increased, however, these results are inconsistent [262].

PYY secreted from the L-cells in the distal ileum and the colon is a major contributor to stimulation of satiety and reducing gastric emptying and intestinal motility [263]. Early PYY increase within two weeks after the RYGB has been documented and these levels are maintained beyond one year [262].

Oxyntomodulin, synthesized in the ileal L-cells, promotes satiety, increases energy expenditure and acts as a GLP-1 receptor agonist. A marked increase in postprandial oxyntomodulin has been reported after RYGB [262]. The impact of GLP-1, oxyntomodulin and PYY has been nicely demonstrated by Behary et al., where an infusion of these three hormones combined induced over 4kg of weight loss and improvement in glycaemic control [264].

Ghrelin, produced in the stomach fundus and the pancreas, when acylated, is the only orexigenic gut hormone. It also stimulates gastric and global gastrointestinal motility and inhibits glucose-dependent insulin production. Its levels are elevated in the fasting and postprandial state in people with obesity when compared to lean subjects. Some, but not all studies have shown a decrease in ghrelin concentration after RYGB [262].

Cholecystokinin (CCK) released from the I-cells in the duodenal mucosa in response to fatty acids or aminoacids, stimulates gallbladder contraction, pancreatic enzymes, insulin, glucagon secretion, slows down gastric emptying and stimulates satiety [265].

1.5.3.2. Bile acids

Research has proved that bile acids do not only facilitate absorption of cholesterol, triglycerides and fat-soluble vitamins but also have a potent impact on glucose and lipids metabolism, gut hormones secretion and satiety. Unconjugated primary bile acids, cholic and

chenodeoxycholic acid are synthetised in the liver from cholesterol. They are then stored in the gallbladder and they subsequently pass via the common bile duct to the duodenum and distal parts of the bowel, where they get transformed into the secondary bile acids (deoxycholic acid, lithocholic acid) through hydroxylation by the gut microbiota. They then are actively reabsorbed via the ileal bile acid transporters (IBAT, i.e. Slc10A2) with only a small proportion of <5% of bile acids recycled via passive diffusion in the proximal small bowel and colon [266]. When they reach the liver, they are transformed into conjugated bile acids by taurine or glycine [267]. By acting on the TGR-5 (the G protein-coupled) receptor in the gut, bile acids stimulate GLP-1 secretion from the L cells in the small intestine and increase energy expenditure in the brown adipose tissue. They also inhibit gluconeogenesis, increase hepatic glycogenesis and improve hepatic and peripheral insulin sensitivity through stimulating the FXR (farnesoid X nuclear receptor) [268, 269]. Stimulation of the FXR also results in raised fibroblast growth factor 19 levels (FGF-19) which in turn increase cholesterol conversion to the bile acids in the liver [267].

RYGB alters nutrient and bile flow which allows for an increased volume of bile acids free of nutrients to reach the distal parts of the intestine. This results in higher volumes of circulating bile acids entering the enterohepatic circulation and consequently increased concentrations in plasma both in animals and in humans. Furthermore, the actual composition of the bile acids is also modified post-operatively with an increase in the secondary bile acids, suggesting involvement of gut microbiota changes in this phenomenon [270]. Interestingly, animal models studies have shown that even an isolated bile flow diversion from the common bile duct to ileum or more proximal small bowel segments results in weight loss with improved glucose homeostasis [271, 272] which confirms the importance of altered bile flow after the RYGB when anatomy is rearranged.

1.5.3.3. Gut microbiota

Significant shifts in the gut microbiome composition as well as metabolites produced by them following RYGB have been identified [273, 274]. The main causes of this process are believed

to be the dietary alterations (both in quantity and in quality), production of gastric secretions and passage, gastric emptying, undigested food entering distal segments of the small intestine or increased oxygen delivery to the usually anaerobic parts of the intestine, which favours abundance of the aerobic species [275]. It has been reported that the ratio of Firmicutes to Bacteroidetes phyla after the RYGB decreases to resemble proportions representative of the lean objects [276, 277]. Increase in the total count of Proteobacteria has been documented in both human [278] [279, 280] and rodent studies [281]. Since gut microbiota interact with the host's energy regulation and glucose homeostasis, these shifts may play a role in metabolic changes after surgery [274]. Confirmation of this theory has been nicely presented by faecal transplantations from post-RYGB mice [274] and from post-RYGB humans [282], both of which induced weight loss in germ-free mice. These phenomena are predominantly attributed to the microbiome-induced changes in mediators such as secondary bile acids, which are formed through deconjugation and dehydroxylation of the primary bile acids by bacteria in the intestinal lumen [283] and short-chain fatty acids. This subcategory of fatty acids, produced by gut microbiota during fermentation of non-digestible and partially digestible polysaccharides in the colon [284], stimulates GLP-1 and PYY secretion via G protein-coupled receptors FFA2. FFA2 receptor stimulation has been shown to protect from hyperphagia, insulin resistance and obesity in rodents on the high-fat diet [285]. Further studies in the field are awaited in order to determine the exact underlying mechanisms of these microbiome-host interactions.

1.6. Importance of the anatomy of the gastric bypass

RYGB results in great outcomes in terms of weight loss (25-35% TBWL) [286] and T2DM remission (40-75%) [132, 198] with a mean reduction in glycated haemoglobin (HbA1c%) of approximately 2% at 1-2 years post-operatively and a reduced number of glycaemia-lowering medications or even a complete cessation of T2DM pharmacotherapy [198, 287, 288]. However, a certain proportion of patients, so-called suboptimal responders, will not achieve such impressive post-operative outcomes. Biliopancreatic diversion (BPD) has been shown to lead to superior rates of T2DM remission when compared to RYGB with up to 95% patients

fulfilling the criteria at 2 years, with an absolute reduction in HbA1c% of 3.9% [198]. Its use, however, is limited due to significant long-term nutritional complications [286]. The main difference between the RYGB and BPD is a much longer biliopancreatic limb and a shorter common channel in the latter. Therefore, multiple bariatric centres have attempted to modify alimentary and biliopancreatic limb lengths in the RYGB in order to optimise its outcomes.

Whilst RYGB has been used as a weight loss and metabolic procedure for over 50 years, no consensus has been reached with regards to the optimal length of the bypassed small bowel segments. Significant variations in the total small bowel length between individuals (3 to 11m) [289, 290] make setting up widely applicable standards even more challenging. Furthermore, a significant heterogeneity exists in the studies reviewing the lengths of the bypassed small bowel limbs, which makes it difficult to compare the results and draw clear-cut conclusions [291]. It has been shown that increasing the length of the alimentary (Roux) limb brings very little or no significant improvement in weight loss [292, 293] or long-term remission of metabolic syndrome-associated diseases [291]. It is only the subgroup of the super-obese who may benefit from the longer alimentary limb [291].

Therefore, more attention has been brought to the length of the biliopancreatic (BP) limb and the common channel. Nergaard et al. compared a standard RYGB (150 cm Roux limb with 60 cm BP limb) to a long BP limb RYGB (200 cm) with a short Roux limb (60 cm) in 187 randomised patients. Over 7 years follow up, an increased long-term weight loss was shown in the long BP limb group. However, no difference in the remission of obesity-related comorbidities was observed and more nutritional deficiencies (iron, calcium, vitamin D) were recorded in this group [294]. The authors speculated that the superiority of the 200 cm biliopancreatic limb in weight loss outcomes was due to the fact that such a long bypass of proximal bowel should allow bypassing most of the foregut, i.e. all of the jejunum, hence the gastrointestinal anastomosis was, in fact, a gastro-ileostomy, not a gastro-jejunostomy. Undigested nutrients entering ileum directly could have a more potent impact on nutrient sensing and eating behaviours and bypassing such a large proportion of foregut could have

stimulated more potent enteroendocrine response and gut hormone secretion. Eighteen non-diabetic patients from this cohort were also recruited to a separate study, where alterations in the enteroendocrine cells were monitored post-operatively [295]. Gastric pouch and jejunal biopsies in the proximity of the gastrojejunal anastomosis were taken at the time of the primary surgery and after 12 months. Enteroendocrine cell density as well as mucosal height were analysed. At one year after the surgery, GLP-1-producing L-cells increased 4.9-fold. This was more pronounced in patients with the 200 cm biliopancreatic limb. GIP-producing K-cells and PYY-producing L-cells increased 2-fold at the same time, without increasing the mucosal height. Increase in the density of GLP-1-producing cells may indicate the influence of the longer biliopancreatic limb on the more potent expression of changes in enteroendocrine cell adaptation post-operatively.

Nora *et al* led a prospective study of 94 patients with obesity and T2DM who underwent RYGB with a 200 cm BP and a 120 cm alimentary limb [296]. The cohort of 40 (43%) patients that completed the 3-year follow up lost 25% body weight, stopped all of their glucose-lowering medications and reduced their HbA1c% by 0.9% (from a baseline of 6.7%), achieving 100% T2DM remission rate. Complication rates (including nutritional complications) were not higher than those reported after a standard RYGB. This study showed that a longer BP limb may be associated with superior outcomes compared to a standard RYGB and achieving 100% T2DM remission rate made it more comparable to the BPD. However, it was a prospective observational study with almost 60% of patients lost to follow up at 3 years, hence reporting bias is possible.

The theory that the bypass of the proximal small bowel has superior and weight loss-independent effects on glucose metabolism compared to the bariatric procedures that do not include an intestinal bypass is based on the fact, that bariatric procedures such as BPD and RYGB have greater clinical effects on glucose control compared to the gastric band and sleeve gastrectomy. This has been demonstrated by clinical and mechanistic studies comparing RYGB to a gastric band and sleeve gastrectomy in both early and late post-operative stages

[213, 297-299]. Furthermore, isolated bypass of the distal duodenum and proximal jejunum, which can be done with endoscopic liner EndoBarrier®, has an impressive metabolic impact. Whilst it causes only a small to moderate weight loss (8-16%) at 6-12 months [300, 301], it results in absolute reductions in HbA1c% of ~1.2-2.4% (starting HbA1c 7.3-9.1%) in the same period of time [302-304].

Altering BP limb length can influence glucose homeostasis and weight loss through several mechanisms. The standard RYGB causes a large release of gut hormones such as GLP-1, oxyntomodulin and peptide YY after eating, leading to reductions in appetite and/or increases in insulin secretion [264, 278, 296, 305-308]. A longer BP limb in RYGB should enable faster delivery of undigested nutrients to the distal jejunum, where a greater number of gut endocrine L cells exists [309]. Therefore, it is expected that it will cause an even greater release of gut hormones that will subsequently drive a higher secretion of postprandial insulin compared to the standard RYGB. Moreover, bypassing a longer segment of the small bowel in the long-BP limb RYGB is expected to result in even higher than in the standard RYGB levels of circulating bile acids, gut microbiota and their metabolites and therefore even more potent effects on T2DM. Long-BP limb RYGB is also expected to increase hepatic and peripheral insulin sensitivity in a similar fashion as the BPD. At the same time, it is not expected to cause the side effects which are the limiting factor in the BPD use.

A review of the human trials in the field, alongside with their key findings is presented in Table 2. It demonstrates conflicting reports on the impact of the biliopancreatic limb length on postoperative outcomes after RYGB. Furthermore, it illustrates a degree of heterogeneity in the design of the studies, with varying biliopancreatic and alimentary limb lengths. Moreover, there is a gap in the field of mechanistic studies which, through investigation of the underlying physiology, would allow a better understanding of mechanisms of action of RYGB. This type of physiological studies could help with improvement of the surgical design in order to optimise outcomes.

Type of study	Sample size	Biliopancreatic +/- Alimentary Limb length(cm)	Follow up (years)	Impact on glycaemic control	Impact on weight
Studies showing no impact of biliopancreatic limb length					
Ruiz-Tovar 2019 [310] RCT	253 vs 253	70 BP + 150 AL 120 BP + 150 AL	5	No difference	No difference
Ramos et al, 2016 [311] Retrospective	20 vs 24 vs 19	50 BP +100 AL 50 BP + 150 AL 100 BP + 150 AL	2	No difference	No difference
Christou et al, 2006 [312] Retrospective	189 vs 83	10 BP + 40 AL 100 BP + 100 AL	>10	No data	No difference
Inabnet et al, 2005 [313] RCT	25 vs 23	50 BP +100 AL 100 BP + 150 AL	1	No data	No difference
Studies showing impact of biliopancreatic limb length on weight or glycaemic control					
Homan et al, 2018 [314] RCT	72 vs 74	150 BP + 75 AL 75 BP +150 AL	4	No difference	Difference in 1-3yrs but not at 4 yrs
Nergaard et al, 2014 [294] RCT	93 vs 94	200 BP + 60 AL 60 BP + 150 AL	7	No difference (T2DM remission 74%)	Difference in excess weight loss (78.4 vs 67.1%)
Patricio et al, 2018 [315] Retrospective	11 vs 9	200 BP 90 BP	4	↑GLP-1 ↓GIP, insulin, c-peptide (non-diabetic)	No difference
Kaska et al, 2014 [316] Retrospective	51 vs 42	50-75 BP 100-150 BP	2	Higher T2DM resolution but no difference in any other glycaemic control markers	No difference
Pinheiro et al, 2007 [317]	57 vs 58	50 BP + 150 AL 100 BP + 250 AL	4	Higher T2DM remission	No difference

RCT	all BMI>50				
Shah et al, 2019 [318] Retrospective	155 vs 230 vs 286	60 BP + 150 AL 200 BP + 60 AL 200 BP + 150 AL	6-10	Higher T2DM remission	Higher WL
MacLean et al, 2001 [319] Retrospective	162 vs 80	10 BP + 40 AL 100 BP + 100 AL	5.5	No data	Higher WL (in superobese only)

Table 2. Literature review of prospective and retrospective studies comparing various biliopancreatic limb lengths.

BP – biliopancreatic limb, AL – alimentary limb, WL – weight loss, T2DM – type 2 diabetes mellitus.

Based on the knowledge of the mechanisms of action of metabolic surgery and some of the promising findings as shown above, a modification of RYGB was designed. Its aim was to optimise the metabolic benefits of surgery, aiming at improved glucose homeostasis similar to the BPD, whilst minimising the negative nutritional impact and keeping the safety profile of the RYGB. This procedure, called a Long Limb RYGB, combines the design of a standard RYGB, but with a longer biliopancreatic limb of 150 cm instead of 50 cm (Figure 2).

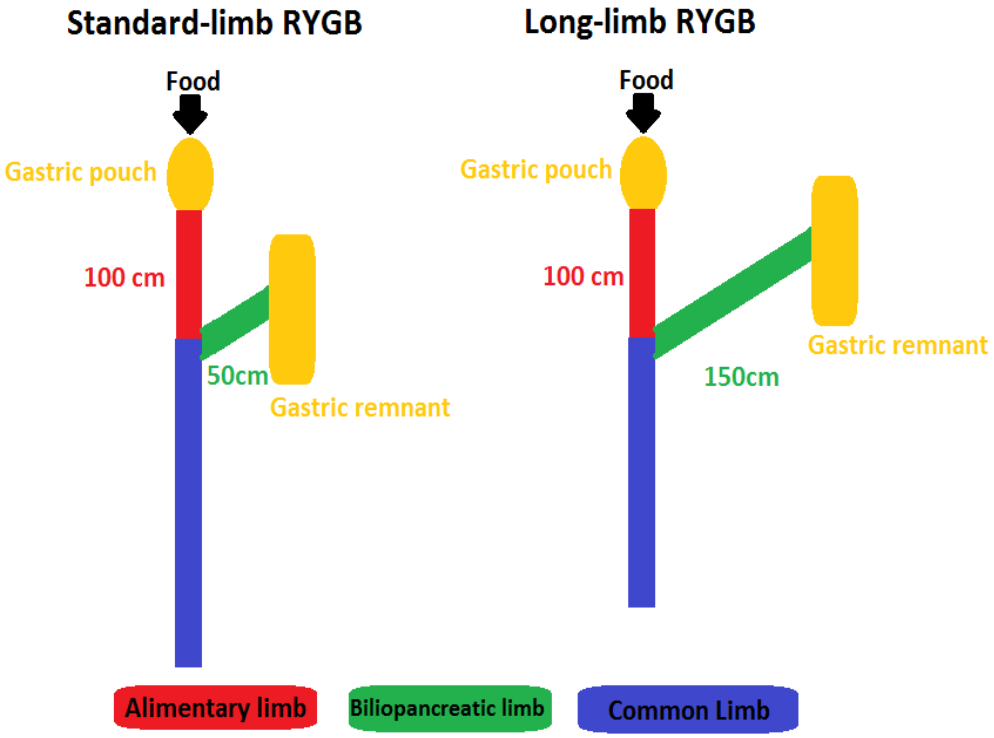


Figure 2. Schematic drawing of the Standard and the Long Limb RYGB.
Small bowel limbs: green – biliopancreatic (50 cm or 100 cm), red – alimentary (100 cm), blue – common channel (length depending on the total small bowel length). Courtesy of Dr Alexander Miras.

1.7. Hypothesis

The aim of the Long Limb Trial was to address the gap in knowledge with regards to the optimal lengths of the RYGB limbs through the understanding of the physiology of glucose regulation after surgery. Therefore, it was designed as primarily mechanistic, not a clinical study.

It was hypothesised that the elongation of the biliopancreatic limb from 50 cm in the Standard Limb to 150 cm in the Long Limb RYGB should result in a longer segment of proximal small bowel being bypassed from nutrient digestion and absorption. Due to this anatomical rearrangement, nutrients should reach the distal small bowel in a shorter period of time and in a less-digested state. The hypothesis of this trial was that:

1. A longer biliopancreatic limb length of 150 cm ('Long Limb') is superior to a standard biliopancreatic limb length of 50 cm ('Standard Limb') in RYGB for the treatment of T2DM.
2. This is associated with an increase in post-prandial secretion of gut hormones such as glucagon-like peptide-1 (GLP-1).
3. Additionally, these are associated with an increase in insulin sensitivity.
4. Both Long Limb and Standard Limb maintain the same safety profile.

1.8. Objectives

The main objectives of this study were to compare the differences between the Long Limb RYGB and the Standard Limb RYGB in several mechanistic and clinical aspects:

1. Fasting and postprandial insulin and gut hormones secretion assessed in a standardised Mixed Meal Tolerance Test (MMTT).
2. Hepatic and peripheral insulin sensitivity evaluated with the hyperinsulinaemic-euglycaemic glucose clamp.
3. Clinical T2DM-related outcomes, including reduction in HbA1c, fasting and postprandial glucose, reduction in glucose-lowering medication use and T2DM remission.
4. Anthropometric measures, including weight, BMI, total body weight loss and changes in body composition.

2. Chapter 2. Methods

2.1. Trial design

This was a prospective double-blinded randomised controlled trial. Fifty surgical candidates were recruited from the Imperial Weight Centre and the King's College Obesity Clinic and randomised to either the Long Limb or the Standard Limb RYGB in 1:1 ratio (see Appendix 1. LONG LIMB Trial Protocol).

2.1.1. Trial registration, ethical approval, funding and regulatory aspects

This project was funded by the Efficacy and Mechanism Evaluation Programme, a Medical Research Council and National Institute for Health Research partnership (EME 13/121/07). The final year of my research was sponsored by the Research Fellowship of the Royal College of Surgeons. The trial was approved by the West London Research Ethics Committee (reference 15/LO/0813) and registered in the International Standard Randomized Controlled Trial Registry (ISRCTN 15283219). Imperial College London was the Sponsor of the study. Written informed consent was obtained from all patients prior to participation by researchers involved in this trial (Information Sheet for Research Participants with Consent Form in Appendix 2).

The trial was coordinated by a Trial Steering Committee (TSC), which consisted of an independent chair and Principal Investigator and Co-Investigators. TSC meetings took place at least every 6 months throughout the duration of the trial in order to review study progress and resolve any outstanding issues. A Data Monitoring and Ethics Committee (DMEC) was also established, with its members being independent of the trial participants and the TSC, while reporting to the TSC. DMEC meetings took place annually in order to monitor the unblinded data and make recommendations to the TSC on any ethical or safety issues as required. Quality Control and Quality Assurance fell under routine auditing process of the NIHR Imperial Clinical Research Facility and Imperial College and King's College London.

The Chief Investigator of the LONG LIMB Trial was Professor Stephen R. Bloom, Professor of Medicine and Head of Department of Investigative Medicine, Imperial College London. Principal Investigator at King's College London was Professor Francesco Rubino. Day-to-day management and coordination of the study was performed by me and other study Co-Investigators and collaborators, including: Dr Alexander Miras, Prof Tricia Tan, Dr Belén Pérez-Pevida, Mr Ahmed R. Ahmed, Mr Sanjay Purkayastha, Mr Krishna Moorthy, Prof Julian Marchesi, Dr Harvinder Chahal, Prof Gary Frost (Imperial College London), Prof Anne Margot Umpleby (University of Surrey), Prof Ameet Patel (King's College London).

2.2. Patient and public involvement

Mrs Georgina Hayman, Humanistic Psychotherapist and Group Leader of British Obesity Surgery Patient Association in West London has supported the development of this trial together with patients from her support group in terms of its design, methodology and arranging practical aspects of the study. She has also helped with patients' retention in the study by creating a separate support group designed specifically for the LONG LIMB Trial participants.

2.3. Inclusion and exclusion criteria

Trial inclusion criteria included age of 18-70, a diagnosis of T2DM treated with glucose-lowering medications (at least one), HbA1c of ≥53.0 mmol/mol (≥7.0%), BMI ≥30 kg/m^2, eligibility for metabolic surgery based on the UK National Institute for Health and Care Excellence guidance [320] and willingness and ability to comply with study requirements and give informed consent. Key exclusion criteria were any surgical, medical or psychological contraindications to metabolic surgery, pregnancy and breastfeeding. Exclusion criteria included history of medical, psychological or other condition, or use of medications, which would either interfere with the study or potentially cause harm to the volunteer; lack of access to a telephone or other factor likely to interfere with ability to participate in the study;

contraindications to bariatric surgery; previous bariatric surgery; type 1 diabetes mellitus; recent blood donation (during the preceding 3 months) or intention to do so before the end of the study; pregnancy or breastfeeding; inability to maintain adequate contraception.

2.4. Trial design

Patients initially underwent a routine assessment in the NHS by the multidisciplinary bariatric team to ensure eligibility for obesity surgery, including medical, psychological and dieticians' assessment. They were then screened by a member of LONG LIMB Trial research team to check eligibility for the trial. Once enrolled in the study (majority of the patients were recruited by the author of this thesis), participants were invited to either the NIHR Imperial Clinical Research Facility or the NIHR King's Clinical Research Facility for a pre-operative mechanistic visit (details below and in Appendix 1. LONG LIMB Trial Protocol).

On the day preceding the operation, participants were randomised by a random sequence generation to either Long Limb or the Standard Limb RYGB surgery in 1:1 ratio using an online randomisation programme (www.randomisation.com) by an independent person not otherwise involved in the study who would then directly inform the operating surgeon about the randomisation allocation. Weight and blood tests were rechecked on the day of surgery or within a week preceding the surgery. Participants were advised to follow a routine post-operative diet (two weeks liquid diet, two weeks puree followed by a gradual introduction of a standard diet as tolerated). Within 2 weeks after the surgery, they attended Clinical Research Facilities at Imperial College or King's College London again to complete a mechanistic visit of identical design as the pre-operative one. The third mechanistic visit was performed when participants achieved 20% of total body weight loss. Additionally, participants underwent a routine clinical assessment led by me or another member of the research team at 3, 6 and 12 months after the surgery or more frequently if the clinical need arose (Figure 3) as detailed below.

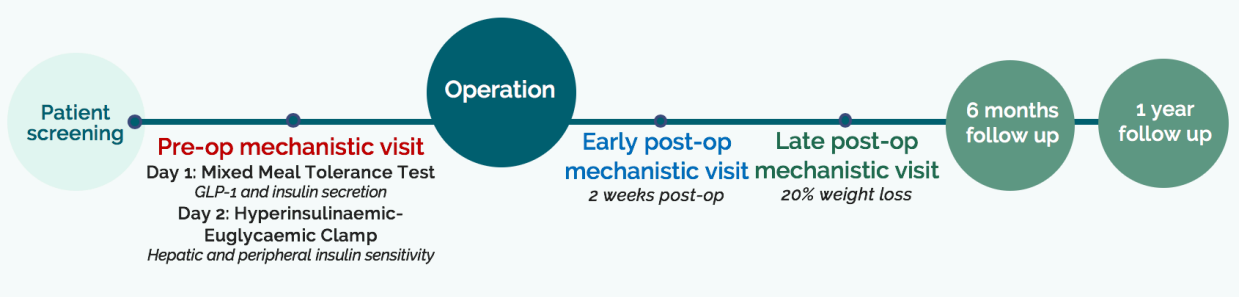


Figure 3: LONG LIMB Trial design.

2.4.1. Screening visit

All candidates for the trial participants were screened by the research team to assess whether they met inclusion criteria. Screening visits took place NIHR Imperial Clinical Research Facility or NIHR King's Clinical Research Facility. After giving informed consent, participants would undergo a detailed assessment including medical history including medications review, physical examination and basic investigations. These included:

1. Bloods tests: full blood count, urea and electrolytes, liver function tests, thyroid function tests, fasting plasma glucose, serum insulin and c-peptide, HbA1c, lipid profile, iron status, vitamin D and B12.
2. Urinalysis for microalbuminuria. All women of child-bearing age were also asked to undergo a pregnancy test.
3. Anthropometric measurements: body weight, height, BMI; waist, hips and neck circumference; body composition using bioelectrical impedance machine Tanita® (adipose tissue percentage and weight, fat free mass, estimated basic metabolic rate).
4. Stool collection for Helicobacter pylori antigen (unless CLO test performed recently). If positive, participants would undergo a routine Helicobacter pylori eradication as per local hospital guidelines.

Following this assessment, patients would be optimised prior to planned surgery, analogous to routine NHS pre-operative pathway, i.e. should any abnormalities be found patients were referred for further investigations and their pharmacotherapy was adjusted by the Trial Consultant Diabetologist. Participants were advised to continue on their usual diet until the

day of surgery with no dietary restrictions until post-operatively. Participants were free to withdraw at any point and where possible were replaced.

2.4.2. Mechanistic visits

Five days prior to the mechanistic visit patients were asked to stop all of their glucose-lowering medications in order to avoid their interference with the mechanistic assessments. When required, an intermediate-acting insulin (Insulatard) was provided and was adjusted by me or the Trial Consultant Diabetologist to reduce the small risk of diabetic ketoacidosis in the days preceding the mechanistic visit. Patients were asked to refrain from alcohol and any strenuous physical activity for 48 hours before the study.

Day 0. Admission

Patients attended either the NIHR Imperial Clinical Research Facility or NIHR King's Clinical Research Facility in the afternoon before the hyperinsulinaemic-euglycaemic clamp procedure (later referred to as clamp). I would check their random plasma glucose on admission and if required, I would prepare and start a continuous infusion of short-acting insulin (Actrapid) at a variable rate as per local hospital guidelines, in order to maintain blood glucose levels between 4.0 to 6.0 mmol/L (Appendix 3, LONG LIMB Trial Mechanistic Visit Standard Operating Procedure). After inserting two venous catheters, I would use the first cannula for infusions and the other one for blood sampling. Participants were asked to consume a standardised meal in the evening, remain fasted on water only from 10 pm onwards and would spend the night at the research facility.

Day 1. Assessment of insulin sensitivity - euglycaemic hyperinsulinaemic clamp

On the morning of the clamp, patients would have their blood tests and anthropometric measurements rechecked. I would follow a validated clamp protocol [321] and its schematic set up is presented in Figure 4. Continuous infusion of short-acting insulin (Actrapid) was continued at a variable rate as per local hospital guidelines to keep blood glucose stable between 4.0 to 6.0 mmol/L. A primed continuous infusion of [6, 6-$^{2}H_{2}$] glucose, a stable

isotope tracer was then started (timepoint -120) and maintained for 6.5 hours. The tracer used in clamps is a naturally occurring metabolite which has been labelled with a stable and non-radioactive label. It was supplied by the Cambridge Isotopes Ltd through their UK suppliers CK Gases Ltd and prepared as sterile solutions suitable for intravenous infusions by the Pharmacy Production Unit at Guys & St. Thomas' NHS Trust.

Two hours later, at timepoint 0, a two-stage hyperinsulinaemic-euglycaemic clamp procedure was started and continued for 4 hours. During the first stage of the clamp procedure, hepatic insulin resistance was assessed. Here, insulin was infused at a low dose of 0.5mU/kg/min for 2 hours. During the second stage of the clamp procedure, peripheral insulin resistance is assessed, and the insulin infusion rate was increased to 1.5mU/kg/min for 2 hours. Euglycaemia will be maintained at the level of 4.0 to 6.0 mmol/L by infusing 20% dextrose (spiked with [6, 6-$^{2}H_{2}$] glucose to prevent a fall in plasma tracer enrichment and underestimation of endogenous glucose production rate) at a variable rate. Rate of exogenous glucose infusion was guided by the blood sampling in 10-minute intervals to measure blood glucose concentration in a YSI 2900 STAT Plus Glucose and Lactate Analyser (YSI 2900®). Such frequent blood glucose monitoring was necessary to ensure safety and avoid the risk of hypoglycaemia during the clamp.

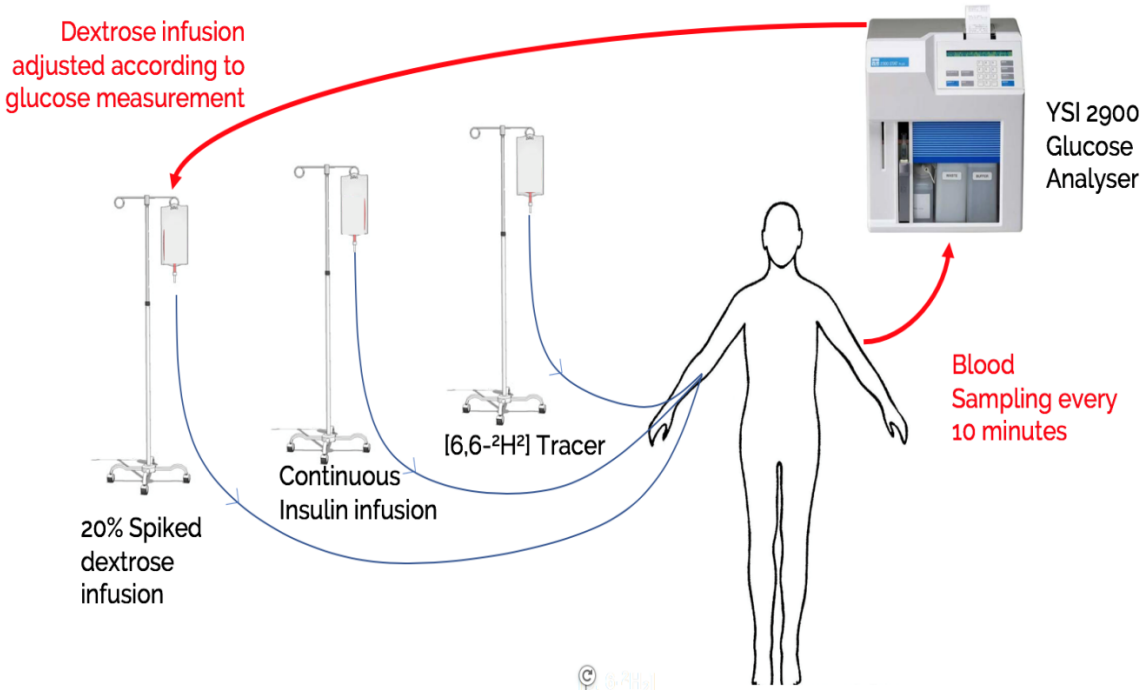


Figure 4. Hyperinsulinaemic-euglycaemic clamp set up

Blood samples were obtained before the start of the tracer infusions (at timepoint -120), every 10 minutes during the final 30 minutes of the basal period and stages 1 and 2 of the clamp procedure and every 30 minutes between these periods to determine glucose enrichment and concentration of insulin and c-peptide. Approximate volume of venesected blood was 180 ml.

At the end of the study, participants were fed a standardised meal and the 20% dextrose infusion continued for a further 30 minutes to prevent post-clamp hypoglycaemia. Patients were then asked to fast from 10 pm and sleep overnight in the Clinical Research Facility.

Day 2: Assessment of fasting and postprandial insulin and gut hormones secretion - Mixed Meal Tolerance Test (MMTT)

Following an overnight fast, a Mixed Meal Tolerance Test (MMTT) was performed as per standard protocol used in human studies in the Department of Investigative Medicine, Imperial College London (Appendix 3, LONG LIMB Trial Mechanistic Visit Standard Operating

Procedure). Standardised liquid meal utilised in this study was a single bottle of Ensure Compact®, 300 kcal in 125 ml (2.4 kcal/ml; 17% protein, 35.1%. fat, 47.9% carbohydrates). Timepoint 0 min in the MMTT was determined by the time of meal consumption. Blood samples were obtained before the meal (at timepoints -30 and 0 min) and for 180 minutes following its consumption (at +15, +30, +60, +120 and +180 min timepoints) to measure fasting and postprandial glucose, insulin, c-peptide and gut hormones (GLP-1, PYY, GIP, ghrelin). At the same timepoints participants had their vital signs monitored. Following the completion of the MMTT, participants were provided lunch of their choice and discharged from the Clinical Research Facility.

The same protocol was followed during further two mechanistic visits, which were performed within 2 weeks after the surgery (Early post-operative visit) and at 20% weight loss (Late post-operative visit).

2.4.3. Surgery

All operations were performed laparoscopically by five Bariatric Consultant Surgeons at either Imperial College Healthcare NHS Trust (Mr Ahmed Ahmed, Mr Krishna Moorthy, Mr Sanjay Purkayastha) or King's College Hospital NHS Foundation Trust (Prof Francesco Rubino, Prof Ameet Patel). Surgical team members were the only ones in the trial who were not blinded to the procedure (the research team and study participants were all blinded). Surgeons followed a standard operating protocol agreed before the trial commenced and the procedures were recorded (Appendix 4, LONG LIMB Trial Surgical Standard Operating Procedure).

After establishing a pneumoperitoneum of 15 mmHg, a total length of the small intestine (excluding duodenum) was measured from the ligament of Treitz to the ileocaecal valve. This length of the jejunum and ileum length will be referred to as the total small bowel length (TSBL). Measurement was performed using 10 cm markers placed on laparoscopic graspers and running the bowel segment by segment along the antimesenteric border (Figure 5). Then a biliopancreatic limb was formed by dividing small bowel at 50 cm (Standard Limb) or 150

cm (Long Limb) depending on treatment allocation, using an endostapler. A further 100 cm small bowel segment from this division formed an alimentary (Roux) limb in both operations. A gastric pouch along the lesser curve of approximately 40ml in volume was formed using endostaplers. The alimentary limb was then brought up to it and gastrojejunostomy was performed (antecolic antegastric), using either a circular stapler or linear stapler and sutures. A leak test was performed to review the integrity of the anastomosis with the alimentary loop occluded and gastro-jejunal anastomosis submerged under saline, distended with oxygen delivered into the gastric pouch via an orogastric tube and with multiple distensions while submerged. Following that, a side-to-side jejuno-jejunostomy was formed between biliopancreatic limb and the 100 cm mark on the alimentary limb by firing the endostapler into the lumen of each and closing the enterotomy with sutures. Mesenteric defects (Petersen's and at jejuno-jejunostomy site) were both closed.

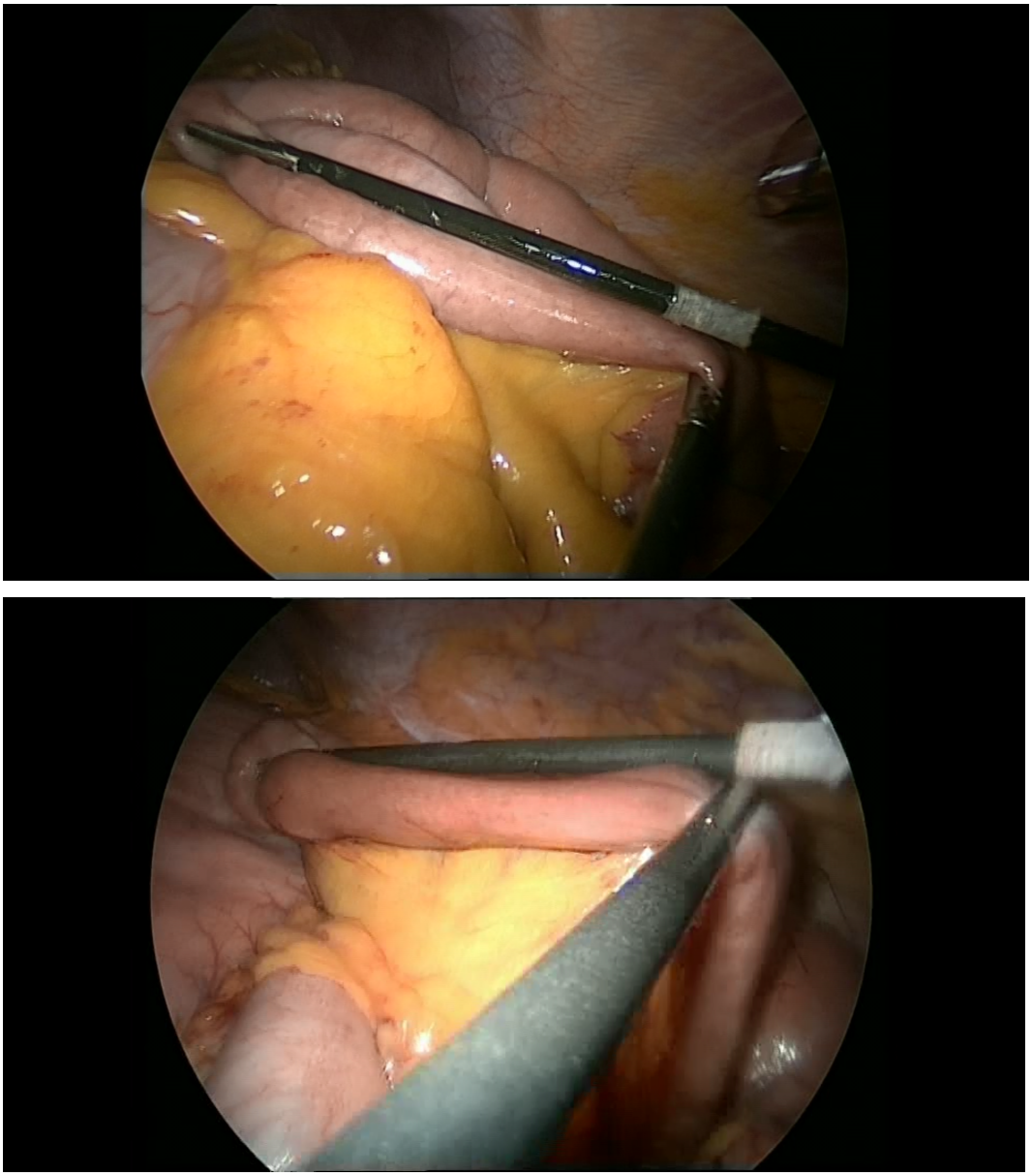


Figure 5. Intraoperative small bowel length measurement

2.4.4. Post-operative care

Standard post-operative protocols were followed in the recovery, with early mobilisation and introduction of liquid low-calorie diet prior to discharge from the hospital. All intra- or post-operative complications were recorded as Adverse Events or Serious Adverse Events (as

defined in section 2.5). Glucose-lowering medications were discontinued when clinically safe, under supervision of the Trial Consultant Diabetologist. Post-operative micronutrient supplementation was based on the British Obesity & Metabolic Surgery Society guidance [322] and included:

- Forceval one capsule once a day
- Cholecalciferol (vitamin D) 20.000 units once a week or 2000 units once a day
- Ferrous fumarate 210 mg once or twice a day (adjusted as required)
- Adcal D3 once or twice a day (adjusted as required)
- Hydroxycobalamin (vitamin B12) 1 mg intramuscular injection once in 3 months.

All patients continued post-operatively on the same pharmacotherapy regimes for hypertension, dyslipidaemia, arthritis and depression as pre-operatively during the trial. Blood pressure-lowering medications were only stopped in cases of hypotension. Remission of hypertension was defined as blood pressure of less than 130/80 mmHg as per the American Heart Association [323] in absence of any pharmacotherapy. Analgesia for arthritis was discontinued as guided by patients' self-reports on pain relief requirements. Absence of analgesia alongside with reported resolution of musculoskeletal pain was defined as resolution of symptoms of arthritis. Study participants continued lipid-lowering agents for 12 months post-operatively regardless of their results and in rare cases where therapy was ceased. If pharmacotherapy was ceased, remission was then defined as total cholesterol <5 mmol/L with non-HDL cholesterol <4 mmol/L and HDL >1 mmol/L [324]. Patients were also advised to continue antidepressants if they had been diagnosed with depression pre-operatively and remission was diagnosed only if pharmacotherapy was ceased and patients reported to be asymptomatic. All patients with Epworth score over 10 pre-operatively were referred for a sleep study in order to rule out obstructive sleep apnoea [325]. Night-time continuous positive airway pressure (CPAP) therapy was commenced in patients with positive sleep study outcomes or the ones who had been diagnosed previously. Post-operatively, remission was defined by patients being asymptomatic (Epworth below 10) in the absence of CPAP, where

treatment cessation was based on recommendations by a sleep apnoea specialist following clinical reassessment.

2.4.5. Clinical visits

Participants underwent clinical assessments (led by me or other members of the research team), that complemented mechanistic assessments, at 3, 6 and 12 months after the surgery. Participants had an open telephone and email access to the research team throughout the trial duration and if required they were reviewed more frequently. Safety blood tests, anthropometric measurements, vital signs and any adverse events were recorded during all the visits. Pharmacotherapy was adjusted throughout the trial by the Trial Consultant Diabetologist, myself or clinicians from the research team who were all blinded to the procedure that had been performed. Additionally, at 12-month follow up (the last visit of the trial), T2DM remission was recorded according to the American Diabetes Association criteria [104]. Partial or complete remission, with HbA1c of <48 mmol/mol, fasting plasma glucose of <5.6 mmol/L and absence of glucose-lowering medication for 12 months was recorded as 'T2DM remission'. During this visit, participants were also requested to bring a three-day dietary record (documented by the participant prospectively in the days preceding admission), fill in a set of standardised psychological questionnaires and provide a stool sample for bomb calorimetry analysis. After completion of the one year follow up, patients were discharged back to continue with the routine NHS follow up.

2.5. Adverse Events

Safety outcomes were assessed by recording of Adverse Events (AEs) and Serious Adverse Events (SAEs) reported from the time of the operation until the last visit of the trial at 12 months following the surgery. Any medical, surgical, nutritional or psychological complications were recorded and reported as adverse events. Records were collected directly from the patients (weekly to bi-weekly contact via the phone or emailed was maintained with each study participant throughout the trial) as well as from the secondary and primary care physicians and healthcare professionals involved in patients' care.

Adverse events were assessed in terms of:

1. Causality – whether they were 'related' or 'unrelated' to the LONG LIMB trial.
2. Whether they were 'expected' or 'unexpected' adverse outcomes in the trial.

AEs were recorded as SAEs if they met any of the following criteria:

1. Led to death.
2. Were life-threatening.
3. Led to hospitalisation or prolongation of existing hospitalisation.
4. Caused persistent or significant disability or incapacity.
5. Were linked to a congenital anomaly or birth defect.
6. Significant AEs that were not immediately life-threatening or did not result in death or hospitalisation but might have jeopardised the subject or might have required an intervention to prevent one of the other outcomes listed above, were also considered to be SAEs.

All SAEs were reported by the author of this thesis or other co-investigators involved in patients' care to the Chief Investigator in the first instance and then submitted to the Joint Research Compliance Office. All AEs and SAEs were actively followed up until their resolution by me, one of the other study co-investigators or a responsible clinician looking after the patient.

Adverse events were also further stratified according to the Clavien-Dindo classification, a commonly utilised method of reporting surgical complications [326].

Grade I:	Any deviation from the normal post-operative course not requiring surgical, endoscopic or radiological intervention. This includes the need for certain drugs (e.g. antiemetics, antipyretics, analgesics, diuretics and electrolytes), treatment with physiotherapy and wound infections that are opened at the bedside.

Grade II	Complications requiring drug treatments other than those allowed for Grade I complications; this includes blood transfusion and total parenteral nutrition.
Grade III	Complications requiring surgical, endoscopic or radiological intervention: Grade IIIa - intervention not under general anaesthetic. Grade IIIb - intervention under general anaesthetic.
Grade IV	Life-threatening complications; including central nervous system complications (e.g. brain haemorrhage, ischaemic stroke, subarachnoid haemorrhage) which require intensive care, but excludes transient ischaemic attacks. Grade IVa - single-organ dysfunction (including dialysis). Grade IVb - multi-organ dysfunction.
Grade V	Death of the patient.

Table 3. Clavien-Dindo classification of surgical complications.

2.6. Trial Outcomes

The primary trial outcome was defined as a change in **peak of active GLP-1** level after the Mixed Meal Tolerance Test within 2 weeks after the surgery.

Secondary outcomes were as follows:

1. Measured at all three mechanistic visits (pre-operative, early post-operative 2 weeks after the surgery and late post-operative at 20% total body weight loss):
    a. Fasting and postprandial plasma concentration of glucose during the MMTT
    b. Fasting and postprandial serum concentration of insulin and c-peptide during the MMTT
    c. Fasting and postprandial plasma concentration of gut hormones (active and total GLP-1, PYY and GIP) during the MMTT
    d. Bile acids, free fatty acids, glucagon during the MMTT*
    e. Visual analogue scales during the MMTT*
    f. Rate of glucose appearance (Ra) and disappearance (Rd) at low and high insulin infusion rate during the hyperinsulinaemic-euglycaemic clamp

   g. Gut microbiota and metabolomics*
   h. Urine and plasma metabolomics*
2. Recorded at all clinical visits (peri-operatively and at 3 and 6 months after the surgery):
   a. glycated haemoglobin (HbA1c) level
   b. total body weight loss (%)
   c. number of glucose-lowering medications
3. Recorded at 12-month clinical follow up:
   a. glycated haemoglobin (HbA1c) level
   b. total body weight loss (%)
   c. number of glucose-lowering medications
   d. anthropometric measurements: body weight, BMI; waist, hips and neck circumference; body composition (adipose tissue percentage and weight, fat free mass, estimated basic metabolic rate).
   e. T2DM remission
   f. Comorbidities
   g. King's Obesity Staging Score
   h. Systolic and diastolic blood pressure
   i. Heart rate
   j. Bowel movements frequency
   k. Fasting blood tests: plasma lipids concentration, plasma glucose, iron profile, vitamins
4. Recorded intraoperatively:
   a. Total small bowel length
   b. Biliopancreatic, alimentary and common channel length
   c. The proportion of biliopancreatic limb and common channel to the total small bowel length
   d. Operating time
   e. Length of in-hospital stay

5. Medical, surgical, nutritional and psychological complications were recorded as AEs or SAEs (as defined in section 2.5) throughout the the trial.

Secondary outcomes marked with * have not been included in this study analysis.

2.7. Blood samples processing

2.7.1. Clinical blood tests

All routine biochemical blood tests such as HbA1c, lipid profile, liver function tests, vitamin levels, were analysed by the local hospital accredited NHS laboratories as part of standard post-operative care (both sites used the same reference ranges for the normal results values). During the mechanistic visits, bloods were processed by me or other members of the research team. Following blood venesection, samples were placed on ice, centrifuged and the separated plasma was kept in -20°C and then transferred to a -80°C freezer for long-term storage. Insulin and c-peptide samples were left in room temperature for 10 minutes to allow clot formation, then serum was centrifuged and stored in the same freezers as all the other samples until further analysis.

Glucose was measured on the ARCHITECT c8200 platform using a hexokinase method in the NHS laboratory. Insulin and c-peptide assays were performed using ARCHITECT i2000SR immunoassay. These particular methods have been chosen as the ones that have been validated and widely utilised in the NHS clinical investigations at the Imperial College.

2.7.2. Gut hormone assays

MILLIPLEX® MAP Human Metabolic Hormone Magnetic Bead Panel (Magpix®) assay was used to measure active GLP-1, PYY and GIP. These gut hormone-measuring kits utilise bead sets coated with a specific capture antibody and requires several steps to conduct the assay. Its intra- and inter-assay precision has been reported as <10% and <15% coefficients of

variation (CV), as reported by the manufacturer. Processing these assays requires a number of steps as listed below:

1. Quality Controls 1 and 2 provided with the kit were reconstituted with 250 μL of deionized water and vortexed.
2. 60 ml of a Wash Buffer was warmed to the room temperature, mixed to bring all salts into a solution and diluted with 540 ml of deionized water.
3. Lyophilized Serum Matrix was mixed with 1 ml of deionized water.
4. Human Metabolic Hormone Standard was reconstituted with 250 μL of deionized water, vortexed and labelled as “Standard 7”. Then 100 μL of this Standard was transferred to a polypropylene microfuge tube with 200 μL of Assay Buffer and labelled as “Standard 6”. 100 μL od Standard 6 was then transferred to a polypropylene microfuge tube with 200 μL of Assay Buffer and labelled as “Standard 5”. The process was analogically repeated through further 4 polypropylene microfuge tubes with 200 μL of Assay Buffer in order to form standards of decreasing concertation towards Standard number 1.
5. Kit plates (8 rows by 12 columns) were washed with 200 μL of Assay Buffer and placed on a shaker for 10 minutes at room temperature, after which Assay Buffer was decanted and residual excess of it was tapped into absorbent towels.
6. 25 μL of each Standard and Control was added to the appropriate wells in the first 3 columns of the plate and Assay Buffer was used as 0 pg/mL standard (background).
7. 25 μL of Assay Buffer was added to the sample wells.
8. 25 μL of matrix solution was added to the background, standards and controls.
9. After the plasma samples were thawed, 25 μL of each sample was placed in a pre-planned well.
10. 25 μL of Mixed Beads were added into each well after vortexing them.
11. Plates were sealed and incubated overnight on a plate shaker (16-18 hours) at 4°C.
12. Following morning, plate contents were gently removed and washed 3 times with 200 μL of the Wash Buffer in an automated plate washer.
13. 50 μL of Detection Antibodies at room temperature was added into each well.

14. Plates were then sealed again, covered with foil and incubated for 1 hour on a plate shaker at room temperature (20-25°C).
15. 50 μL of Streptavidin-Phycoerythrin was added to each well and the plate was again sealed and covered with foil and incubated on a shaker for 30 minutes at room temperature.
16. Plate contents were gently removed and washed 3 times with 200 μL of the Wash Buffer in an automated plate washer.
17. 100 μL of Drive Fluid was added to all wells and beads were resuspended by placing the plates on a shaker for 5 minutes.
18. Following that final step, plates were run on MAGPIX® software to obtain the readings of active GLP-1, GIP and PYY.

Northern Lights Mercodia® ELISA immunoassay was used to measure total GLP-1 in order to validate results of active GLP-1 obtained from the MILLIPLEX® MAP Human Metabolic Hormone Magnetic Bead Panel (Magpix®) assay. The antibody pair used in this assay measures GLP-1 (7-36) and (9-36) and has no significant cross-reactivity with GLP-2, GIP, Glucagon and Oxyntomodulin. The sensitivity of this assay is 1.5 pM and the approximate range of this assay is 4.1 to 1,000 pM. The intra- and inter-assay CVs were ≤2% and ≤12% respectively, as reported by the manufacturer. Mercodia® ELISA immunoassay assay requires the following steps:

1. Preparation of enzyme conjugate and wash buffer solution at room temperature.
2. The working solution was prepared by mixing equal volumes of Substrate Reagent A and B (5 ml each).
3. 25 μL of the Calibrators and Controls provided in the kit as well as plasma samples (after thawing) was pipetted into pre-planned wells.
4. 50 μL of enzyme conjugate was added into each well.
5. Plates were sealed and incubated on a shaker (700-900 rpm) for 2 hours at room temperature.

6. They were then washed in an automated plate washer with 700 μL of wash buffer in 6 cycles.
7. 100 μL substrate working solution was added into each well and plates were sealed, foiled and incubated at room temperature for 15 minutes.
8. Microplate reader for chemiluminescence was used to obtain the readings of total GLP-1 by computerised data reduction of the relative light units.

2.7.3. Glucose isotopic enrichment from hyperinsulinaemic-euglycaemic clamps

Glucose isotopic enrichment was measured by Gas Chromatography-Mass Spectrometry on a HP 5971A MSD (Agilent Technologies, Wokingham, Berks, UK) at the Wolfson Centre for Translational Research, Postgraduate Medical School, University of Surrey. The process of plasma glucose derivatisation and its analysis with Gas Chromatography-Mass Spectrometry is conducted in the stages listed below.

Plasma glucose samples:

1. Samples and Quality Control samples were thawed and mixed by vortexing, then centrifuged for 10 minutes at 4°C, at 2500 rpm to spin down any proteins.
2. 50 μl of each plasma sample was pipetted into small glass test tubes.
3. After 500 μl of ethyl alcohol was added to the plasma samples, they were vortexed, then centrifuged at 4°C, 2500 rpm for 10 mins.
4. The supernatant was transferred to ½ dram vial using a glass Pasteur pipette.

Spiked dextrose infusate samples:

5. Duplicate dilutions of each sample were performed 1:300 (10 μl dextrose in 3 ml of water).
6. 50 μl diluted dextrose was pipetted into ½ dram vial and 500 μl ethyl alcohol was added.

All Samples

7. All samples were blown dry under Oxygen-Free Nitrogen at 50°C.

8. Methoxyamine hydrochloride was freshly made in pyridine 2% (0.02g/1ml) by adding methoxyamine hydrochloride powder into a 20ml screw glass bottle using the tared weighing method and the pyridine in the fume hood. 100 μl of the solution was then added to each sample.
9. Tubes were capped, vortexed and then heated at 90°C for 2 hours.
10. After cooling, 50 μl BSTFA (N,O-bisTrimethylsilyl-trifluoroacetamide with 1% Trimethylchlorosilane) was added, samples were re-capped, vortexed and heated at 120° C for 15 mins.
11. They were then cooled, dried under Oxygen-Free Nitrogen at room temperature and reconstituted with 500 μl decane.
12. Final dilution for mass spectrometry was made of 50 μl of the above sample with 500 μl decane in an autosampler vial, which was capped and vortexed and subsequently transferred to the Gas Chromatography-Mass Spectrometry.

Rates of glucose appearance (Ra) and disappearance (Rd) from plasma then were calculated by Professor Anne Margot Umpleby using non-steady-state equations proposed by Steele and modified for stable isotopes [327].

2.8. Sample size calculations

GLP-1, which has been elected as the primary outcome of this trial, is an incretin which has been shown to increase after RYGB. The majority of studies in the field have shown that peak active GLP-1 concentrations are approximately 2 fold greater after Standard Limb RYGB [65, 328] compared to pre-operatively. In this trial, an estimation of tripling peak of active GLP-1 levels after Long Limb RYGB within 2 weeks after the surgery was made. The LONG LIMB Trial was powered to detect a statistically significant difference in peak active GLP-1 of 10.0 pmol/L between the group means, assuming a standard deviation of 10.8 pmol/L within each group. With a sample size of 20 completers in each arm, statistical power was 80% to detect this difference at α=0.05. Assuming a 20% drop-out rate based on our department's previous experience in trials on obesity surgery, 25 patients were planned to be recruited into each arm. The rationale for chosing a mechanistic rather than a clinical primary outcome was that

this approach would allow to investigate an underlying physiology and based on this to review whether any signal for change in clinical outcomes exists.

2.9. Statistical analysis

Continuous variables are summarised using the number of data-points, with mean and standard deviation (SD) if the normal distribution of these variables was confirmed. Continuous variables not found to be normally distributed are summarised by the number of data-points, median and inter-quartile range. Categorical variables are presented as the frequency and percentage of values in each category. All the analysis is based on the intention-to-treat principle. The analysis of the primary outcome, i.e. postprandial peak of active GLP-1 concentration during the MMTT at the early mechanistic post-operative visit within 2 weeks after the surgery, was performed using Analysis of Covariance (ANCOVA). The peak of postprandial active GLP-1 concentration at this visit was considered as the outcome measure, whilst the peak of active GLP-1 recorded at the pre-operative mechanistic visit was included as a covariate. The baseline adjusted difference in outcome values between groups were reported, along with a corresponding 95% confidence interval.

Secondary outcomes measured on a continuous scale, with a baseline measurement, were analysed using a similar approach to the one outlined for the primary outcome. The data from each post-operative time point in the ANCOVA analysis was analysed in a separate analysis. For continuous secondary outcomes with no baseline measurement, the two groups were compared using the unpaired t-test. Alternatively, the Mann-Whitney test was used if the data were not normally distributed. All outcome distributions were assessed with D'Agostino-Pearson omnibus normality test.

Furthermore, all continuous variables were also analysed with a mixed-effects model with Bonferroni adjustment for multiple comparisons to assess within group changes and confirm differences between the groups. The mixed-effects model contains both fixed and random effects. This statistical model is of a similar design to the repeated measures analysis of

variance (ANOVA) however its major advantage is that adjusts for missing values (provided they are missing at random).

Binary and nominal outcomes were compared between the two study groups using either the Chi-square test or Fisher's exact test if the number of responses in some categories was low. Ordinal outcomes were analysed using the Mann-Whitney test to allow for the natural ordering of the response categories. Association between outcomes was performed using Pearson correlation. Alternatively, Spearman's rank correlation was used if the Pearson correlation assumptions (e.g. non-linear relationship, both variables non-normally distributed) were not met. Statistical significance was defined as a p-value of $p<0.05$. The data analyses were performed using the statistical software packages GraphPad PRISM (version 8), Stata (version 15.1) and SPSS (version 25).

2.9.1. Derived variables

Peak

For each outcome, the peak was defined as the maximum post-meal concentration (i.e. from time 15 onwards) per patient, regardless of at which timepoint that peak concentration was achieved.

Area Under the curve (AUC)

Outcomes of the mixed-meal tests recorded at multiple timepoints (-30, 0, +15, +30, +60, +120, +180) were summarised by the Area Under the Curve (AUC) calculations. AUCs were calculated using the trapezoid rule. For outcomes with measurements at time -30, the first value used in the calculation will be the mean of -30 and 0 timepoints. When there was no -30 value, the time 0 value was used as the first measurement in the calculation.

Absolute changes from baseline

Absolute changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the outcome timepoint.

Percentage changes from baseline

Percentage changes from baseline, such as total body weight loss percentage, were calculated by subtracting the individual subject's baseline value from the value at the outcome timepoint, dividing this sum by the baseline value and multiplying by 100.

3. Chapter 3. Clinical outcomes

Sixty-three candidates were screened for eligibility and of whom 53 (84%) were recruited into the LONG LIMB Trial. Of these, 26 were randomised to the Long Limb and 27 to the Standard Limb RYGB. Due to significant intraabdominal adhesions secondary to previous surgeries, one patient from the Long Limb and one from the Standard Limb group could not receive the allocated procedure and instead underwent vertical sleeve gastrectomy and one-anastomosis gastric bypass respectively. Furthermore, there was one participant in each group with difficult venous access which precluded their participation in the mechanistic visits. However, these two patients continued in the trial with clinical outcomes being recorded. Only one trial patient was lost to follow up, due to relocating abroad (Standard Limb participant). Details are presented in the CONSORT Flow Diagram (Figure 6).

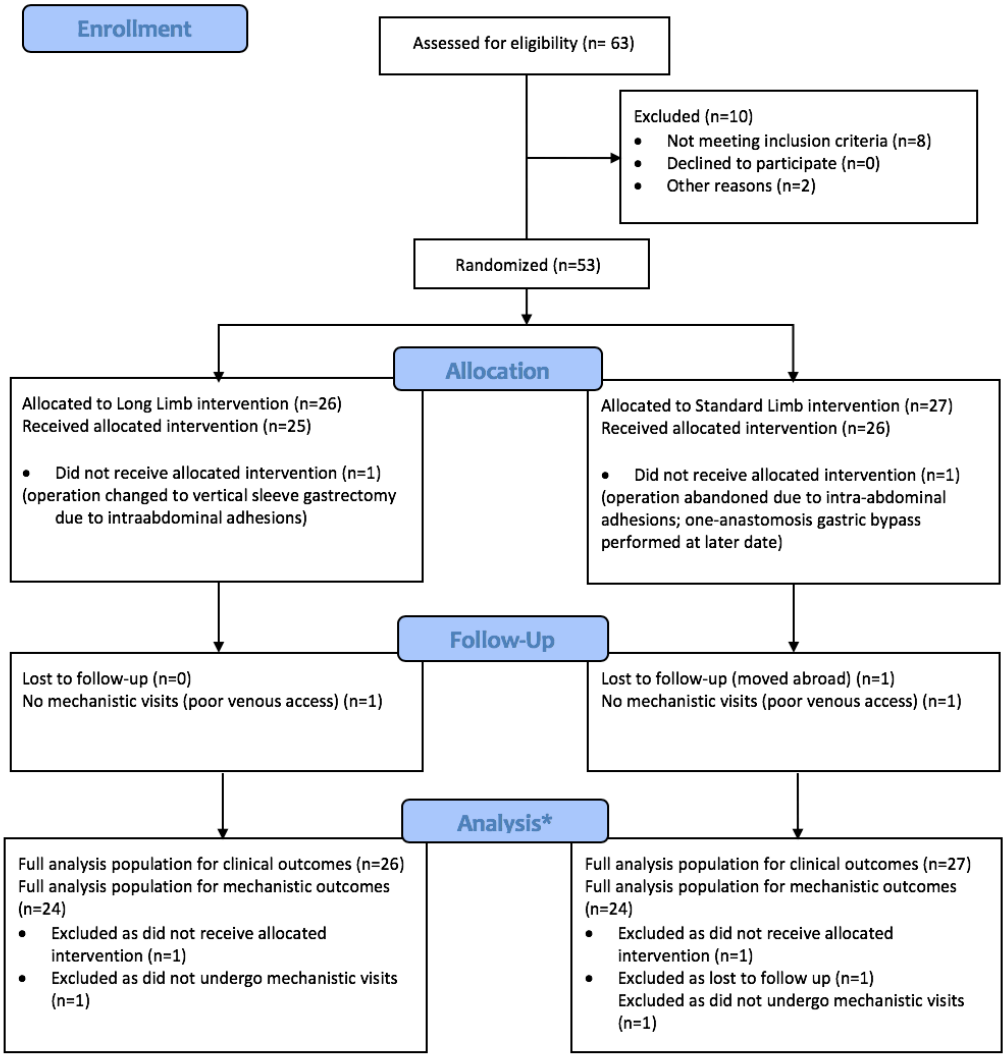


Figure 6. CONSORT Flow Diagram

3.1. Baseline characteristics

Baseline characteristics, which were recorded within 7 days preceding the surgery, were balanced well between the groups (Table 3). The majority of the patients were middle-aged White European females. The mean peri-operative BMI was 43 ± 8 kg/m^2 in the Long Limb and 42 ± 6 kg/m^2 in the Standard Limb group. Participants from the Long Limb group presented

with a mean HbA1c of 76 ± 16 mmol/mol, the median duration of T2DM of 8 (6-9) years and were taking a median number of 3 (2-3) glucose-lowering medications. Patients from the Standard Limb group had a mean HbA1c of 73 ± 17 mmol/mol, the median duration of T2DM of 8 (6-10) years and were taking a median number of 3 (2-3) glucose-lowering medications.

Characteristic	**Long Limb** **Baseline** **n=26**	**Standard Limb** **Baseline** **n=27**
Gender % (n)	69% Female (18)	59% Female (16)
Ethnicity % (n)	69% White (18) 23% Asian (6) 8% Afro-Caribbean (2)	85%White (23) 7.5% Asian (2) 7.5% Afro-Caribbean (2)
Age (years)	48 ± 9	49 ± 10
Weight (kg)	121 ± 28	117 ± 18
BMI (kg/m^2)	43 ± 8	42 ± 6
Duration of T2DM (years)	8 [6-9]	8 [6-10]
Number of glucose-lowering medications	3 [2-3]	3 [2-3]
HbA1c (mmol/mol)	76 ± 16	73 ± 17
Hypertension	68% (17)	69% (18)
Dyslipidaemia	76% (19)	69% (18)
Obstructive sleep apnoea	28% (7)	50% (13)
Arthritis	20% (5)	24% (6)
Depression	36% (9)	46% (12)

Table 3. Key clinical parameters at baseline.

Categorical data presented as percentage (n). Continuous data presented as mean ± SD when normally distributed or median [interquartile range] when non-normally distributed. BMI: Body Mass Index, T2DM – type 2 diabetes mellitus. HbA1c – glycated haemoglobin.

3.2. Weight loss and body composition

Within 14 days after the surgery patients in both groups lost a similar amount of total body weight (Standard Limb 6.2 ± 2.3% vs. Long Limb 6.1 ± 1.6%, p=0.97). As per protocol, both groups were studied again at matched 20% weight loss; this occurred on average 4.5 months after surgery (recorded weight at the late post-operative mechanistic visit: Long Limb 20.6 ± 2.7% and Standard Limb 21.5 ± 2.8%). There were no differences in total body weight loss percentage between the groups at any time point post-operatively including records at 3 months (19 ± 4% vs 19 ± 4%), 6 months (24 ± 4% vs 26 ± 6%) and 12 months (29 ± 8% vs 30 ± 8%; ANCOVA p=0.52) in Long Limb vs Standard Limb respectively however, total body weight loss was significant at all postoperative timepoints when compared to baseline within each group (mixed-effects model p<0.001; Figure 7). Changes in anthropometric records and resting metabolic rate at 1 year are presented in Table 4.

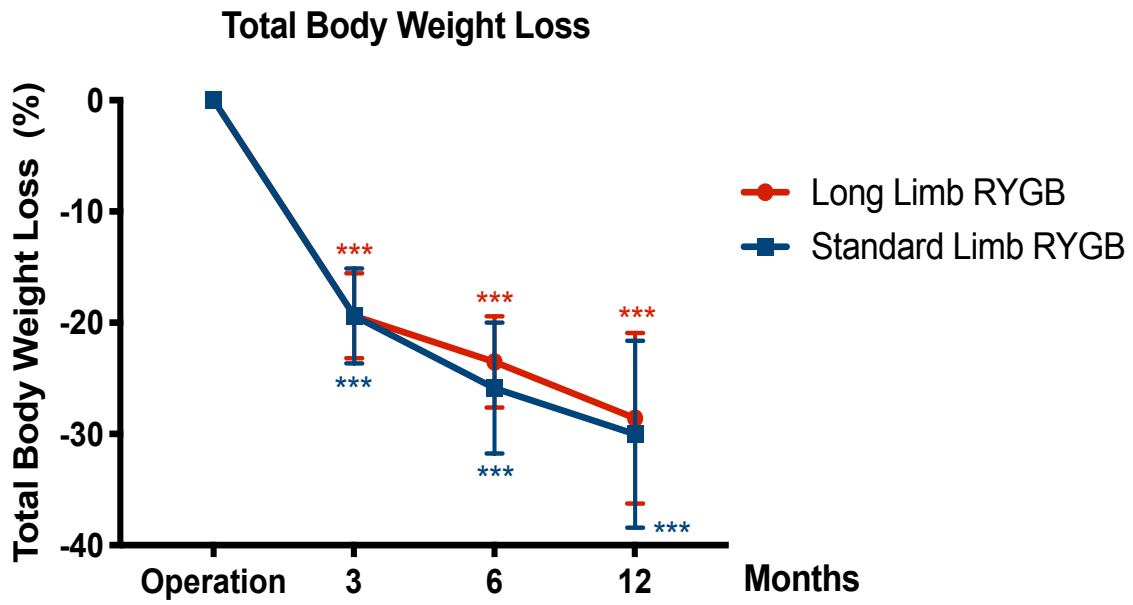


Figure 7. Total body weight loss within the first post-operative year.

Data presented as mean ± standard deviation. N=26 in each group. Statistical test used: mixed-effects model with Bonferroni adjustment for multiple comparisons in post-hoc analysis. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group; ***p<0.001. No statistically significant difference between the groups.

Variable	**Group**	**Day of surgery**	**12 months post-op**	**Mixed-effects analyses**						**Within group post hoc analysis**
				Group effect		**Visit effect**		**Group*Visit interaction**		
				(DFn, DFd) = F value	**P value**	**(DFn, DFd) = F value**	**P value**	**(DFn, DFd) = F value**	**P value**	**p value**
Weight (kg)	Long Limb	121 ± 28	87 ± 24	(1, 53) = 0.7	0.41	(1, 52) = 512	<0.001	(1,52) = 0.2	0.65	<0.001
	Standard Limb	117 ± 18	82 ± 13							<0.001
BMI (kg/m^2)	Long Limb	43 ± 8	31 ± 7	(1, 53) = 1.2	0.28	(1, 52) = 581.7	<0.001	(1, 52) = 0.8	0.77	<0.001
	Standard Limb	42 ± 6	29 ± 5							<0.001
Waist circumference (cm)	Long Limb	128 ± 14	100 ± 16	(1, 53) = 0.1	0.92	(1, 49) = 333.8	<0.001	(1, 49) = 1.2	0.27	<0.001
	Standard Limb	130 ± 12	97 ± 11							<0.001
Hips circumference (cm)	Long Limb	134 ± 16	111 ±15	(1, 51) = 2.1	0.15	(1, 47) = 279.5	<0.001	(1,47) = 0.5	0.47	<0.001
	Standard Limb	130 ± 11	105 ±7							<0.001
Neck circumference (cm)	Long Limb	44 ± 6	37 ± 5	(1, 54) = 0.1	0.82	(1, 49) = 245.2	<0.001	(1,49) = 0.1	0.77	<0.001
	Standard Limb	44 ± 4	37 ± 4							<0.001
Body fat (%)	Long Limb	44 ± 30	30 ± 9	(1, 53) = 1.4	0.25	(1, 48) = 305.6	<0.001	(1,48) = 1.2	0.28	<0.001

	Standard Limb	43 ± 7	27 ± 8							<0.001
Fat mass (kg)	Long Limb	55 ± 16	27 ± 14	(1, 53) = 2.3	0.14	(1, 47) = 474.2	<0.001	(1,47) = 0.01	0.92	<0.001
	Standard Limb	50 ± 11	22 ± 7							<0.001
Fat free mass	Long Limb	67 ± 15	56 ±12	(1, 52) = 0.03	0.86	(1, 46) = 167.4	<0.001	(1, 46) = 1.8	0.19	<0.001
	Standard Limb	64 ± 12	56 ± 9							<0.001
Resting metabolic rate (kcal/day)	Long Limb	2201 ± 535	1760 ± 420	(1, 52) = 0.3	0.60	(1, 41) = 175.9	<0.001	(1,41) = 0.7	0.41	<0.001
	Standard Limb	2040 ± 384	1727 ± 272							<0.001

Table 4. Anthropometric parameters and resting metabolic rate at baseline and 12 months.

Continuous data presented as mean ± standard deviation when normally distributed or median [interquartile range] when non-normally distributed. N=26 in the Long Limb group; N=27 in the Standard Limb group pre-operatively and N=26 in the Standard Limb group at 12 months post-operatively. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. No statistically significant differences between the groups; p-values refer to comparing outcomes within Long Limb or Standard Limb at 12-month follow-up. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom. BMI – Body Mass Index.

As expected, all patients had a significant reduction in their weight, BMI, waist, hips and neck circumference (however with no differences between the study arms). Significant changes in patients' weight also influenced shifts in body composition, with a reduction in fat tissue being more excessive than the fat free mass loss, despite some reduction in the resting energy expenditure. These finding are consistent with published outcomes following obesity surgery [329].

3.3. Type 2 diabetes mellitus – related clinical outcomes

There were no significant differences in glycated haemoglobin, HbA1c between the Long Limb and the Standard Limb groups at any time point post-operatively including at 12 months (Standard Limb 43 ± 10 mmol/mol vs. Long Limb 41 ± 5 mmol/mol, p=0.20; Table 5). HbA1c reduction within Long Limb and Standard Limb groups was statistically significant at 3, 6 and 12 months (Figure 8).

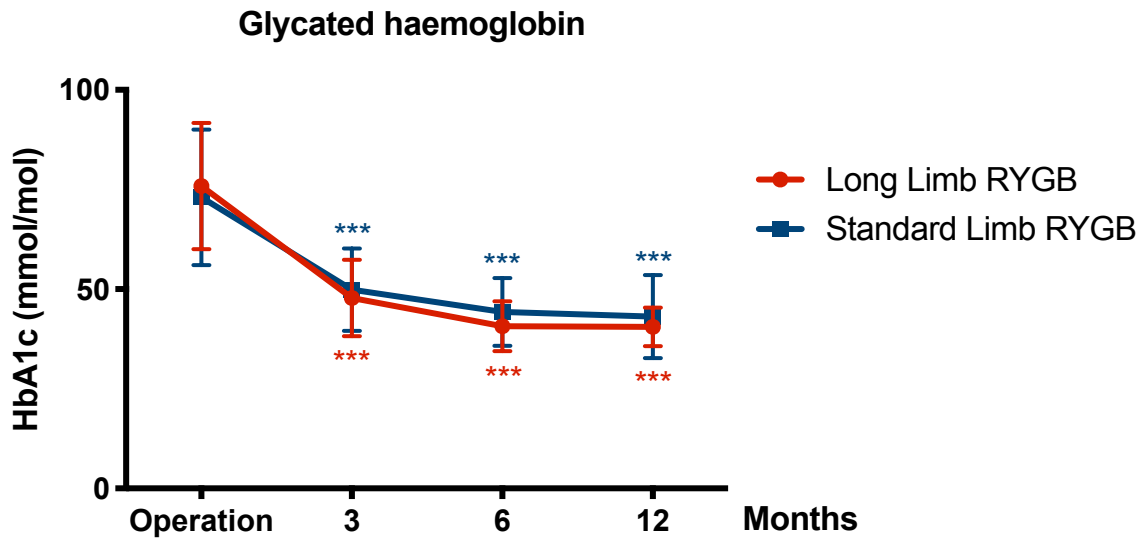


Figure 8. Changes in the glycated haemoglobin within the first post-operative year.

Data presented as mean ± standard deviation. N=26 in each group. Statistical test used: mixed-effects model with Bonferroni adjustment for multiple comparisons in post-hoc analysis. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group; ***p<0.001. No statistically significant difference between the groups.

Variable	**Group**	**Day of surgery**	**12 months post-op**	**Mixed-effects analyses**						**Within group post hoc analysis**
				Group effect		**Visit effect**		**Group*Visit interaction**		
				(DFn, DFd) = F value	**P value**	**(DFn, DFd) = F value**	**P value**	**(DFn, DFd) = F value**	**P value**	**p value**
HbA1c (mmol/mol)	Long Limb	76 ± 16	41 ± 5	(1, 49) = 0.4	0.53	(3, 145) = 159.3	<0.001	(3, 145) = 1.5	0.22	<0.001
	Standard Limb	73 ± 17	43 ± 10							<0.001
Fasting plasma glucose (mmol/L)	Long Limb	10.3 ± 2.7	5.4 ± 0.9	(1, 49) = 2.0	0.17	(1, 49) = 144.5	<0.001	(1, 49) = 0.7	0.42	<0.001
	Standard Limb	11.3 ± 3.2	5.6 ± 1.4							<0.001
Fasting serum insulin (mU/L)	Long Limb	17 ± 11	6 ± 3	(1, 53) = 0.1	0.82	(1, 53) = 55.3	<0.001	(1, 53) = 0.03	0.88	<0.001
	Standard Limb	17 ± 13	6 ± 3							<0.001
Serum c-peptide (nmol/L)	Long Limb	1258 ± 623	604 ± 183	(1, 49) = 0.03	0.87	(1, 48) = 74.2	<0.001	(1,47) = 0.3	0.56	<0.001
	Standard Limb	1200 ± 603	666 ± 265							<0.001

Table 5. Type 2 diabetes mellitus – related outcomes at baseline and 12 months.

Continuous data presented as mean ± standard deviation when normally distributed or median [interquartile range] when non-normally distributed. N=26 in the Long Limb group; N=27 in the Standard Limb group pre-operatively and N=26 in the Standard Limb group at 12 months post-operatively. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. No statistically significant differences between the groups; p-values refer to comparing outcomes within Long Limb or Standard Limb at 12-month follow-up. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom. HbA1c – glycated haemoglobin.

Fasting state hyperglycaemia was reduced by half 12 months after the RYGB, with mean readings reduced to normal levels below 6 mmol/L. As a result, hyperinsulinaemia present in the fasting state pre-operatively was reduced almost three-fold at the end of the follow-up period and serum c-peptide concentration was roughly halved in each group. Whilst these results were significant within groups, no differences between the study arms were observed. Changes of glycaemic homeostasis postprandially will be discussed further in Chapter 4.

There were no significant differences in the percentage of patients achieving glycaemic remission as per ADA criteria for partial or complete remission at 12 months between both groups: Standard Limb 62% vs. Long Limb 77%, p=0.23.
Throughout the trial, the use of glucose-lowering medications decreased similarly in both groups as illustrated in Figure 9.

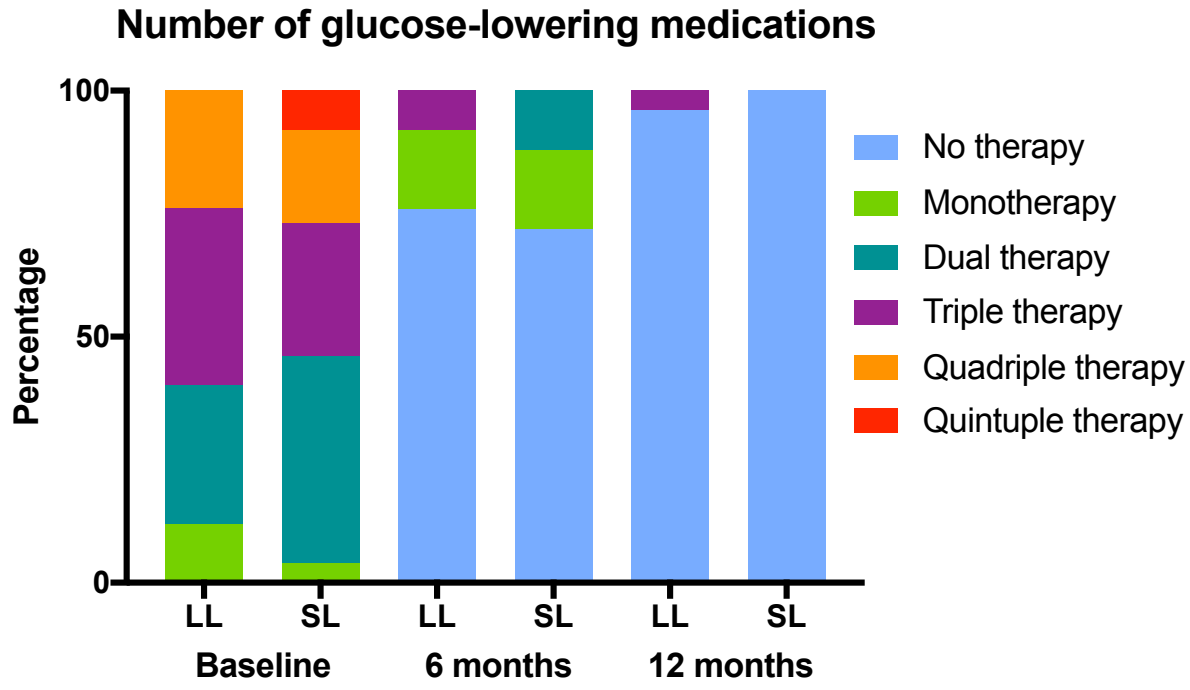


Figure 9. Changes in glucose-lowering medications use in the first post-operative year.
Number of medications refers to the number of classes of glucose-lowering medications used.

The majority of patients required dual or triple pharmacotherapy for T2DM pre-operatively, with almost all being on Metformin. Of 4 patients from each group who were on insulin pre-operatively, all managed to stop it post-operatively (Table 6). All but one participant from the Long Limb stopped all glucose-lowering medications 9 months after the surgery at the latest, therefore HbA1c results presented above reflect on glycaemic control in the absence of medications. Following one year follow up, based on HbA1c and fasting plasma glucose levels, glucose-lowering medications were restarted in 7 participants from the Standard Limb but none (apart from the participant who continued on diabetic pharmacotherapy throughout the year) in the Long Limb group.

Characteristic	**Long Limb RYGB Baseline n=26**	**Standard Limb RYGB Baseline n=27**	**Long Limb RYGB 12 months post-operatively n=26**	**Standard Limb RYGB 12 months post-operatively n=26**
Number of glucose-lowering medications (classes)				
1	11% (3)	4% (1)	0	0
2	27% (7)	44% (12)	0	0
3	35% (9)	26% (7)	1	0
4	27% (7)	19% (5)	0	0
5	0% (0)	7% (2)	0	0
Classes of medications:				
Biguanides	92% (24)	93% (25)	1	0
SGLT-2 inhibitors	54% (14)	56% (15)	1	0
Sulfonylurea	50% (13)	48% (13)	0	0
GLP-1 agonists	35% (9)	15% (4)	1	0
DPP-4 inhibitors	31% (8)	52% (14)	0	0
Insulin	15% (4)	15% (4)	0	0
Total	**72**	**75**	**3**	**0**

Table 6. Glucose-lowering medications use pre-operatively and

Categorical data presented as percentage (n). RYGB – Roux-en-Y gastric bypass. SGLT-2 – sodium-glucose transport protein-2. GLP-1 – glucagon-like peptide-1. DPP-4 – dipeptidyl peptidase-4 inhibitor.

Variable	Group	N	Day of surgery	12 months post-op	Between group comparison p value
HbA1c (^) (mmol/mol)	Long Limb	6	80 ± 12	45 ± 5	0.04
	Standard Limb	10	71 ± 7	53 ± 9	
Change in HbA1c (^^) (%)	Long Limb	6		-43 ± 9	0.009
	Standard Limb	10		-25 ± 13	

Table 7. HbA1c and its change analysis in participants not achieving T2DM remission at 12 months post-operatively.

Continuous data presented as mean ± standard deviation when normally distributed. (^) Analysis using ANCOVA. (^^) Analysis using the unpaired t-test. HbA1c – glycated haemoglobin.

Interestingly, when analysing the small population of patients who had not achieved T2DM remission, those 16 patients had an average duration of T2DM pre-operatively of 10.3 ± 6.1 years which was longer than T2DM duration in patients who achieved remission (6.8 ± 3.9 years; p = 0.01). Standard Limb patients had worse outcomes when compared to the Long Limb participants in terms of absolute and percentage reduction of HbA1c at 1 year post-operatively (Table 7). However, the impact of such difference and whether it is caused by differing biliopancreatic limb in the two study arms cannot be established within this study as longer follow up needs to be conducted. No difference in the duration of T2DM prior to the surgery was observed in neither of these subgroups.

3.4. Comorbidities

There were no differences in comorbidities rates between the groups neither pre- nor post-operatively (Table 8). None of the comorbidities rates has increased following the surgery. Statistically significant remission of obstructive sleep apnoea within both groups was

observed, as most patients were able to stop night time CPAP therapy within the first post-operative year following a sleep study.

Variable	**Group**	**Day of surgery**	**12 months post-op**	**Within group comparison p value**	**Between groups comparison p value**
Hypertension	Long Limb	69% (18)	50% (13)	0.16	0.58
	Standard Limb	70% (19)	58% (15)	0.24	
Dyslipidaemia	Long Limb	77% (20)	65% (17)	0.85	0.86
	Standard Limb	67% (18)	65% (17)	0.92	
Obstructive sleep apnoea	Long Limb	27% (7)	0% (0)	0.005	0.08
	Standard Limb	48% (13)	12% (3)	0.004	
Arthritis	Long Limb	19% (5)	15% (4)	0.71	0.52
	Standard Limb	22% (6)	23% (6)	0.94	
Depression	Long Limb	35% (9)	31% (8)	0.77	0.39
	Standard Limb	48% (13)	42% (11)	0.67	

Table 8. Comorbidities at baseline and at 12 months after the surgery.

Categorical data presented as percentage (n). Statistical test used: chi-square. N=26 in the Long Limb group; N=27 in the Standard Limb group pre-operatively and N=26 in the Standard Limb group at 12 months post-operatively.

3.5. Blood results and microalbuminuria

Patients were monitored throughout the trial for micro- and macro elements deficiencies and if required, they were corrected by adjusting supplements accordingly throughout the follow-up period. It was very encouraging to see that the overall mean levels of plasma albumin, haematinics and vitamins were unchanged or even often significantly improved compared to the pre-operative levels (Table 9). This is likely to be due to strict adherence to the supplementation regime post-operatively by study participants.

Improvement in lipid profile, with a decrease in total cholesterol and triglycerides and increase in HDL levels is expected with weight loss and has been widely reported as one of the positive impacts of bariatric surgery [330].

Small degree increases in plasma bilirubin (although still within the normal range) was observed within both groups at one year, which could be the result of the increased enterohepatic bile circulation. Statistically significant decreased levels of ALT and GGT in the Standard Limb cohort could reflect on the improvement in the fatty liver infiltration. The reason why this, not the Long Limb group, achieved significant change is most likely incidental and could be due to the fact that baseline levels of these enzymes were higher pre-operatively, hence the degree of their reduction could have been more pronounced. However, no routine radiological or histological liver assessments were performed to verify these changes.

Even though a trend towards a decrease in microalbuminuria was observed, it was not statistically significant.

Full spectrum of the blood results monitored pre-operatively and at 12 months after the surgery are presented in Table 9.

Variable	**Group**	**Day of surgery**	**12 months post-op**	**Mixed-effects analyses**						**Within group post hoc analysis**
				Group effect		**Visit effect**		**Group*Visit interaction**		
				(DFn, DFd) = F value	**P value**	**(DFn, DFd) = F value**	**P value**	**(DFn, DFd) = F value**	**P value**	**p value**
Hb (g/L)	Long Limb	131 ± 16	135 ±12	(1, 53) = 0.05	0.83	(1, 53) = 1.4	0.29	(1,53) = 1.7	0.20	0.10
	Standard Limb	134 ± 13	133 ± 9							0.86
Iron (μmol/L)	Long Limb	13 ± 5	16 ± 6	(1, 50) = 0.8	0.38	(1, 48) = 22.3	<0.001	(1, 48) = 0.3	0.57	0.004
	Standard Limb	13 ± 5	18 ± 6							0.001
Transferrin saturation (%)	Long Limb	19 ± 8	24 ± 10	(1, 51) = 0.2	0.63	(1, 50) = 15.9	<0.001	(1, 50) = 0.01	0.91	0.008
	Standard Limb	19 ± 8	25 ± 9							0.006
Vitamin B12 (ng/mL)	Long Limb	390 ± 170	521 ± 364	(1, 54) = 0.1	0.72	(1, 53) = 9.4	0.003	(1,53) = 0.01	0.93	0.03
	Standard Limb	373 ± 139	497 ± 278							0.04
Vitamin D (ng/mL)	Long Limb	61 ± 27	70 ± 26	(1, 53) = 2.3	0.14	(1, 52) = 6.2	0.016	(1,52) = 0.5	0.48	0.22
	Standard Limb	67 ± 30	83 ± 34							0.03
Folate (ng/mL)	Long Limb	9 ± 5	10 ± 4	(1, 53) = 0.9	0.34	(1, 51) = 0.9	0.36	(1,51) = 0.01	0.92	0.56

	Standard Limb	10 ± 5	11 ± 5							0.47
Total cholesterol (mmol/L)	Long Limb	4.7 ± 1.2	4.2 ± 0.9	(1, 50) = 0.3	0.58	(1, 50) = 14.7	<0.001	(1,50) = 0.1	0.74	0.005
	Standard Limb	4.6 ± 1.0	4.0 ± 0.8							0.02
LDL (mmol/L)	Long Limb	2.6 ± 0.9	2.5 ± 0.7	(1, 51) = 0.8	0.37	(1, 49) = 3.9	0.06	(1, 49) = 0.07	0.79	0.24
	Standard Limb	2.5 ± 1.0	2.2 ± 0.7							0.11
HDL (mmol/L)	Long Limb	1.0 ± 0.3	1.2 ± 0.2	(1, 53) = 4.1	0.05	(1, 52) = 84.3	<0.001	(1,52) = 1.7	0.20	<0.001
	Standard Limb	1.1 ± 0.2	1.3 ± 0.3							<0.001
TG (mmol/L)	Long Limb	2.7 ± 2.6	1.1 ± 0.3	(1, 53) = 0.9	0.34	(1, 53) = 25.3	<0.001	(1,53) = 0.8	0.38	<0.001
	Standard Limb	2.2 ± 1.1	1.1 ± 0.6							0.005
Albumin (g/L)	Long Limb	39 ± 3	40 ± 3	(1, 52) = 0.3	0.57	(1, 52) = 2.2	0.15	(1,52) = 0.7	0.4	0.11
	Standard Limb	38 ± 4	39 ± 3							0.66
Bilirubin (g/L)	Long Limb	9 ± 6	13 ± 10	(1, 53) = 0.005	0.95	(1, 53) = 40.5	<0.001	(1,52) = 0.1	0.73	<0.001
	Standard Limb	9 ± 5	13 ± 5							<0.001
ALP (IU/L)	Long Limb	93 ± 24	106 ± 50	(1, 53) = 1.2	0.27	(1, 53) = 3.7	0.06	(1,53) = 0.9	0.36	0.05
	Standard Limb	88 ± 24	93 ± 31							0.48

ALT (IU/L)	Long Limb	33 ± 15	35 ± 38	(1, 54) = 1.0	0.33	(1, 53) = 3.7	0.06	(1,53) = 5.2	0.03	0.80
	Standard Limb	50 ± 35	30 ± 15							0.005
GGT	Long Limb	53 ± 51	34 ± 47	(1, 54) = 0.3	0.60	(1, 53) = 12.5	0.001	(1,53) = 3.1	0.08	0.21
	Standard Limb	78 ± 104	24 ±13							0.001
Urine albumin /creatinine ratio	Long Limb	6.4 ±10.2	3.1 ± 8	(1, 54) = 2.7	0.12	(1, 53) = 2.5	0.12	(1,53) = 0.9	0.36	0.08
	Standard Limb	2.8 ± 3.8	1.9 ± 2.7							0.64

Table 9. Blood tests and microalbuminuria results at baseline and 12 months.

Continuous data presented as mean ± standard deviation when normally distributed or median [interquartile range] when non-normally distributed. N=26 in the Long Limb group; N=27 in the Standard Limb group pre-operatively and N=26 in the Standard Limb group at 12 months post-operatively. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. No statistically significant differences between the groups; p-values refer to comparing outcomes within Long Limb or Standard Limb at 12-month follow-up. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom. Hb – haemoglobin, LDL – low density lipoprotein, HDL – high density lipoprotein, TG – triglycerides. ALP – alkaline phosphatase, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase.

3.6. Intraoperative results

Lengths of alimentary and biliopancreatic limbs were meticulously assessed in all patients. Total small bowel length assessment was performed in all 25 participants who had the Long Limb RYGB but only in 21 out of 26 Standard RYGB patients due to technical challenges such as intraabdominal adhesions which precluded safe measurements all the way to the ileocaecal valve. The median total small bowel length (TSBL) in the Standard Limb group was 615 cm (IQR 470-678, range 320-740) and in the Long Limb group 610 cm (IQR 555-685cm, range

520-910). The median common channel length in the Standard Limb group was 465 cm (range 170-590) and in the Long Limb group 360 cm (range 250-660). The differences between the groups in the TSBL as well as the common channel were not statistically significant (p=0.10 and p=0.12). The median biliopancreatic limb/ TSBL ratio in the Standard Limb group was 8% (range 7-16) and in the Long Limb group 25% (range 16-29; Table 10) which was a statistically significant difference ($p < 0.001$).

There were no significant differences in the operative times between the two procedures (p=0.17) or the in-hospital stay (2 ± 0.7 days in both groups).

	Long Limb RYGB n=25	**Standard Limb RYGB n=21**
Common channel length (cm)	360 [305-435] (250-660)	465 [320-528] (170-590)
Total small intestinal length (cm)	610 [555-685] (520-910)	615 [470-678] (320-740)
Biliopancreatic limb/total small intestinal length ratio (%)	25 [22-27] (16-29)	8 [7-11] (7-16)
Common channel/total small intestinal length ratio (%)	59 [55-64] (48-73)	76 [68-78] (53-80)
Operating time (minutes)	164 ± 51 (59-241)	146 ± 42 (79-250)

Table 10. Intra-operative small bowel length measurements and operative time

Continuous data presented as mean ± standard deviation when normally distributed or median [interquartile range] when non-normally distributed.

3.6.1. Correlation between intestinal limb lengths with baseline characteristics and key clinical and mechanistic outcomes

Significant interindividual variability in TSBL existed in the studied population: as mentioned above, overall it ranged from 320 to 910 cm with a mean of 610 ± 106 cm, with a common channel of 170 to 660 cm (405±107 cm). Since reports of bowel length variability potentially influencing the outcomes of the RYGB exist, I have performed a separate analysis, pooling all patients together regardless of their randomisation arm in order to investigate correlations between the TSBL and baseline characteristics as well as the post-operative outcomes. Across all the Long Limb Trial patients, TSBL showed weak correlation with pre-operative BMI which was borderline statistically significant (r=0.3, p=0.05) – longer TSBL was found in patients with a higher BMI (Figure 10).

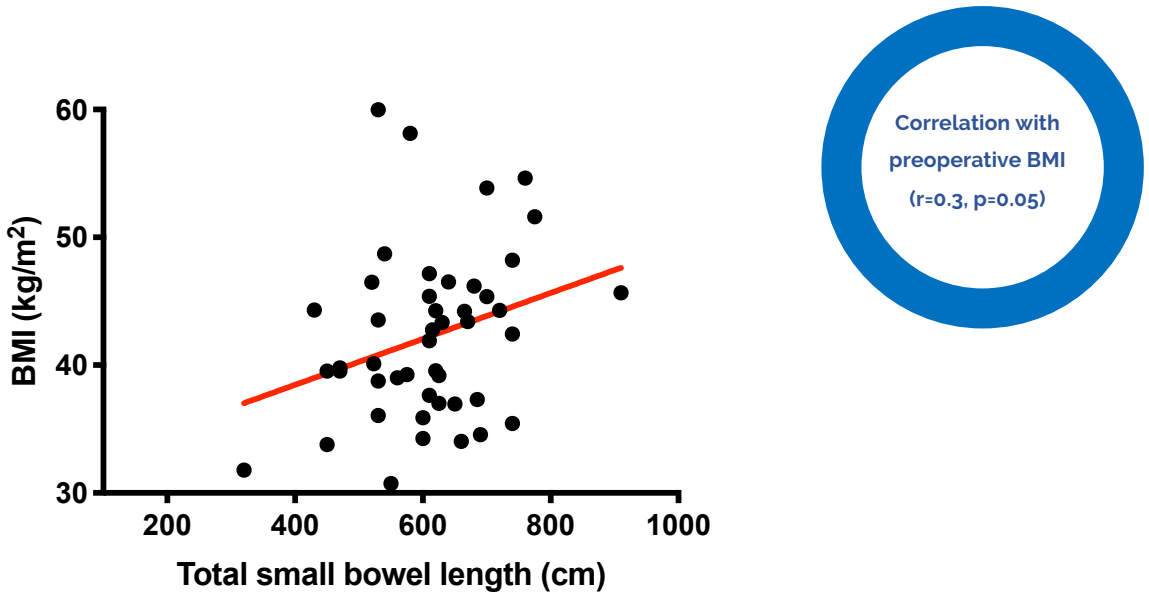


Figure 10. Association between the total small bowel length and pre-operative BMI.

N=25 in the Long Limb group; N=21 in the Standard Limb group. Statistical test used: Pearson's correlation coefficient. BMI – Body Mass Index.

Furthermore, a weak correlation between the pre-operative fasting plasma glucose and the TBSL was found (r=0.04, p=0.02; Figure 11).

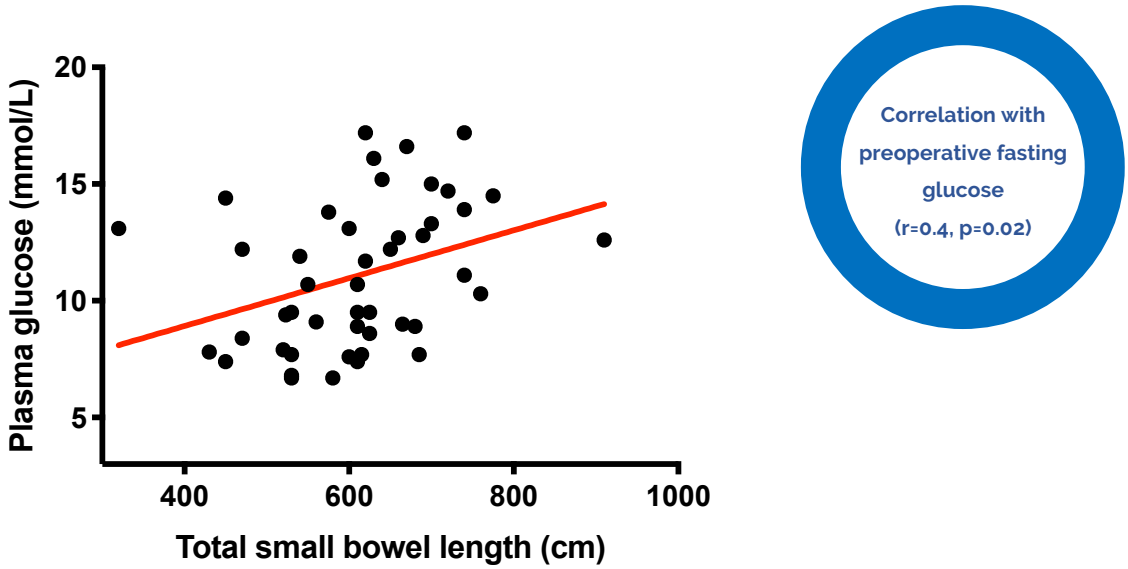


Figure 11. Association between the total small bowel length and pre-operative fasting plasma glucose.

N=25 in the Long Limb group; N=21 in the Standard Limb group. Statistical test used: Pearson's correlation coefficient.

However, no association between the TSBL and other baseline characteristics such as age, gender, height, pre-operative weight, HbA1c, fasting insulin, haemoglobin, plasma iron, transferrin saturation, vitamin D, vitamin B12 or albumin was found. At 12 months post-operatively, TBSL showed a weak positive correlation with albumin (r=0.34, p=0.02) but no other correlations between the TSBL and any of the post-operative clinical outcomes, including weight, BMI, total body weight loss, frequency of bowel motions, HbA1c and change in HbA1c percentage, fasting glucose and insulin, T2DM remission, haemoglobin, transferrin saturation, vitamin D, vitamin B12 were found. There was also no correlation between the BMI and fasting plasma glucose (r=-0.1, p = 0.52).

Both the absolute common limb length (Figure 12) and its ratio of the TSBL (Figure 13) showed a weak positive correlation with plasma iron levels measured 12 months after the surgery. Otherwise, there were no correlations between the post-operative outcomes listed above with the length of the biliopancreatic limb and the common limb (cm) as well as with their ratios to the TSBL.

Likewise, no correlations between the bowel lengths and mechanistic outcomes (which will be described in detail in the next two chapters) from hyperinsulinaemic-euglycaemic clamps and mixed meal tolerance tests were found.

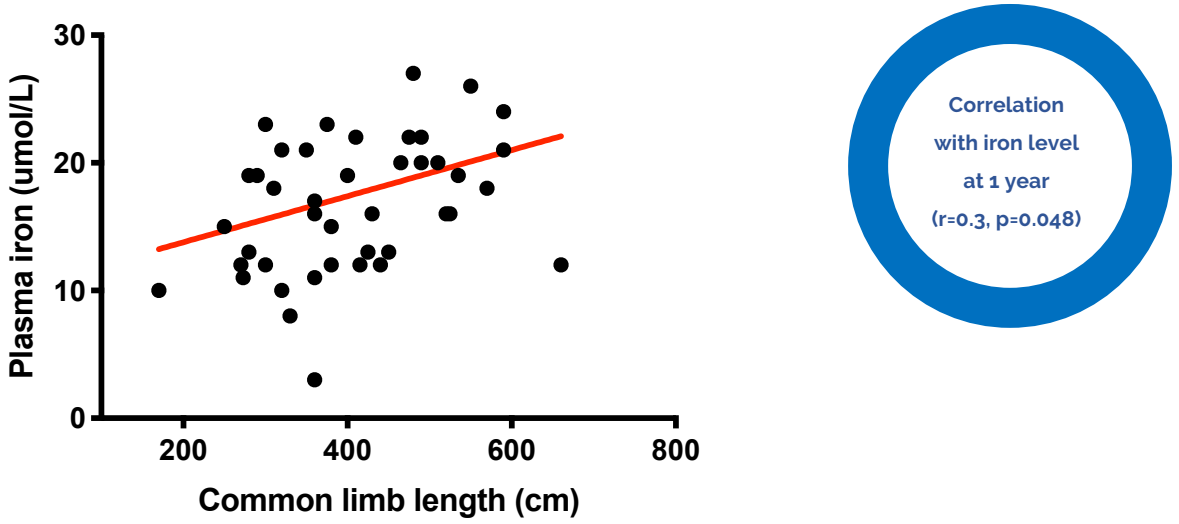


Figure 12. Association between the common limb length and plasma iron 1 year after the RYGB.

N=25 in the Long Limb group; N=21 in the Standard Limb group. Statistical test used: Pearson's correlation coefficient.

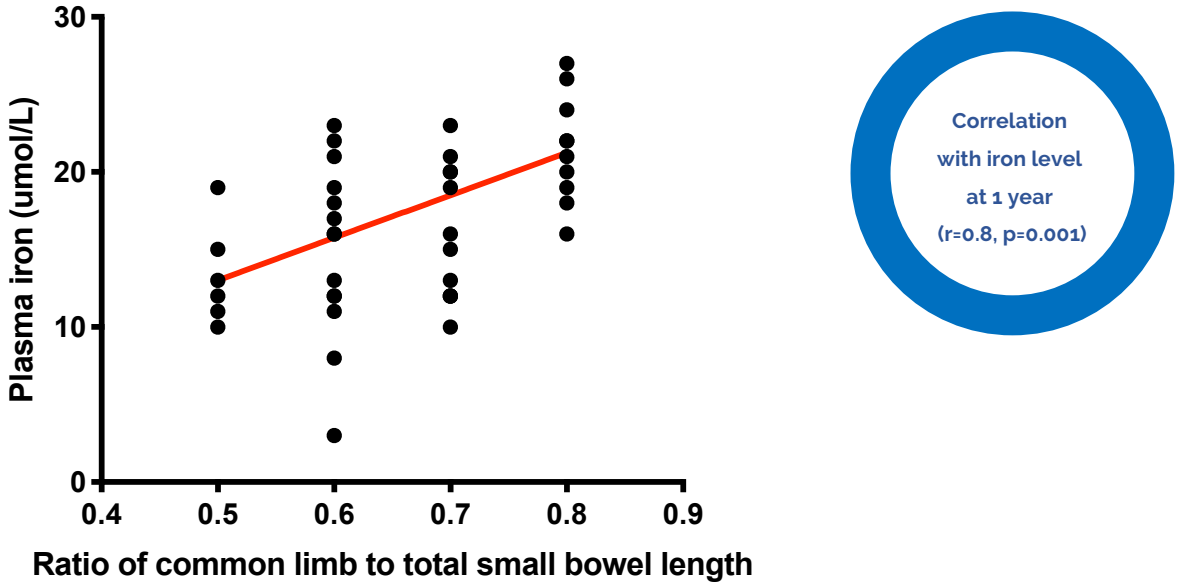


Figure 13. Association between the common limb length to total small bowel length ratio and plasma iron 1 year after the RYGB.

N=25 in the Long Limb group; N=21 in the Standard Limb group. Statistical test used: Pearson's correlation coefficient.

3.7. Recorded Adverse Events

In total, 23 AEs were recorded in 14 participants from the Long Limb group. A further 20 AEs were recorded in 13 participants from the Standard Limb group. Of these, 5 events were SAEs recorded in 3 Long Limb participants and 4 were SAEs recorded in 4 Standard Limb participants. Adverse events were then further classified according to Clavien-Dindo classification, with the majority being Class I or II (Table 11). Four readmissions within 30 days from the surgery were recorded (viral tonsillitis, constipation, dehydration and anastomotic ulcer). AEs were equally distributed between the two participating sites.

Adverse Event	**Long Limb RYGB n=26**	**Standard Limb RYGB n=27**
Cardiovascular	0	0
Gastrointestinal		
Anastomotic stricture	1*	0
Anastomotic ulcer	0	1
Peri-operative bleeding	2	0
Gallstones	1	0
Abdominal pain	1	0
Laparotomy for purulent peritonitis	1*	0
Gastritis	1	0
Diarrhoea	1	2
Constipation	0	1
Infections		
Wound infection	4	2
Pneumonia	4	2
Viral tonsillitis	1	0
Soft tissue and musculoskeletal		
Incisional hernia	1*	0
Limb fracture	0	1
Nutritional and metabolic		
Intravenous treatment for dehydration	0	1
Acute kidney injury	0	2
Anaemia	2	2
Vasovagal	1	2
Hypoglycaemic episode	2	4
***Adverse Events leading to hospitalisation**	5 (in 3 participants)	4 (in 4 participants)

Clavien-Dindo classification of complications (grades)		
I	6	11
II	14	9
III a	1	0
III b	1	0
IV	1	0
V	0	0
Total	23	20

Table 11. Post-operative adverse events and complications.

*related sequence of events in one study participant

The majority of recorded adverse events were related to infection requiring antibiotic therapy, namely wound infections and pneumonia, which are common and expected early post-operative complications after routine obesity surgery. Most of the events were surgery-related with several, such as limb fracture due to the fall or Campylobacter diarrhoea not related to the intervention but happening within the trial period. There were no deaths in the trial.

4. Chapter 4. Gut hormones and insulin secretion

Insulin and gut hormones secretion in fasting and post-prandial state were assessed with a mixed meal tolerance test (MMTT), as described in the Methods chapter. The first of these mechanistic assessments took place pre-operatively and the second one within two weeks after the RYGB surgery (assuming time period short enough not to allow for a significant weight loss but also long enough to allow some recovery after the operation). Patients in both groups lost similar amounts of body weight: Standard Limb 6.2 ± 2.3% vs. Long Limb 6.1 ± 1.6% ($p=0.97$). This reduction in TBWL was already statistically significant within both groups when compared to their weight on the day of surgery ($p<0.001$ in both groups). The last mechanistic visit was planned for the matched weight loss of 20%, with actual recorded TBWL being 21.5 ± 2.8% in the Standard Limb and 20.6 ± 2.7% in the Long Limb with no difference between the groups. These visits took place approximately 4.5 months after the RYGB (range 2 to 8 months).

4.1. Gut hormones

The primary outcome of the study was postprandial peak in active glucagon peptide-1 (GLP-1) plasma concentration at two weeks after the RYGB. It has increased significantly within both groups from 16 ± 13 pmol/L pre-operatively to 62 ± 31 pmol/L at the early post-operative visit after the RYGB in the Long Limb group ($p<0.001$) and from 24 ± 33 pmol/L to 78 ± 41 pmol/L ($p<0.001$) but no significant differences between the groups were found (Figure 13, Table 12). A similar pattern was found in the AUCs of active GLP-1 which increased from 1274 ± 1347 (pmol/L)x mins to 4528 ± 1764 (pmol/L)x mins in the Long Limb ($p<0.001$) and from 2330 ± 3832 (pmol/L)x mins to 5357 ± 3864 (pmol/L)x mins in the Standard Limb ($p<0.001$) with no difference between the study arms.

At 20% weight loss, significant increases compared to the baseline in the post-prandial peaks and AUC of active GLP-1 concentration within both groups were sustained: 68 ± 22 pmol/L and 71 ± 31 pmol/L in the Long and Standard groups respectively ($p<0.001$ in both) and AUC

of 3812 ± 1327 (pmol/L)x mins and 5259 ± 3613 (pmol/L)x mins in the Long and Standard groups respectively ($p<0.001$ in both). However, no significant differences between the groups were found (Table 12, Figure 13).

The total GLP-1 assay that was run to crosscheck the results of the active GLP-1 confirmed the same patterns within both groups, with a significant increase in peak and AUC within two weeks after the surgery in each group ($p<0.001$). That increase was maintained when 20% weight loss was achieved (Table 12).

There were no differences between the groups in peaks or AUCs of the other incretin, glucose-dependent insulinotropic polypeptide (GIP) nor the polypeptide YY (PYY) (Table 12).

There were also no correlations between the biliopancreatic limb length or its proportion to the total small bowel length and peaks or AUCs of active GLP-1, total GLP-1, GIP and PYY.

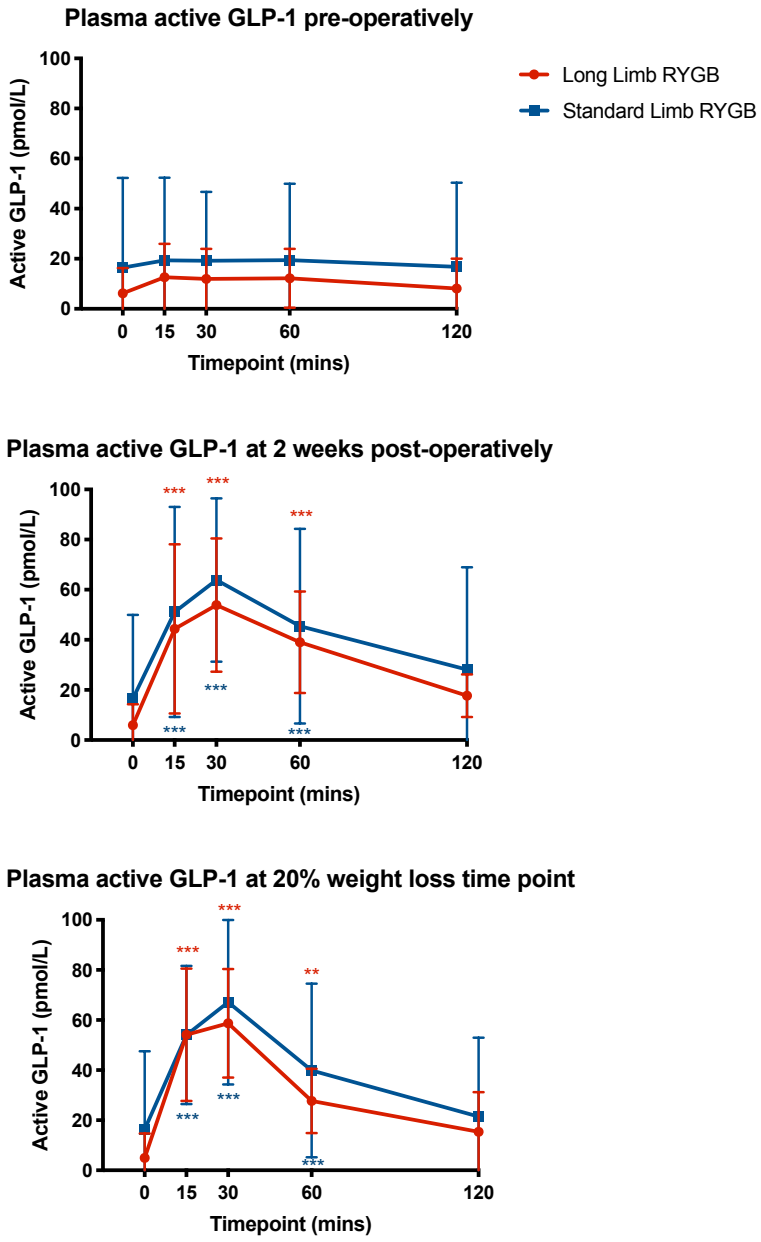


Figure 13. Active GLP-1 response during the mixed meal tolerance test.

Data plotted as means ± SD. N=24 in each group. GLP-1 – glucagon-like peptide-1. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. *p<0.05, **p<0.01, ***p<0.001

Variable	**Group**	**Pre-op visit**	**2 weeks post-op**	**20% weight loss**	**Mixed-effects analyses**						**Post hoc comparisons p value**	
					Group effect		Visit effect		Group * Visit		Pre-op vs 2 weeks post-op	Pre-op vs 20% weight loss
					(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value		
Peak of plasma active GLP-1 (pmol/L)	Long Limb	16 ± 13	62 ± 31	68 ± 22	(1, 48) = 1.7	0.20	(2, 96) = 100.7	<0.001	(2, 96) = 1.4	0.26	<0.001	<0.001
	Standard Limb	24 ± 33	78 ± 42	71 ± 31							<0.001	<0.001
AUC of plasma active GLP-1 (pmol/L) x mins	Long Limb	1274 ± 1347	4528 ± 1764	3812 ± 1327	(1, 48) = 1.8	0.18	(2, 79) = 104.8	<0.001	(2, 79) = 0.6	0.56	<0.001	<0.001
	Standard Limb	2330 ± 3832	5357 ± 3864	5259 ± 3613							<0.001	<0.001
Peak of plasma total GLP-1 (pmol/L)	Long Limb	15 ± 11	99 ± 37	112 ± 38	(1, 48) = 0.1	0.70	(2, 96) = 154.1	<0.001	(2, 96) = 0.3	0.75	<0.001	<0.001
	Standard Limb	13 ± 6	105 ± 49	117 ± 57							<0.001	<0.001

AUC of plasma total GLP-1 (pmol/L) x mins	Long Limb	1044 ± 542	6487 ± 1987	6039 ± 2555	(1, 48) = 0.005	0.95	(2, 96) = 149.1	<0.001	(2, 96) = 0.3	0.74	<0.001	<0.001
	Standard Limb	1018 ± 394	6190 ± 2566	6281± 2586							<0.001	<0.001
Peak of plasma GIP (pmol/L)	Long Limb	107 ± 95	135 ± 88	109 ± 86	(1, 48) = 0.9	0.36	(2, 96) = 3.2	0.05	(2, 96) = 2.5	0.09	0.86	1.0
	Standard Limb	91 ± 72	141 ± 122	173 ± 135							0.18	0.007
AUC of plasma GIP (pmol/L) x mins	Long Limb	8276 ± 6990	7242 ± 3893	7300 ± 6233	(1, 46) = 0.006	0.94	(2, 83) = 0.4	0.68	(2, 83) = 1.3	0.29	1.0	1.0
	Standard Limb	5973 ± 5132	7700 ± 8157	9262 ± 6832							1.0	0.28
Peak of plasma PYY (pmol/L)	Long Limb	44 ± 31	110 ± 40	93 ± 29	(1, 50) = 2.9	0.09	(2, 80) = 63.9	<0.001	(2, 80) = 0.4	0.69	<0.001	<0.001

	Standard Limb	63 ± 50	130 ± 57	118 ± 52							<0.001	<0.001
AUC of plasma PYY (pmol/L) x mins	Long Limb	7135 ± 3550	11943 ± 4465	8178 ± 2588	(1, 30) = 2.2	0.15	(2, 33) = 18.6	<0.001	(2, 33) = 0.2	0.82	0.12	0.09
	Standard Limb	8745 ± 6194	13856 ± 6847	12335 ± 6061							<0.001	0.001

Table 12. Postprandial glucose excursion and insulin secretion in the mixed meal tolerance test.

Continuous data presented as mean ± standard deviation. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. p-values refer to comparing outcomes within Long Limb or Standard Limb at 2 weeks post-op visit and 20% weight loss visit. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom. GLP-1 – glucagon-like peptide-1, PYY – peptide YY. GIP – gastric inhibitory polypeptide. AUC – area under the curve, calculated from time point 0 to 120 min

4.2. Insulin secretion

The postprandial peak of insulin secretion increased significantly within the first two post-operative weeks within each group and was roughly doubled at the timepoint when 20% TBWL was achieved. However, an increase in insulin AUC during the 180 minutes after the meal did not reach statistical significance within or between the groups (Table 13, Figure 14).

Variable	**Group**	**Pre-op visit**	**2 weeks post-op**	**20% weight loss**	**Mixed-effects analyses**						**Post hoc comparisons p value**	
					Group effect		Visit effect		Group * Visit		Pre-op vs 10-14 days post-op	Pre-op vs 20% weight loss
					(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value		
AUC of plasma glucose	Long Limb	2639 ± 659	1957 ± 653	1428 ± 430	(1, 48) = 1.3	0.27	(2, 96) = 96.1	<0.001	(2, 96) = 1.2	0.29	<0.001	<0.001
	Standard Limb	2876 ± 617	1938 ± 562	1642 ± 551							<0.001	<0.001
Peak of plasma glucose (mmol/L)	Long Limb	16.5 ± 4.1	13.9 ± 4.3	11.3 ± 2.4	(1, 48) = 1.9	0.17	(2, 96) = 32	<0.001	(2, 96) = 1.2	0.29	0.008	<0.001
	Standard Limb	17.5 ± 3.7	14.1 ± 3.4	13.4 ±4.0							<0.001	<0.001
AUC of serum insulin	Long Limb	5128 ± 2833	6028 ± 3405	5657 ± 2831	(1, 48) = 0.3	0.59	(2,95) = 4	0.02	(2, 95) = 0.4	0.64	0.20	0.81
	Standard Limb	5280 ± 2464	6259 ± 3088	6433 ± 3059							0.15	0.07
Peak of serum insulin	Long Limb	43.4 ± 27.0	76.1 ± 51.4	81.8 ± 38.3	(1, 48) = 0.4	0.54	(2, 95) = 40.5	<0.001	(2, 95) = 0.3	0.72	<0.001	<0.001

(mU/L)	Standard Limb	43.9 ± 23.0	84.1 ± 44.0	90.6 ± 45.7							<0.001	<0.001

Table 13. Postprandial glucose excursion and insulin secretion in the mixed meal tolerance test.

Continuous data presented as mean ± standard deviation. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. p-values refer to comparing outcomes within Long Limb or Standard Limb at 2 weeks post-op visit and 20% weight loss visit. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom.

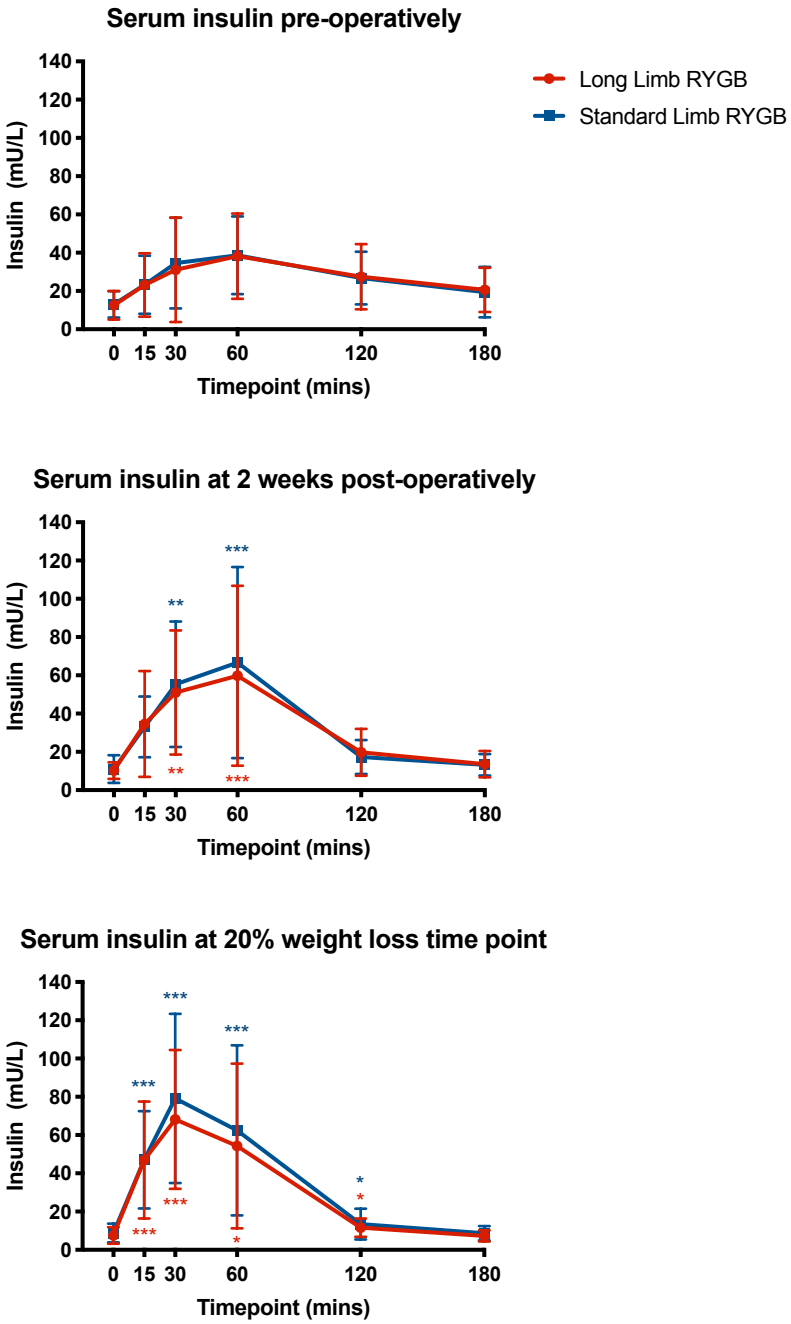


Figure 14. Serum insulin excursion during the mixed meal tolerance test.

Data expressed as means ± SD. N=24 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. *p<0.05, **p<0.01, ***p<0.001

Glucose excursions during the mixed meal tolerance test showed lower peaks and decreased AUC as compared to the pre-operative records (Table 12, Figure 15).

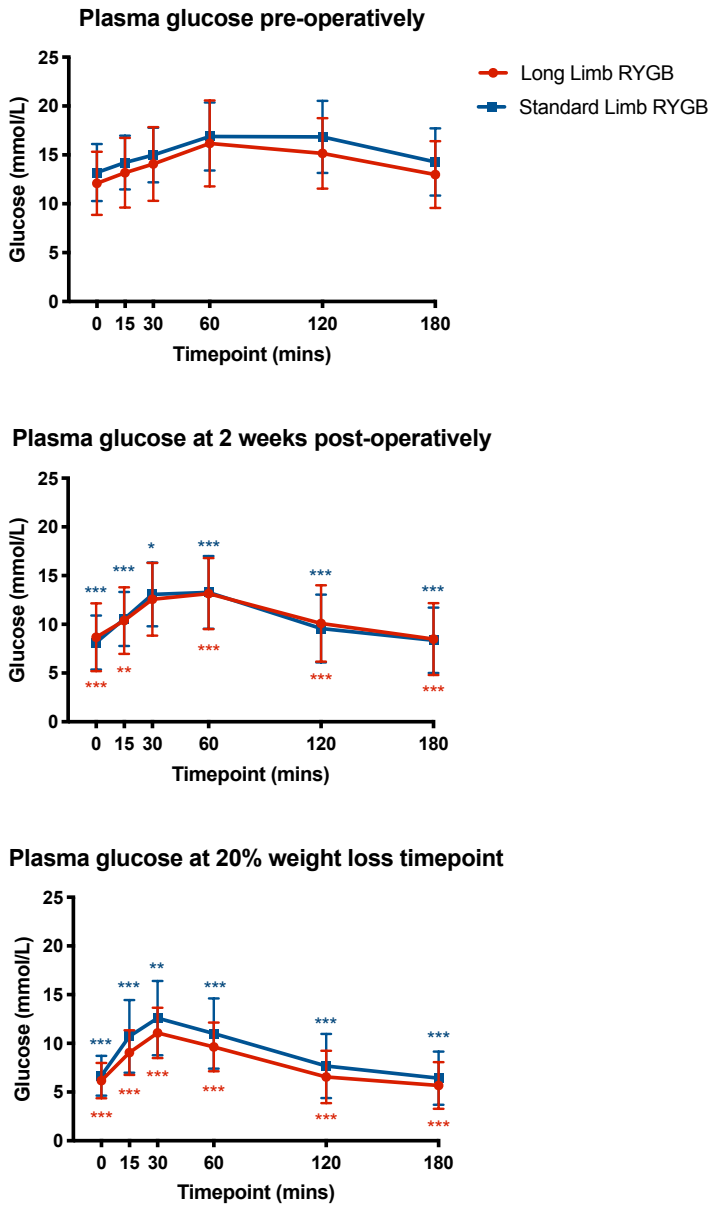


Figure 15. Plasma glucose excursion during the mixed meal tolerance test.

Data expressed as means ± SD. N=24 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. *p<0.05, **p<0.01, ***p<0.001

β-cell function was assessed with Insulinogenic Index which is a measure of the insulin first phase secretion in response to an oral stimulus at 30 minutes postprandially. It did not show statistically significant improvement within or between the groups (Figure 16).

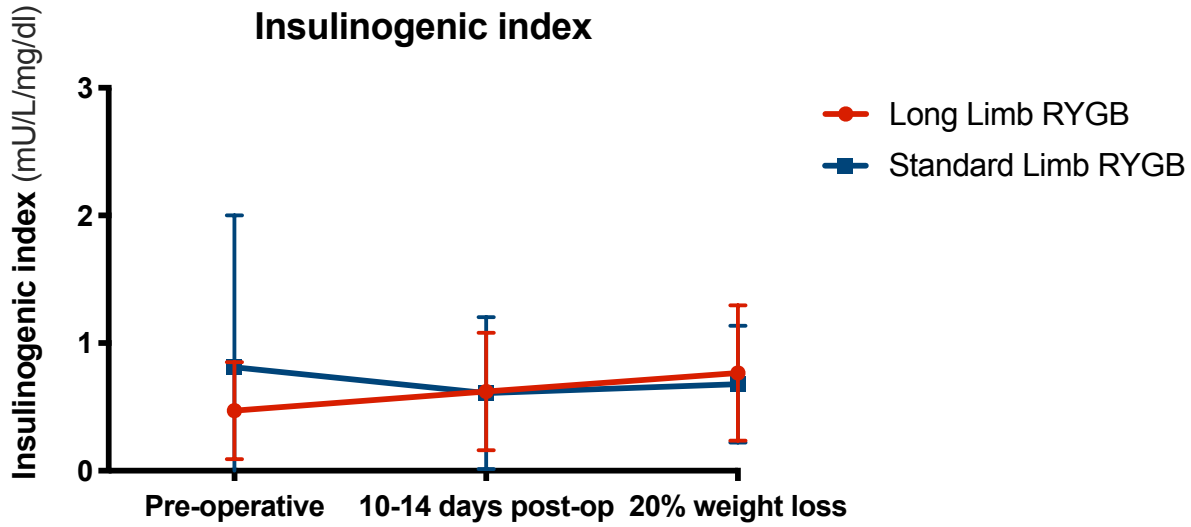


Figure 16. Insulinogenic index derived from the first 30 minutes of the mixed meal tolerance test.

Data expressed as means ± SD. N=24 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. No statistically significant differences between or within the study arms were found.

Disposition index, an indicator of β-cell function throughout the mixed meal tolerance test, derived as a product of insulin sensitivity over the amount of glucose secreted in response to the blood glucose levels, has shown statistically significant increase (i.e. improvement) in the Long Limb but not the Standard Limb group at both post-operative visits (Figure 17).

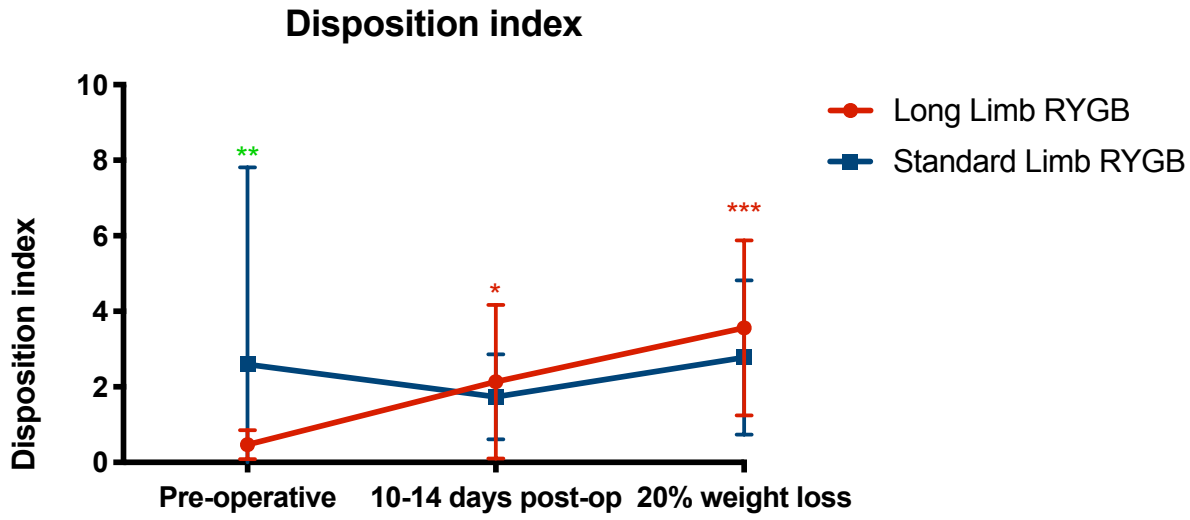


Figure 17. Disposition index derived from the mixed meal tolerance test.

Data expressed as means ± SD. N=24 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. Green stars – comparison between the groups. *p<0.05, **p<0.01, ***p<0.001

5. Chapter 5. Insulin Sensitivity

Insulin sensitivity was assessed with a gold-standard methodology in the field, the hyperinsulinaemic-euglycaemic clamp, as described in the Methods chapter. The assessments took place during the same visits as the mixed meal tolerance test, i.e. pre-operatively, early post-operatively within 14 days after the surgery and late post-operatively at matched 20% weight loss, as described above. There were no differences in the TBWL between the groups at any of the mechanistic visits.

Rate of plasma glucose appearance (Ra), which refers to the endogenous (hepatic) glucose production, reflects on the hepatic insulin sensitivity during the low-dose infusion of the insulin. The dose of insulin in the first, low-dose phase of the clamp, is set at a level sufficient to suppress endogenous glucose production in a subject with undisturbed insulin sensitivity. Significant decrease in Ra was recorded within both groups at the 14 days timepoint and at the point of matched 20% weight loss (Figure 18, Table 14), but there were no significant differences between the Standard and Long Limb groups.

Rate of plasma glucose disappearance (Rd) reflects on the peripheral insulin sensitivity by quantifying the rate of glucose uptake into the peripheral tissues (predominantly muscle). It is best represented at the high dose of the insulin infusion (i.e. the second phase of the clamp), when endogenous glucose production should have been completely suppressed. Rd increased significantly compared to baseline within both groups (Figure 18, Table 13), both within two weeks after the surgery and at the point of matched 20% weight loss, but there were no significant differences between the Standard and Long Limb groups (Table 14).

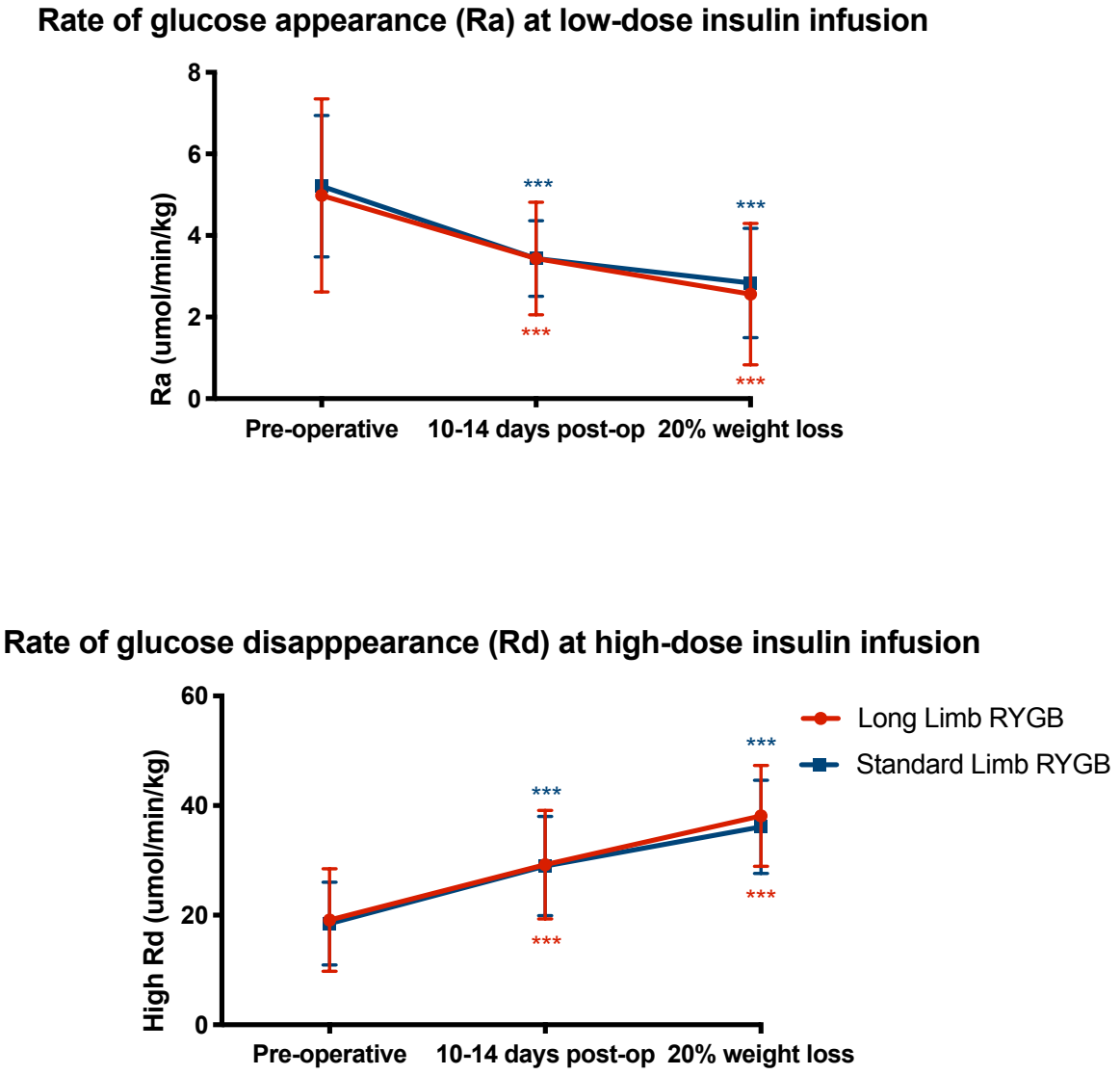


Figure 18. Rate of glucose appearance at a low-dose insulin infusion and rate of glucose disappearance at a high-dose glucose infusion during the hyperinsulinaemic-euglycaemic clamp.

Data plotted as means ± SD. N=23 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. *p<0.05, **p<0.01, ***p<0.001

Variable	**Group**	**Pre-op visit**	**10-14 days post-op**	**20% weight loss**	**Mixed-effects analyses**						**Post hoc comparisons p value**	
					Group effect		Visit effect		Group * Visit		Pre-op vs 10-14 days post-op	Pre-op vs 20% weight loss
					(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value	(DFn, DFd) = F value	P value		
Ra in low dose insulin infusion (umol/min/kg)	Long Limb	4.99 ± 2.37	3.43 ± 1.38	2.56 ± 1.73	(1, 46) = 0.3	0.60	(2, 92) = 43.0	<0.001	(2, 92) = 0.1	0.92	<0.001	<0.001
	Standard Limb	5.21 ± 1.73	3.44 ± 0.93	2.84 ± 1.34							<0.001	<0.001
Ra in high dose insulin infusion (umol/min/kg)	Long Limb	1.17 ± 1.99	0.67 ± 1.70	-0.97 ± 1.29	(1, 47) = 2.5	0.12	(2, 93) = 20.2	<0.001	(2, 93) = 1.2	0.30	0.88	<0.001
	Standard Limb	1.99 ± 2.37	0.65 ± 1.69	-0.03 ± 1.84							0.02	<0.001
Corrected for insulin concentration Ra in low dose (umol/min/kg)	Long Limb	191.7 ± 83.3	115.6 ± 55.8	82.5 ± 59.4	(1, 46) = 0.64	0.43	(2, 92) = 59.5	<0.001	(2, 92) = 0.4	0.96	<0.001	<0.001
	Standard Limb	204.6 ± 66.0	121.1 ± 34.9	91.5 ± 49.6							<0.001	<0.001
Corrected for insulin	Long Limb	128.6 ± 237.2	85.3 ± 209.5	-89.4 ± 135.2	(1, 47) = 1.8	0.18	(2, 93) = 19	<0.001	(2, 93) = 1.5	0.23	1.00	<0.001

concentration Ra in high dose (umol/min/kg)	Standard Limb	231.5 ± 277.6	67.8 ± 190.7	-3.7 ± 175.3							0.007	<0.001
Rd in low dose insulin infusion (umol/min/kg)	Long Limb	10.58 ± 3.56	13.56 ± 4.21	16.48 ± 4.06	(1, 47) = 2.9	0.10	(2, 93) = 68.6	<0.001	(2, 93) = 0.6	0.57	<0.001	<0.001
	Standard Limb	9.79 ± 1.65	11.76 ± 2.90	15.24 ± 2.82							0.02	<0.001
Rd in high dose insulin infusion (umol/min/kg)	Long Limb	19.13 ± 9.37	29.21 ± 9.90	38.13 ± 9.18	(1, 47) = 0.3	0.61	(2, 93) = 84.8	<0.001	(2, 93) = 0.2	0.83	<0.001	<0.001
	Standard Limb	18.49 ± 7.56	28.96 ± 9.08	36.12 ± 8.51							<0.001	<0.001
Corrected for insulin concentration Rd in low dose (umol/min/kg)	Long Limb	0.29 ± 0.14	0.44 ± 0.22	0.52 ± 0.18	(1, 47) = 1.8	0.19	(2, 93) = 81.3	<0.001	(2, 93) = 3.0	0.05	<0.001	<0.001
	Standard Limb	0.26 ± 0.10	0.34 ± 0.10	0.50 ± 0.14							0.01	<0.001
Corrected for insulin concentration	Long Limb	0.17 ± 0.08	0.30 ± 0.13	0.42 ± 0.17	(1, 47) = 0.07	0.79	(2, 93) = 90.7	<0.001	(2, 93) = 0.04	0.97	<0.001	<0.001

Rd in high dose (umol/min/kg)	Standard Limb	0.16 ± 0.08	0.30 ± 0.12	0.41 ± 0.11							<0.001	<0.001
Insulin clearance in low-dose insulin infusion (L/kg/min)	Long Limb	92.8 ± 22.6	101.8 ± 26.3	87.2 ± 18.9	(1, 48) = 0.001	0.97	(2, 95) = 6.9	0.002	(2, 95) = 1.2	0.31	0.10	0.37
	Standard Limb	93.4 ± 33.2	96.6 ± 28.5	90.1 ± 28.1							1.0	1.0
Insulin clearance in high-dose insulin infusion (L/kg/min)	Long Limb	89.0 ± 16.9	97.6 ± 25.2	90.7 ± 24.0	(1, 48) = 0.2	0.66	(2, 95) = 2.8	0.07	(2, 95) = <0.001	1.0	0.39	1.0
	Standard Limb	91.6 ± 26.1	100.4 ± 38.5	92.8 ± 21.6							0.36	1.0

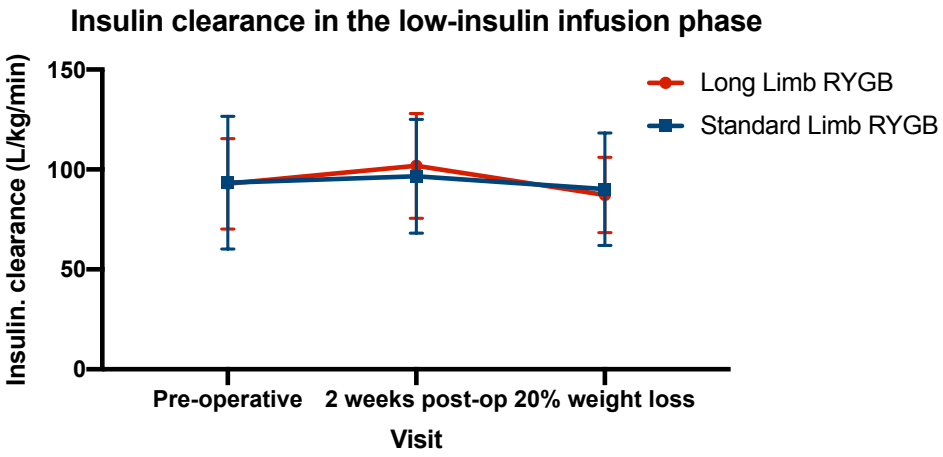
Table 14. Within groups comparisons of the rate of glucose appearance (Ra) and disappearance (Rd) in hyperinsulinaemic-euglycaemic clamps.

Continuous data presented as mean ± standard deviation. N=23 in each group. Statistical test used: mixed-effects analysis with Bonferroni adjustment for multiple comparisons in post-hoc analysis. p-values refer to comparing outcomes within Long Limb or Standard Limb at 2 weeks post-op visit and 20% weight loss visit. DFn – numerator degrees of freedom; DFd – denominator degrees of freedom. Ra: rate of glucose appearance. Rd: rate of glucose disappearance. Low: measured in the phase of low-dose insulin infusion. High: measured in the phase of high-dose insulin infusion.

Despite no recorded increase in the insulin clearance post-operatively (Figure 19), mean insulin concentrations in the steady-state of each clamp phase decreased post-operatively (Table 15), therefore all Ra and Rd records were also adjusted for insulin concentration in the steady-state of the related phase of the clamp. No difference between the groups was shown in the analysis of the adjusted values (Table 14, Figure 20).

Clamp phase	**Baseline visit**	**2 weeks post-op visit**	**20% weight loss visit**
Low insulin infusion (L/min/kg)	43	36	32
High insulin infusion (L/min/kg)	120	108	94

Table 15. Mean serum insulin concentration in the low and high insulin infusion rate steady state of hyperinsulinaemic-euglycaemic clamp across all three mechanistic visits for all patients.



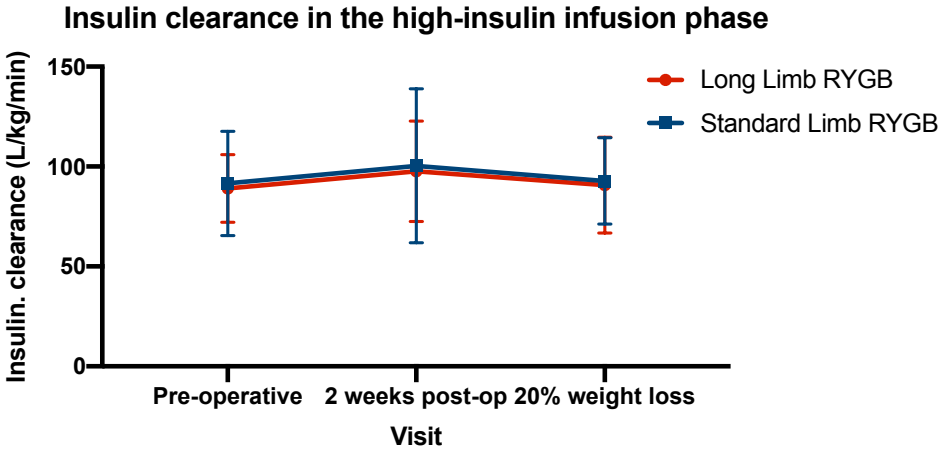


Figure 19. Insulin clearance in the low and high dose insulin infusion across three hyperinsulinaemic-euglycaemic clamps.

Data plotted as means ± SD. N=23 in each group. Mixed-effects model analysis with Bonferroni correction for multiple comparisons. No differences between or within the groups.

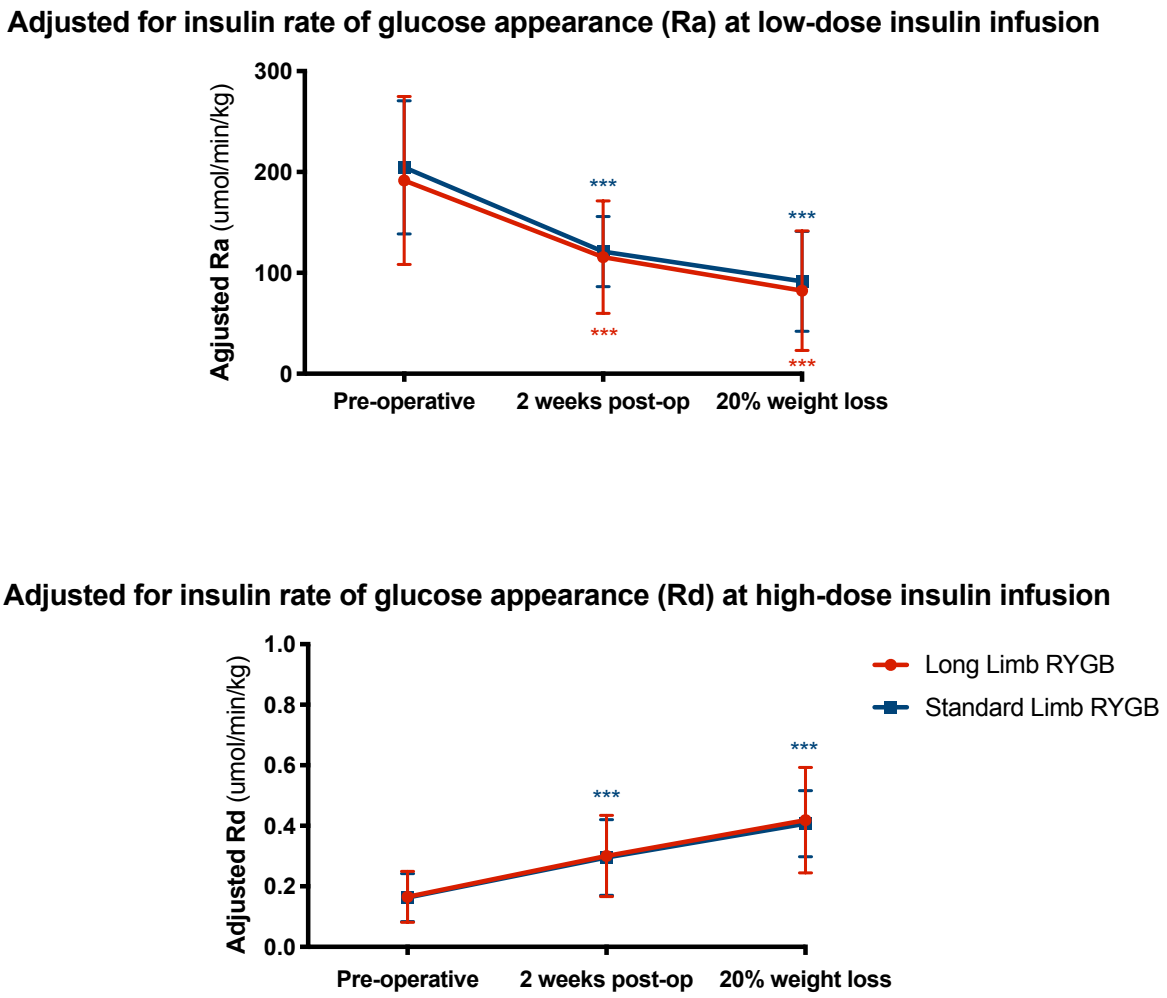


Figure 20. Rate of glucose appearance and disappearance adjusted for insulin concentration in the steady-state in the low and high dose insulin infusion across three hyperinsulinaemic-euglycaemic clamps.

Data plotted as means ± SD. N=23 in each group. Mixed-effects model analysis with Bonferroni adjustment for multiple comparisons. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative value within each group. *p<0.05, **p<0.01, ***p<0.001

When insulin sensitivity was assessed with more simple tools utilising fasting state readings, such as HOMA2 %S or its opposite, HOMA IR (to assess insulin resistance), the results were consistent with the ones obtained from the clamps, i.e. insulin sensitivity increased, insulin resistance decreased at one year when compared to baseline. These changes were significant within each group with no difference between them (Figure 21).

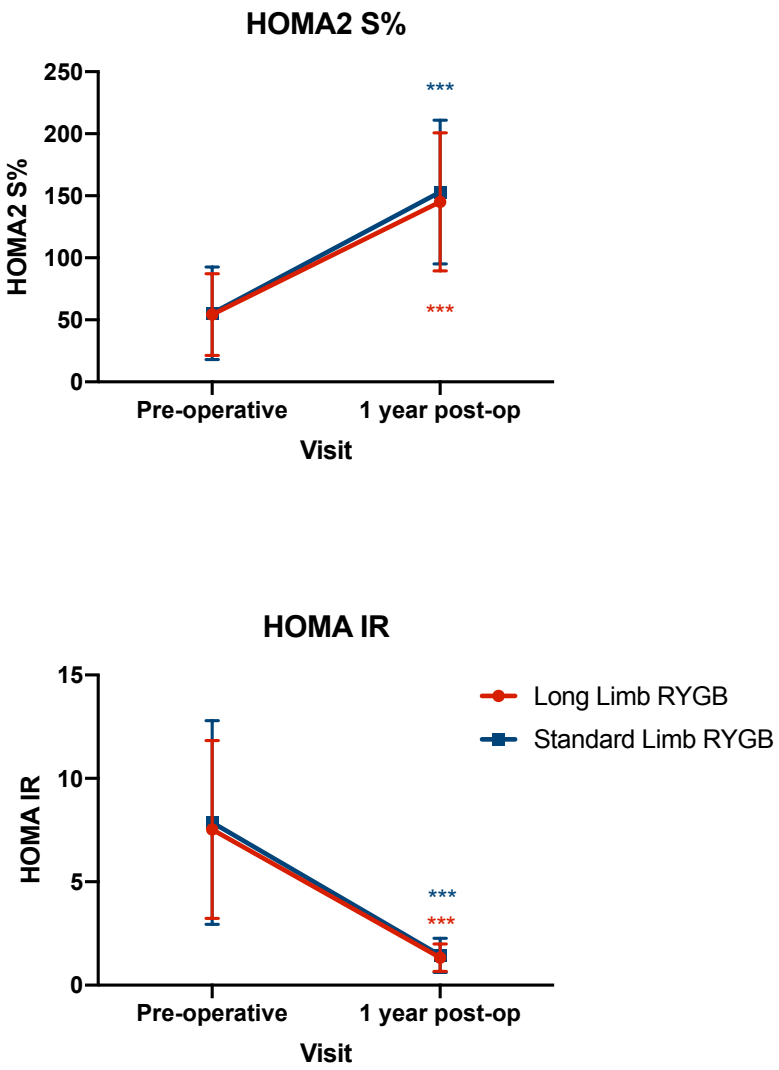


Figure 21. Assessment of insulin sensitivity (HOMA2 S%) and resistance (HOMA IR) at the operation day and one year.

Data plotted as means ± SD. N=26 in the Long Limb; N=27 in the Standard Limb group pre-operatively and N=26 at 1 year. Mixed-effects model analysis with Bonferroni correction for multiple comparisons. No differences between the groups. Stars in red and blue compare the Long and Standard Limb values respectively at each timepoint to the pre-operative visit within each group. ***p<0.001

6. Chapter 6. Discussion and Conclusions

6.1. Overview and strengths of the study

Obesity surgery is the most effective and durable treatment available for type 2 diabetes mellitus (T2DM), obesity and associated comorbidities of the metabolic syndrome. RYGB has a profound impact on glucose homeostasis resulting in remission of T2DM in approximately half of the cases. However, many patients will not achieve remission after RYGB. The enthusiasm after initial superb reports of the RYGB outcomes such as 84% remission rate of T2DM [199], has been later somewhat moderated following the reports of other RCTs with a thorough long-term follow up, where only 30% [137] to 51% of patients fulfilled ADA criteria for partial T2DM remission [330]. Furthermore, of the ones who do achieve T2DM remission in the early post-operative years, a proportion will relapse in the years to come. Also, publications with follow up beyond 2 years post-operatively consistently prove the tendency to gradual weight regain [330]. Post-operative relapse of T2DM and weight regain, as well as abdominal pain, internal hernias, anaemia and nutritional deficiencies, increased risk of bone fractures, psychological issues are the main problems of RYGB surgical therapy that limit its positive therapeutic impact on the long-term morbidity and mortality that arises from obesity and T2DM. Therefore, the need for optimising outcomes through modification of the existing procedures or developing new procedures exists, both as primary and secondary surgical interventions. One of the main trends here is to do it through altering the lengths of the bypassed small intestine during the surgery.

The Long Limb Trial is the first double-blinded RCT comparing 50 cm versus 150 cm biliopancreatic limb RYGB by scrutinizing an underlying physiological impact of the two procedures as well as monitoring clinical outcomes for one year. This is this first such trial in the field, that used the combination of robust mechanistic methodology to assess glucose homeostasis after this particular surgical modification. It utilised the gold-standard tool in assessing the insulin sensitivity, a hyperinsulinaemic-euglycaemic clamp, and mixed meal tolerance test to assess postprandial glucose homeostasis after RYGB with the two different biliopancreatic limb lengths. The LONG LIMB Trial has demonstrated that RYGB with a biliopancreatic limb of 150 cm is not superior to 50 cm with regards to fasting and postprandial glycaemia, GLP-1 secretion, insulin secretion or insulin sensitivity. In keeping with these mechanistic measurements, no difference between the two groups in terms of HbA1c reduction, T2DM remission or weight loss at one year was found. The fact that no mechanistic or clinical

difference between the procedures was found, is a very important piece of evidence to the ongoing scientific debate on the optimal length of the bypassed bowel lengths in the RYGB.

The main clinical outcomes at 12 months are consistent with what has been reported in the RYGB-related literature [167]. Both Long Limb and Standard Limb have induced a profound weight loss of 29 ± 8% and 30 ± 8% respectively (p=0.52). Weight loss was predominantly attributed to the fat mass loss, which was approximately halved in both groups, allowing to achieve total body fat content of 27 ± 14% and 22 ± 7% in the Long and Standard Limb groups respectively. Partial or complete criteria for the T2DM remission were achieved by 69% of participants in total (Standard Limb 62% vs. Long Limb 77%, p=0.23). Significant weight loss enabled stopping night time CPAP therapy and remission of obstructive sleep apnoea in most patients from both groups. Remission rates of hypertension, dyslipidaemia, arthritis and depression at 1 year were not significant within the groups which is not consistent with the literature [152] however, it could be due to strict remission criteria applied in this study and a relatively short post-operative follow-up. No differences in the prevalence of nutritional deficiencies or any other adverse events were found between the two procedures, which confirmed an unchanged safety profile of the Long Limb RYGB. Clinical results compatible with the literature in the field support the notion that mechanistic outcomes reported in this trial can be generalised to the overall population with T2DM and obesity undergoing RYGB. A small caveat in applying any trials results to a wider population may be the so-called Hawthorne effect [331], when study participants alter their behaviour due to the fact that they are being monitored by the research team. This phenomenon may be contributing to very good outcomes reported in the studied cohort. For example, an intense and frequent (in relation to the routine NHS-delivered) follow up and open access to the research team over the phone and email 7 days a week was implemented during the Long Limb Trial. It has certainly led to a minimal dropout rate (only one patient lost to follow up at one year due to moving abroad). Furthermore, self-reported participant adherence to the post-operative micronutrient supplementation was excellent and resulted in improved plasma levels of iron, transferrin saturation, vitamins D and B12 as compared to pre-operative readings, which is opposite to what has been reported in the bariatric surgical population followed up routinely in the public healthcare system. Hence a possibility exists that the Hawthorne effect potentially enhanced positive study outcomes however, even if that took place, this study outcomes are applicable to other subjects with similar baseline characteristics.

There was no difference in the safety profile between the Long and the Standard Limb procedures. Adverse events reported in this trial (43 in total) seem to be higher than expected

after routine bariatric surgery. There are several explanations for it. Firstly, what is reported is not restricted to surgery-related complications. Any adverse events taking place from the point of recruitment to the trial to the last visit of the trial 12 months post-operatively have been recorded. Therefore, several were isolated and unrelated incidents, such as a limb fracture. Secondly, many of the published data are restricted to the peri-operative (during the hospital stay related to the surgery) or the 30-day morbidity, whereas here I presented detailed records from 12 months post-operatively. Thirdly, a certain proportion of complications may go unreported, especially in the large cohort retrospective studies [152] and under-reporting may be taking place. Throughout the three years of the Long Limb Trial's duration, I have been contacting patients on a weekly to biweekly basis, therefore even the mildest adverse events have been recorded and the risk of missing any incidents was minimised. Double blinding throughout the duration of the study prevented any bias in frequency of contact with any of the patients.

6.2. Importance of the small bowel limb lengths

Studies investigating the elongation of the alimentary limb in the RYGB failed to demonstrate any major benefits [332] [293] apart from a selected patient population with the highest spectrum of obesity, where some advantage was shown [291]. Therefore, surgeons and researchers' attention has shifted towards investigating the biliopancreatic limb. Several clinical human studies have shown the benefit of the elongating of the biliopancreatic limb in terms of postoperative impact on glucose homeostasis, weight loss or both. On the other hand, some trials showed quite opposite results, with no advantage of the longer biliopancreatic limb shown. A review of the human trials in the field, alongside with their key findings has already been presented in Table 2 in the Introduction. Only a handful of studies attempted to scrutinize the mechanistic impact of lengthening the biliopancreatic limb in animal models [333] and humans [315], and the results have been so far inconclusive.

Direct comparison of the trials presented in Table 2 to the Long Limb Trial is hindered by three major issues:

1. Lengths of the analysed biliopancreatic limbs in presented trials vary between 10 cm and 200 cm.
2. Majority of the studies amend not only the biliopancreatic but also the alimentary limb length, which makes conclusions more convoluted.
3. Ratios of the biliopancreatic limb to the TSBL and the TSBL itself are rarely reported.

The largest retrospective analysis in the field was conducted by Gisslasson and Nergard's Scandinavian group [318]. In this study, 671 patients with an average BMI of 50 kg/m^2 underwent RYGB with either a 60 cm biliopancreatic and 150 cm alimentary, 200 cm biliopancreatic and 60 cm alimentary or 200 cm biliopancreatic and 150 cm alimentary limb and were followed up for 6 to 10 years. The authors conclude that 200 cm biliopancreatic limb provides superior outcomes in terms of the excess weight loss, less weight regain and comorbidities remission. They believe that a greater weight loss is achieved through the shortening of the total alimentary channel, i.e. summative length of the alimentary and common limb. Therefore, they advise BP limb of 200 cm and AL of 100 cm in order to achieve optimal outcomes. However, this is a retrospective review which does not support the clinical outcomes with any mechanistic data which could explain the physiology of such events. To the contrary, an impressively large RCT of over 500 patients led by Ruiz-Tovar [310] showed no difference in weight loss, remission of T2DM, hypertension or dyslipidaemia between 70 cm and 120 cm biliopancreatic limb RYGB at any timepoint in the 5-year follow up. Design of this trial was very similar to the Long Limb Trial, with 50 cm difference between the two biliopancreatic limb lengths and a constant alimentary limb length (here 150 cm). Furthermore, all the trials showing the same results as the Long Limb Trial investigated a biliopancreatic limb no longer than 120 cm with the most frequent design involving only 50 cm difference in its length between the two study arms (it was 100 cm in our trial). Of the trials reporting superiority of the longer biliopancreatic limb many included biliopancreatic length of 200 cm, with a wider length difference between the study arms. These results may mean that:

1. Extending standard biliopancreatic limb length by 50-100 cm may not be sufficient to elicit any mechanistic or clinical impact.
2. A longer biliopancreatic limb of 200 cm may be the optimal length.
3. Given many contraindicating results, perhaps assessing the ratios, not the absolute limb lengths would be more appropriate.

Another possibility of why the Long Limb RYGB was not superior to the Standard Limb RYGB is that the foregut theory might be applicable only to the very limited segment of the proximal small intestine. That means that it might be sufficient to bypass the duodenum and the very proximal segments of the jejunum and extending the length of the biliopancreatic limb any further may not bring any additional metabolic benefit. This theory works in interventions such as duodenal mucosal resurfacing (DMR) or the EndoBarrier.

Our study confirmed that significant inter-individual variability in TSBL exists in patients with T2DM and obesity. TBSL ranged from 320 to 910 cm with a mean of 610 ± 106 cm, with

a common channel of 170 to 660 cm (405±107 cm). These findings are consistent with the literature [290] [334]. Stefanidis et al.'s review [292] advocates concentrating on optimising the common channel length in order to improve outcomes, however introducing its reduction by 100 cm in the Long Limb Trial settings has not made any impact on outcomes.

With such a wide range of bowel lengths, some authors advocate routine measurement of the TSBL intraoperatively and deciding on the alimentary, biliopancreatic and common channel limb lengths based on their ratio of the TSBL. However, no consensus on the optimal ratios has been agreed yet. Even when analysed the ratios of the limb to the TBSL, no meaningful correlations with any of the mechanistic or clinical outcomes have been found.

6.3. Intestinal remodelling

Of the positive correlations of the TSBL with male gender and height as well as HbA1c and plasma glucose previously reported in the literature [290] [334], only the last one has been confirmed in the Long Limb Trial. Furthermore, I found a weak correlation between the TSBL and BMI across all the study population. These interesting findings inevitably bring up the question of whether these correlations are contributing to the development of obesity and T2DM (for example, the larger intestinal surface might be allowing for more glucose and other nutrient absorption) or whether it was the intestinal remodelling that happened as a consequence of these diseases. Due to anatomical rearrangement, rapid absorption of glucose takes place after the RYGB which results in the early postprandial spike in plasma glucose and insulin concentration [65].

Intestinal remodelling has certainly been showed to take place after the RYGB. Enteroendocrine cells are expected to proliferate and increase in density. However, no increase in mRNA in these cells means that the cell may increase in number but their secretory function is unchanged [295]. If an increase in the GLP-1 is due to increased L-cells density in the intestine, it is surprising to see how quickly this process occurs, with the peak rising 3 to almost 4-fold within two weeks after the surgery. Peak readings continue to rise beyond that point but when reviewed four months later that increase is of much lesser extent following the early post-operative visit. Even though no histological sampling was performed in the Long Limb Trial, its findings confirm that enteroplasticity was taking place in both study groups, however most likely it was not more pronounced in the Long Limb cohort.

The higher peak of active GLP-1 in the Long Limb RYGB was not demonstrated 2 weeks after the surgery (primary outcome) nor at 20% TBWL which took place 4.5 months post-operatively on average. A possibility exists that the intervals between when these visits were performed were too short to examine the full impact of intestinal remodelling on the mechanistic outcomes. An alternative explanation of the lack of change in gut hormones secretion between the study arms is that the biliopancreatic limb of 150 cm might not be long enough to induce them and perhaps the difference might be demonstrated if it was elongated to at least 200 cm, as in Patricio's study where such difference was observed [65].

The absence of either an earlier or higher peak in post-prandial GLP-1 concentrations after the Long Limb RYGB also challenges the hypothesis that faster delivery of less digested nutrients to more distal segments of the small intestine, with a high density of the enteroendocrine cells, triggers the enhanced secretion of incretin and anorexigenic hormones like GLP-1 and PYY. One explanation of this rather unexpected finding is that there is no linear relationship between GLP-1 secretion and the length of intestine exposed to ingested nutrients, as previously suggested. Perhaps the optimal length of the bypassed proximal bowel has already been achieved with the Standard Limb RYGB, hence extending its length further and by this reducing the common channel length will result in further enhancement of the GLP-1 response. An alternative hypothesis is that secretion of GLP-1 from the L-cells is not exclusively stimulated by their contact with intraluminal nutrients, but also by neural or hormonal mechanisms that have not been discovered yet. Human and animal studies demonstrating similar patterns of GLP-1 increase after the sleeve gastrectomy and after the RYGB seem to support this way of reasoning [241]. Thirdly, just as mentioned above, the difference of 100 cm in biliopancreatic limb length (50 cm vs 150 cm) might have not been long enough to trigger the enhanced secretion of gut hormones and a longer biliopancreatic limb length of 200 cm or above should have been introduced.

6.4. Type 2 diabetes mellitus remission

Consistent with the increased secretion of GLP-1, at the 12-month mark, both the Long Limb and Standard Limb groups have been equally successful in improving glycaemia as judged by HbA1c levels: from 76 ± 16 mmol/mol to 41 ± 5 mmol/mol in the Long Limb group versus a change from 71 ± 15 mmol/mol to 43 ± 10 mmol/mol in the Standard Limb group (treatment effect of Long Limb vs Standard Limb -3 [95% CI -8 to +2), p=0.20). No difference between the groups is consistent with results of the YOMEGA Trial, where 2-year outcomes following a one anastomosis gastric bypass were similar to RYGB with similar improvement in T2DM and weight loss [178].

The majority of the Long Limb Trial participants had such profound improvement in their glucose homeostasis early post-operatively that they were able to stop all of their glucose-lowering medications before leaving the hospital after the surgery, which has been the standard practice at both operating sites. This is consistent with the evidence reporting that 30-100% of patients have normalized fasting blood glucose within days after the surgery and at only 1-2% TBWL [65]. It is assumed that such a rapid process is due to the weight-independent mechanisms however a marker caloric restriction also takes place during the early post-operative days, from the estimated 3000-6000 kcal/day pre-operatively to as little as 300 kcal/day [65].

All but one patient had all glucose-lowering medications withdrawn at 9 months after the surgery at the latest. This was done to look for evidence of diabetes remission, i.e. glycated haemoglobin levels in the nondiabetic range of <48 mmol/mol and fasting glucose of <6.9 mmol/L without any treatment. Partial or complete criteria for T2DM remission were achieved by 69% of participants in total (Standard Limb 62% vs. Long Limb 77%, p=0.23). However, after the closure of the trial, seven patients in the Standard Limb group have had to restart their diabetes treatment compared to only one in the Long Limb group. These are relatively small numbers nevertheless it may mean that a longer follow up is required in order to reveal differences between the Standard and the Long Limb in case the latter does not make a more potent impact on the glucose metabolism in the first post-operative year but it may minimise the rates of diabetes relapse in the future years. This hypothesis is supported by other studies (not all available at the time we initiated the trial) which suggest that the Long Limb surgery has longer-lasting effects than Standard Limb on sustained weight loss and glycaemic improvement [294, 296, 316, 317, 335].

HOMA-IR, a crude marker of insulin resistance in the fasting state has been showed to decrease within days after obesity surgery with this effect maintained beyond one year [65] and this has been confirmed in our study. A similar pattern was observed in the HOMA2 S%.

Hyperinsulinaemic-euglycaemic clamps conducted pre-operatively, within 2 weeks after the surgery and at 20% matched total body weight loss have clearly demonstrated an increase in both hepatic and peripheral insulin sensitivity at all visits within the groups (with no between-group difference). As presented in Table 1 in the Introduction, early and late increase in hepatic liver sensitivity after RYGB is expected and has been well documented in the literature. Improvement in the skeletal muscle insulin sensitivity is believed to be a predominantly weight loss-dependent phenomenon, which takes place after a moderate weight loss of 10-15% and

then doubles after 20% TBWL is achieved [65]. What is interesting, however is that our cohort of patients also had an early increase in peripheral insulin sensitivity (regardless of the study arm allocation), which so far has been reported predominantly after the BPD. Out of studies utilising clamps with an isotope, only Gastadelli et al. found an increase in hepatic insulin sensitivity at one week after the RYGB [213]. Recorded weight loss of 5kg at the time of the postoperative assessments was similar to the one in the Long Limb. The Long Limb Trial employed longer clamps than the majority of reviewed studies presented in Table 1. Furthermore, our clamps consisted of two 2-hour phases with a low and high dose infusion in each stage. It is possible that also the dose of insulin used in other studies was too low to detect changes in the peripheral insulin sensitivity that are already present within early post-operative days. Most of the studies apply equivalent of 1.0 mU/kg/min whereas we infused 1.5 mU/kg/min in the second phase of the clamp. Another explanation could be that the 6% TBWL recorded at two weeks in our cohort could be sufficient enough to trigger the weight-dependent mechanisms of insulin sensitivity improvement. These could be the reasons why the other studies detect the increase in peripheral insulin sensitivity only at the later post-operative stages when weight-dependent mechanisms bring additional impact to its improvement.

Assessment of endogenous glucose production (i.e. hepatic insulin sensitivity) was derived from the basal stage of the clamps in most studies, hence it was calculated during the isotope loading phase prior to the introduction of insulin infusion. These calculations were not possible in the Long Limb Trial, as patients were so profoundly insulin resistant, that they required a separate insulin infusion at variable rate throughout the morning preceding the clamp and throughout the isotope loading phase in order to maintain their blood glycaemia below 6 mmol/L. Nevertheless, the isotope dilution technique allowed a thorough assessment of endogenous glucose production. Its decrease, relating to the improvement in the hepatic insulin sensitivity, was present at early and late post-operative stages both in the Long Limb Trial as well as in all the other studies.

Changes in insulin secretion and β-cell function after the RYGB in the Long Limb Trial are slightly complex. Firstly, the postprandial peak of insulin secretion increased significantly within the first two post-operative weeks within each group and was roughly doubled at the timepoint when 20% TBWL was achieved. Also, the peak has shifted from 60 to 30mins after the oral stimulus, which indicated improvement in the first phase of insulin secretion. There are several reports of postprandial insulin secretion AUC decreasing after the RYGB due to improved insulin sensitivity, hence reduction in hyperinsulinaemia. However, the Long Limb Trial participants did not show any statistically significant changes in this field. If anything, there was a trend towards an increase

in insulin AUC during the 180 minutes after the meal which could relate to improved β-cell secretory function.

The lack of difference between the study arms in the post-prandial insulin secretion both early post-operatively and at 20% weight loss could be due to a similar reason as alluded to above. Bypassing just the very proximal segments of the small intestine might be enough to elicit maximal insulin secretory response and extending it further will not deliver any superior outcomes. Or, on the other hand, 150 cm biliopancreatic limb might have been too short to demonstrate the difference.

6.5. Limitations

Apart from its undeniable strengths, the Long Limb Trial has several limitations. First, one year may be a relatively short period of the post-operative follow up to fully appreciate the difference in the impact of both surgeries. As shown by several trials and observational studies [317] [294] [335] [296] [316], changes in weight, glycaemic homeostasis and comorbidities resolution dynamically evolve beyond the first post-operative year. Second, the trial was powered to detect a difference in the postprandial peak of GLP-1 within 14 days post-operatively, which may have been too short a period of time to allow for the full intestinal adaptation to take place. Third, powering the study for mechanistic outcomes may mean that the cohort of just over 50 patients would not be adequate to detect any major clinical differences. Fourth, as already discussed above, the difference of 100 cm between the biliopancreatic limbs (50 cm in the Standard and 150 cm in the Long Limb RYGB) may not be sufficient to demonstrate differences in the impact of their lengthening. Fifth, also as mentioned earlier, in view of such variability of the TSBL, using fixed measurements of the biliopancreatic limbs may be less practical than estimating their proportions of the total small bowel length. Sixth, this trial refers to 50 cm biliopancreatic limb as the “standard”, which may actually not be the standard at all the bariatric surgery centres since a substantial variation in practice exists. Seventh, even though the technique of the intra-operative small bowel measurement was standardised and pre-specified in the trial Standard Operating Procedures, an inter-surgeon (and inter-procedure) variability may have taken place, thus introducing a certain level of heterogeneity in the actual limb lengths assessed. Finally, it was impossible to assess all the mechanisms that are involved in glycaemic and weight control regulation following the metabolic surgery within the time and financial constraints of this thesis, therefore the ones that were believed to be most likely to describe metabolic differences between the Standard and Long Limb RYGB were chosen. Plasma bile acid levels, gut microbiota, plasma,

stool and urinary metabolomics and free fatty acids samples have been collected throughout the trial and will be analysed in due course alongside dietary records and psychological questionnaires. Furthermore, no routine testing for the small intestinal bacterial overgrowth was performed in the studied cohort. Since this is a recognised complication of RYGB that could affect clinical outcomes it will be considered in the future, alongside the investigations listed above.

6.6. Future directions

Based on the trial findings and existing evidence in the field, a trial extension study has been applied for with the aim to repeat the mixed meal tolerance test after the second post-operative year. This may reveal a change in the GLP-1 secretion profile after a sufficient amount of time would have been allowed for intestinal adaptation to take place. Furthermore, clinical follow up will be extended to 5 years in order to make outcomes reporting more robust. Outstanding results such as gut microbiome and metabolomics, plasma bile acids will be analysed in due course. Dietary records and psychological questionnaires will also be scrutinized in collaboration with the dietetic and psychology team.

If further trials looking into the impact of the biliopancreatic limb lengths were designed, I would certainly advocate:

1. A wider difference between tested biliopancreatic limb lengths.
2. Measuring the total small bowel length and assessing the proportions, not the absolute lengths of the small bowel limbs bypassed.
3. If possible, validating the small bowel length measurements by repeating them twice intraoperatively by two different surgeons.
4. Taking intra-operative and late (i.e. after 1-2 years) post-operative small bowel biopsies: jejunal from the region of the gastrojejunal anastomosis (easily accessible through the upper gastrointestinal endoscopy) and ileal from the terminal ileum via colonoscopy. This would enable evaluation of the enteroendocrine cells' proliferation and density and assessment of the impact of biliopancreatic limb lengths on the intestinal adaptation.
5. Powering study for clinical outcomes, i.e. aiming for a larger cohort.

However, the LONG LIMB Trial might have narrowed down the number of intestinal segments that can be modified during the RYGB in an attempt to improve its impact on glycaemic control. Future clinical and mechanistic studies could investigate the role of the common channel and explore novel mechanisms through which the intestine regulates glycaemia.

Recent findings from humans and animal models have demonstrated that the common channel is the intestinal segment where the majority of ingested glucose uptake takes place after surgery [243]. This process is dependent on the interaction of glucose and the sodium content of bile with the sodium-dependent glucose co-transporter 1. Thus, RYGB with a common channel short enough to selectively reduce the absorption of glucose, but not other nutrients, could prove to be superior to the standard RYGB design for patients with T2DM.

However, one must remember that perhaps RYGB has achieved its maximum potential in the current design and manipulating bowel limbs any further will not provide any metabolic improvements.

6.7. Conclusions

In conclusion, the Long Limb Trial has scrutinised important aspects of the physiology after the RYGB. It has clearly confirmed a potent impact of the RYGB on improved glucose homeostasis from the early post-operative days through increasing hepatic and peripheral insulin sensitivity. This translated into a significant reduction of fasting plasma glucose, insulin and HbA1c as well as impressive T2DM remission rates of 69% across the entire cohort at one year. Total body weight loss of approximately 30% at 12 months, with more of it attributed to the fat mass than the lean mass reduction, was also observed.

The lack of difference in both mechanistic and clinical outcomes in the first post-operative year has so far demonstrated that there is no metabolic rationale to lengthen the biliopancreatic limb in RYGB from 50 cm to 150 cm. Longer-term follow up is required in order to confirm these findings.

Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
BMI	Body Mass Index
BMR	Basic Metabolic Rate
BOSPA	British Obesity Surgery Patient Association
BPD	Biliopancreatic Diversion
DS	Duodenal Switch
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CPAP	Continuous Positive Airway Pressure
DMEC	Data Monitoring and Ethics Committee
EECs	Enteroendocrine Cells
EWL	Excess Weight Loss
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-Like Peptide-1
HbA1c	Haemoglobin A1c (glycated haemoglobin)
HDL	High Density Lipoprotein
HOMA 2 %S	Homeostasis Model Assessment for Insulin Sensitivity
LDL	Low Density Lipoprotein
LOCF	Last Observation Carried Forward
MCR	Metabolic Clearance rate of Glucose
MMTT	Mixed Meal Tolerance Test
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PPI	Public and Patient Involvement
PYY	Peptide YY
Ra	Rate of glucose appearance
Rd	Rate of glucose disappearance
RCT	Randomised Controlled Trial

RYGB	Roux-en-Y Gastric Bypass
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
T2DM	Type II Diabetes Mellitus
TSC	Trial Steering Committee
TSBL	Total Small Bowel Length
VAS	Visual Analogue Scale
WHO	World Health Organization
WL	Weight Loss

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Appendices

Appendix 1. LONG LIMB Trial Protocol

Appendix 2. LONG LIMB Trial Information Sheet for Research Participants

Appendix 3. LONG LIMB Trial Mechanistic Visit Standard Operating Procedure

Appendix 4. LONG LIMB Trial Surgical Standard Operating Procedure

Imperial College London

Department of Investigative Medicine
Imperial College London

6th Floor Commonwealth Building
Imperial College London at Hammersmith Campus
Du Cane Road, London W12 ONN, UK
Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320

Study Protocol

Comparison of the effects of the long-limb to the standard-limb gastric bypass on type 2 diabetes mellitus

The LONG LIMB trial

Background and rationale

The most effective and durable treatment for both obesity and type 2 diabetes mellitus (T2DM) remains bariatric surgery. Prospective case-controlled studies and randomised controlled clinical trials have shown that bariatric surgery causes a mean body weight loss of 20-40% and a mean absolute reduction in glycated haemoglobin (HbA1c) of ~2% at 1-2 years post-operatively in the context of reduced diabetes medications [336-339]. Despite initial optimism that this operation would cause long term remission for the majority of patients with moderate diabetes, disappointingly, <40% achieve euglycaemia without diabetic medications with the 'gold standard' standard-limb RYGB [132].

Alternative surgical techniques have been sought to improve the rates of T2DM remission. An RCT performed by our co-investigator, Professor Francesco Rubino [336], showed that standard-limb RYGB and biliopancreatic diversion (BPD) performed on obese patients with T2DM led to a matched total body weight loss of 33% at 2 years post-operatively. However, after this weight loss, BPD was superior in its glycaemic improvements, with a clinically meaningful absolute reduction in HbA1c of 3.9% compared to a 2.2% after standard-limb RYGB. Unfortunately, the BPD procedure has the distinct disadvantage of a substantially higher risk of developing severe nutritional complications, and this has limited its use [340]. To improve the glucose-lowering efficacy of standard-limb RYGB, whilst avoiding the high risk of complications with the BPD procedure, the long-limb RYGB has been devised as a hybrid operation that combines the standard design of standard-limb RYGB, but with a longer biliopancreatic limb.

Nora *et al* reported on the results of a prospective study of obese T2DM patients who underwent an long-limb RYGB with a 200 cm biliopancreatic limb and a 120 cm alimentary limb [341]. The cohort of 40 patients that completed the 3 year follow up lost 25% body weight, stopped all of their glucose-lowering

medications and reduced their HbA1c by 0.9% (from a baseline of 6.7%). A longer biliopancreatic limb is therefore associated with superior glycaemic control (100% diabetes remission) when compared to that usually achieved by standard-limb RYGB, <40% [132, 342]. The rates of any complications, including nutritional, were not higher than those reported after standard-limb RYGB.

The standard-limb RYGB causes a large release of gut hormones such as GLP-1, oxyntomodulin and peptide YY after eating, leading to reductions in appetite and/or increases in insulin secretion [278, 306, 343, 344]. As the long-limb RYGB enables the faster delivery of un-digested nutrients to the distal jejunum, where there is a greater number of gut endocrine L cells [237, 345], we expect that there will be an *even greater* release of gut hormones that will drive a higher secretion of insulin immediately after eating compared to the standard-limb RYGB.

In the long-limb RYGB, the biliopancreatic limb is longer than the standard-limb RYGB (150 vs. 50 cm). We therefore expect that the long-limb RYGB will be similar to the BPD and that both hepatic and peripheral insulin sensitivity will be increased *before* weight loss has taken place. We also expect that the increased insulin sensitivity will persist in the longer term and will be more powerful in reducing glucose levels than standard-limb RYGB.

In this trial, we wish to confirm the superior efficacy on T2DM of the long-limb RYGB over the standard-limb RYGB, and investigate the mechanisms underlying their differences.

Hypotheses

The main anatomical difference between long-limb RYGB and standard-limb RYGB is that the segment of the bypassed proximal intestine, the biliopancreatic limb, is longer (150 vs. 50cm respectively). This means that in the long-limb RYGB the common channel is shorter, and as a result nutrients reach the

distal small bowel faster and in a less-digested state. The physiological mechanisms through which these changes in anatomy can alter glucose homeostasis are not currently known.

We hypothesize that the long-limb RYGB is better for T2DM because:

1. It increases the immediate post-prandial insulin secretion significantly more than the standard-limb RYGB, by enhancing the post-prandial secretion of gut hormones, and in particular glucagon-like peptide (GLP) -1, over that seen with the standard-limb RYGB.

2. It increases insulin sensitivity significantly more than the standard-limb RYGB, before *and* after weight loss has taken place.

Objectives

Our objectives are to compare the standard and the long-limb RYGB in terms of the differences in:

1. Insulin and gut hormone secretion, following a standardised mixed meal tolerance test.
2. Insulin sensitivity (hepatic and peripheral), using the two-step euglycaemic-hyperinsulinaemic clamp method.
3. Changes in plasma bile acid levels.
4. Gut bacterial diversity and their metabolite profile.

Trial team

The Chief Investigator is Professor Stephen R. Bloom, Professor of Medicine and Head of Department of Investigative Medicine, Imperial College London. Co-investigators and collaborators are Prof Francesco Rubino (King's College London), Mr Ahmed Ahmed, Dr Tricia Tan, Dr Alexander Miras, Prof Gary Frost, Prof Jeremy Nicholson, Prof Elaine Holmes, Prof Margot Umpleby (University of Surrey), Prof Ameet Patel (Kings College London), Mr Sanjay Purkayastha, Mr Krishna Moorthy, Miss Avril Chang (King's College London), Dr Harvinder Chahal and Dr Julian Marchesi.

Trial design

This will be a prospective double-blinded randomised controlled clinical trial. Fifty patients will be recruited from the Imperial Weight Centre and the King's College Obesity Clinic, and randomised to either the long-limb or the standard-limb RYGB surgery.

Inclusion criteria

- Male or female participants

- Aged between 18-70 years
- Diagnosed with T2DM according to WHO 2006 and 2011 criteria
- HbA1c ≥7.0% (≥53.0 mmol/mol) on screening
- Body mass index (BMI) ≥ 30 kg/m^2 and eligible for bariatric surgery based on NICE guidance
- On glucose-lowering medications
- Willing to comply with study requirements and able to give informed consent

Exclusion criteria

- History of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer.
- Without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.
- Specific contraindications to bariatric surgery
- Previous bariatric surgery
- Diagnosed with Type 1 diabetes mellitus
- Donated blood during the preceding 3 months or intention to do so before the end of the study
  Current pregnancy or breastfeeding
- Inability to maintain adequate contraception

Screening visit

All participants will be screened to assess whether they meet inclusion criteria and this process will comprise a medical history, routine physical examination, basic investigations (full blood count, urea and electrolytes, liver function tests, thyroid function tests, fasting plasma glucose, fructosamine, HbA1c, lipid profile, iron indices, vitamins, minerals and metabolites, urinalysis for dipstick and albuminuria, and electrocardiogram) and psychological/quality of life questionnaires. All women of child-bearing age will also be asked to undergo a pregnancy test.

Baseline visit before surgery

Day 1: Assessment of insulin sensitivity - euglycaemic hyperinsulinaemic clamp

On the days prior to the visit patients' glucose-lowering medications will be adjusted by a research nurse or a clinician in order to avoid their interference with the measurements. Patients will also be asked to refrain from alcohol and strenuous physical activity for 48 hours before the study. A non-invasive device (e.g. pedometer) may be used to monitor their physical activity levels. Patients will attend the research facility in the evening before the clamp procedure. Two venous catheters will be inserted. The first

cannula will be used for infusions and the other for blood sampling. They will be asked to consume a standardised meal, remain fasted from 10pm onwards, and commenced on an insulin infusion to keep their blood glucose stable between 4.0-6.0 mmol/l. On the morning of the clamp a primed continuous infusion of [6, 6-$^{2}H_2$] glucose, a stable isotope tracer, will be started and maintained for 7 hours. Two hours later a two-stage hyperinsulinaemic-euglycaemic clamp procedure will be started and continued for 5 hours. During stage 1 of the clamp procedure, in which hepatic insulin resistance is assessed, insulin will be infused at a low dose (depending on patient's weight/body surface area) for 2 hours. During stage 2 of the clamp procedure, in which peripheral insulin resistance is assessed, insulin will be increased to a higher dose (depending on patient's weight/body surface area) for 3 hours. Euglycaemia will be maintained by infusing 20% dextrose at a variable rate. Blood samples will be taken every 5-10 minutes to measure blood glucose concentration and the dextrose infusion will be adjusted accordingly. The exogenous glucose infusion will be enriched with 6, 6 $^{2}H_2$ glucose to prevent a fall in plasma tracer enrichment and underestimation of endogenous glucose production rate. Regular glucose monitoring is necessary to ensure safety and avoid the small risk of hypoglycaemia.

Blood samples will be obtained before the start of the tracer infusions, every 10 min during the final 30 min of the basal period and stages 1 and 2 of the clamp procedure and every 30 minutes between these periods to determine glucose enrichment and concentration, free fatty acid, insulin, c-peptide, glucagon, gut hormones, bile acids and metabolite concentrations. At the same time points participants will be asked to complete appetite visual analogue scales.

At the end of the study, participants will be fed a standardised meal and the glucose infusion continued for a further 20 minutes to prevent hypoglycaemia. The maximum amount of venesected blood will be 180 mls. Patients will be asked to remain fasted and sleep overnight in the Clinical Research facility.

Blood samples will be centrifuged and the separated plasma kept in a -20°C or -80°C freezer. The isotopic enrichment of plasma glucose will be determined by gas chromatography mass spectrometry (GCMS) at the Wolfson Centre for Translational Research, Postgraduate Medical School, University of Surrey.

The stable labelled isotope tracer [6, 6 $^{2}H_2$] glucose is not a drug, but a naturally occurring metabolite which has been labelled with a stable and non-radioactive label. Stable isotope tracers are widely and safely used in metabolic research by groups throughout the UK and worldwide. All labelled isotope tracers are ordered from Cambridge Isotopes Ltd through their UK suppliers CK Gases Ltd. They are prepared as sterile solutions suitable for intravenous use by the Pharmacy Production Unit at Guys & St. Thomas' NHS Trust to ensure they are safe for the participants. The products are supplied with the appropriate

certificate of analysis and MSDS. We have used the same manufacturer to ensure the quality of the products and the supporting documentation.

Day 2: Assessment of insulin and gut hormone secretion - mixed meal tolerance test

On the morning of day 2 the fasted patients will be given a standardized mixed-meal followed by measurements of glucose, insulin, gut hormones, bile acids and metabolites at t= -30, -15, 0, 15, 30, 60, 90, 120 and 180 minutes, where time zero is the time of administration of the meal. At the same time points they will be asked to rate their appetite, and have their BP and pulse measured. They will then be discharged from the clinical research facility.

Additional assessments

The following assessments will also take place on or around the baseline visit:

- glycaemia - fasting plasma glucose, glycated haemoglobin (HbA1c) and fructosamine
- body weight and body composition using bioelectrical impedance machines
- plasma lipids - total, low-density and high-density lipoprotein cholesterol, and triglycerides
- 24 hour collection of faeces for bomb calorimetry analysis
- Blood, urine and faecal samples collection for microbiomic and metabolomic analyses
- total caloric intake and macronutrient composition will be assessed through the use of food diaries
- frequency of hypoglycaemic episodes
- adverse events

Surgery

The patient, the research and clinical team, except for the operating surgeon, will be blinded to the type of operation that has been performed, unless clinical need and urgency dictates the un-blinding of the clinical team (e.g. development of a surgical complication). The procedures will be filmed in order to allow the data monitoring and ethical committee to audit procedures and ensure the consistency of the surgical technique amongst the operating surgeons. Filming of surgery takes place as part of routine NHS care for clinical governance purposes. In brief, the total length of the small intestine will be measured from the ligament of Treitz to the terminal ileum. A completely isolated proximal gastric pouch 15-30ml in volume will be created using endostaplers. Next the ligament of Treitz will be exposed and a loop of small bowel taken up to the gastric pouch (antecolic) with the alimentary limb on the patient's right and a 50 cm biliopancreatic limb in the standard-limb RYGB or 150 cm biliopancreatic limb in the long-limb RYGB, on patients' left. The alimentary limb will be anastomosed with a stapler to the gastric pouch. A leak test will be performed with the Roux loop occluded; the gastro-jejunal anastomosis will be submerged under

saline, distended with oxygen via an orogastric tube and with multiple distensions while submerged. Next the alimentary limb will be measured to 100 cm. Then a side-to-side entero-enterostomy will be performed by stapling the biliopancreatic limb to the 100 cm mark on the alimentary limb making parallel antimesenteric enterotomies and firing the endostapler into the lumen of each. The enterotomy will be closed and the procedure completed. Following surgery patients in both groups will be advised to consume the same standard post-operative low-calorie diet.

Early post-operative visit

This will take place 7-14 days after surgery and before substantial weight loss has taken place. The same assessments and procedures as in the baseline visit will be followed.

Late post-operative visit

This will take place when patients in both groups achieve a total body weight loss of 20% of their pre-operative weight. The same assessments and procedures as in the baseline visit will be followed.

Yearly visit

This will take place 1 year after surgery and will involve clinical assessments including the following:

- Body weight and body fat
- Blood pressure and pulse
- Blood tests: full blood count, urea and electrolytes, liver function tests, thyroid function tests, fasting plasma glucose, HbA1c, lipid profile, iron indices, vitamins, minerals and metabolites, and urinalysis for dipstick and albuminuria
- Psychological/quality of life questionnaires
- Medical, surgical, nutritional and psychological complications, including length of inpatient stay and number of outpatient consultations
- Number of medications

Clinical assessment and follow-up: Patients will be assessed clinically as part of routine NHS care. Patients in both groups and both hospitals will receive protocol-driven medical care. After surgery patients will be followed-up at ~10 days, 3, 6, 12 months and yearly thereafter, unless clinical need dictates more frequent consultations. The data obtained from these clinical assessments will be used to compliment the data from the mechanistic studies.

Primary outcome change in peak GLP-1 level after the mixed meal tolerance test

Secondary outcomes change from baseline in:

- plasma levels of glucose, insulin, c-peptide, gut hormones, bile acids, FGF-19 and 21 after the mixed meal tolerance test
- rate of glucose appearance (Ra) and disposal (Rd) in the euglycaemic hyperinsulinaemic clamp
- faecal caloric content
- blood, urine and faecal microbial diversity and metabolomics
- total caloric intake and macronutrient composition
- HbA1c
- total number of medications
- rates of patients achieving diabetes remission
- body weight
- systolic, diastolic blood pressure and pulse
- serum fasting lipids
- medical, surgical, nutritional and psychological complications
- adverse events

Sample size calculations

The majority of published studies have shown that peak active GLP-1 concentrations are ~2-fold greater after standard-limb RYGB (11, 20) compared to pre-operatively. We have estimated that that peak active GLP-1 levels after long-limb RYGB will be tripled at 1-2 weeks after surgery. We have powered this study to detect a statistically significant difference in peak active GLP-1 of 10.0 pmol/L between the group means assuming a SD of 10.8 pmol/L within each group. A power calculation shows that a sample size of 20 completers in each arm will have a statistical power of 80% to detect this difference at α=0.05. We plan to recruit 25 patients to each arm, assuming a 20% drop-out rate based on our own experience with our previous patient cohorts undergoing standard-limb RYGB and metabolic testing after surgery.

Drop-outs

Subjects will be free to withdraw at any point. If a subject withdraws from the study before they have completed their last visit, they will be replaced.

Trial Closure

The end of the clinical trial is defined as the last visit of the last patient.

Data analysis plan

The primary outcome will be compared between treatment groups using a linear model, incorporating stratifying factors and adjusting for relevant baseline covariates. Bayesian estimates of the mean difference, with 95% credible intervals, will also be derived. Other data will be summarized using

appropriate descriptive statistics, and exploratory linear models may also be used to compare mean values of continuous variables, or to compare categorical outcomes, between the two treatment groups.

Procedure for emergency un-blinding

This is a randomised, double-blinded study. The randomisation lists will be created and held by Dr Victoria Salem, Clinical Lecturer in Endocrinology, Imperial College London, in a secure area within the centre (this copy to be held as code-break envelopes).

In the case of a medical emergency or in the event of a serious medical condition, when knowledge of treatment allocation is essential for the clinical management or welfare of the subject, an investigator or other physician managing the subject may decide to un-blind that subject's treatment code. They should therefore request and obtain the relevant code-break envelope.

The investigator must sign and date the open un-blinding envelope, as soon as is reasonably possible, and at the very least within 24 hours of the code break. The reason for the code break must be documented on the envelope. The Investigator will also record the date and reason for revealing the blinded treatment assignment for that subject in the CRF and in the subject's medical notes.

Patient and public involvement

Mrs Georgina Hayman runs a very successful support group for obese patients undergoing bariatric surgery (British Obesity Surgery Patient Association West London) and she will help with patient retention by creating a "belonging to a family" environment. Her group have already contributed to the development of this application, starting from its design, undertaking the research, choice of research topic and eventually dissemination of the study findings through her patient support group.

Definitions of Adverse Events and Reactions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs

All such events, whether expected or not, should be recorded.

Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to non-obesity or diabetes related causes, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs should be reported to the REC where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs

SAEs must be reported to the Chief investigator and the Sponsor within 24hrs of becoming aware of the event:

CI details: Fax: 0208 383 8320, attention of: Prof Sir Stephen Bloom

Sponsor details: Fax: 0203 311 0203 or email: jrco.ctimp.team@imperial.ac.uk

Please send SAE forms to: Section of Investigative Medicine, Division of Diabetes, Endocrinology & Metabolism, Imperial College London

Tel: 0208 383 3242 (Mon to Fri 09.00 – 17.00) or 07751236735 (24 hours, 7 days a week).

Follow-up of AEs and SAEs

After the initial AE report, the Chief Investigator or appropriately qualified designee will proactively follow the subject at subsequent visits and contacts. Follow up information about a previously reported SAE must be reported to the Trial Management Group and Sponsor within 24 hours of receiving it. AEs and SAEs will be followed until they resolve, stabilise to a level acceptable to the Investigator or delegates even after the reporting period or the subject is lost to follow-up. Additional measures may be carried out by the Investigator to elucidate as fully as possible the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations or consultation with other health care professionals. In the event that a subject becomes pregnant, the follow-up period will be deemed to have ended when the health status of the child has been determined on its birth.

Monitoring

A risk assessment will be completed by the Sponsor and the monitoring frequency will ensue from this. The monitoring will be performed by members of the JRCO.

Regulatory issues

Ethics and regulatory approvals: The study must be submitted for Site Specific Assessment (SSA) and approval by the research and development (R&D) department at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before recruitment of participants from the NHS Trust in question into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent: The study will be conducted in accordance with applicable regulatory requirements, with International Conference on Harmonization "Good Clinical Practice" (GCP), with all applicable subject privacy requirements, and with the guiding principles of the Declaration of Helsinki. Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Confidentiality: The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Indemnity: Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Sponsor: Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

Funding: The National Institute for Health Research (NIHR) and Medical Research Council is funding this study through its Efficacy and Mechanism Evaluation programme.

Audits: The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, and other also by other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

Study management, data monitoring and ethics

The trial will be coordinated by a Trial Steering Committee (TSC), which will consist of an independent chair and members, the Principal Investigator, co-investigators, the Project Manager and a patient representative. A Data Monitoring & Ethics Committee will also be established. All its members will be independent of the applicants and of the TSC, while reporting to the TSC. They will meet at least annually and their role will be to monitor the unblinded data and make recommendations to the TSC on whether there are any ethical or safety reasons why the trial should not continue. The committee will consist of Professor Carel W le Roux (Metabolic Physician), Mr Richard Welbourn (Bariatric Surgeon) and Dr Les Huson (statistician). The day-to-day management and coordination of the study will be performed by Dr Alexander Miras.

Quality Control and Quality Assurance

The trial will be adopted by the NIHR/Wellcome Trust Clinical Research Facilities at both Imperial and King's College London and will fall under their QC/QA regime.

Imperial College London

Department of Investigative Medicine
Imperial College London
6th Floor Commonwealth Building
Imperial College London at Hammersmith Campus
Du Cane Road, London W12 ONN, UK
Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

You will be given a copy of this information sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

Comparison of the effects of the long-limb to the standard-limb gastric bypass on type 2 diabetes mellitus. The LONG LIMB trial

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Please be assured that if you decide not to take part this will not affect your clinical care in any way.

WHAT IS THE PURPOSE OF THE STUDY?

Obesity is the main cause of the world wide epidemic of diabetes. Weight loss, or 'bariatric', surgery produces major and sustained weight loss and is being increasingly used to treat obese diabetic patients. There was initial optimism that these procedures might "cure all diabetes". However, the gold-standard operation, standard gastric bypass, effectively cures diabetes in only 4 out of 10 patients.

To design a safer and more successful procedure we need to understand how bariatric surgery works to improve diabetes. Hormones from the gut are released when we eat food. They control how the body uses the food it absorbs. For example they release the sugar-lowering hormone insulin, and also greatly reduce appetite, which is why one feels less hungry after eating a meal. We have discovered that the good effects of bariatric surgery, and in particular the gastric bypass, are mainly due to increased release of gut hormones, reducing patient's appetite and improving the release of insulin.

In this project we will be testing a new procedure called the long-limb gastric bypass which involves one change in the design of the standard-limb gastric bypass. We want to find out whether it is better than the currently available standard-limb gastric bypass for sugar diabetes (type 2 diabetes mellitus) and if so what are the mechanisms. This is a new procedure so we also need to find out more about its safety and whether it is associated with the same, fewer or more complications than the standard-limb gastric bypass.

WHY HAVE I BEEN INVITED?

You have been invited because you are eligible to undergo the gastric bypass, and you have type 2 diabetes mellitus which is not well controlled.

You should not take part in this study if you:

- have significant medical, surgical or psychiatric conditions, or take any medicine that may affect the trial or harm you
- have undergone bariatric surgery previously or there are specific technical reasons why you cannot undergo bariatric surgery
- have Type 1 diabetes mellitus.
- are currently pregnant.
- are unable to maintain adequate contraception.
- do not have access to a telephone
- have donated blood in the last 3 months or intent to do so by the end of the study

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

Screening: If you agree to volunteer for this study you will first have a consultation with a doctor from the team that will ensure that you meet the inclusion criteria for the study and take a medical history, examine you, take basic blood tests (for example to look at your kidney and liver function), take a urine sample and perform an electrocardiogram to look at the structure/function of your heart. You will be asked to answer psychological/quality of life questionnaires that will take approximately 45 minutes to complete and provide a urine sample for a pregnancy test if you are a woman of child-bearing age.

Baseline visit: This will take place before your surgery. A few days before you attend for the study visit you may be asked to stop some of your diabetes medicines and may have to temporarily replace them with insulin. This is so that they do not affect the results of the studies. You will also be asked to avoid alcohol and strenuous exercise. Your exercise levels may be monitored through a non-invasive device (e.g. pedometer). You will be invited to attend the clinical research facility at Imperial or King's College London on a pre-arranged evening and have something to eat. A nurse or doctor will then insert one cannula in each arm and start an overnight infusion of insulin depending on your blood sugar. The nursing staff will be testing your blood sugar frequently during the night to ensure that it remains between 4.0-6.0. You will not be allowed to eat or drink anything other than water overnight.

On the day of the first study we will measure how sensitive your body is to insulin using the "clamp" test. It is called a clamp study because we keep, or "clamp", the blood glucose stable at a fixed level. In addition, an isotope, together with insulin and dextrose will be given through an infusion in the first cannula whilst blood will be taken from the other one for testing. This isotope is not radioactive and will not affect you in any negative way. We will aim to keep your blood sugar in the normal range. In the very unlikely event that your blood sugar goes low, we will be able to correct this promptly. Blood will be taken multiple times during the study for analysis of glucose, insulin, c-peptide, lipids, hormones and metabolites. In total up to 180 mls of blood will be taken (~36 teaspoonfuls). The test can take up to 8 hours to complete. At the end of the study you will be given something to eat and drink, but you will remain fasted overnight.

On the day of the second study we will measure how much insulin and hormones your body is producing using the mixed meal test. You will be asked to eat a liquid meal which is contains protein, fat and sugar and is similar to having a milkshake. Blood will be taken 8 times during the test which will last approximately 3 hours. You will also be asked how hungry and how full you are during the test and have your blood pressure and pulse taken. The doctor or nurse will be looking after you during

the entire test. You will not be given any medications to take. When the test ends, the cannulae will be removed and you can go home.

During/around the time of your stay with us we will also:

- measure your body weight and body fat
- collect stool samples for 24 hours in order to measure the amount of calories in them
- collect a blood, urine and stool sample to analyse it for bacteria and their by-products
- ask you to complete a food diary
- ask you to report any unwanted effects you have experienced recently including low blood sugars (hypos)

Surgery: Following your baseline visit you will be randomised (allocated randomly) to undergo either the standard or the long-limb gastric bypass. This will be performed by a bariatric surgeon at either Imperial or King's College London. The only difference between the long-limb and the standard limb gastric bypass is that in the former, the length of the small intestine that is bypassed (i.e. will not "see" food anymore) is 150 cm compared to 50 cm in the latter. Only the operating surgeon and a member of our research team will know which of the two procedures you underwent. It is not the surgeon that decides which procedure you will have. This will be determined by which procedure you are allocated to randomly. Following surgery you will be discharged from hospital and advised to consume a standard low-calorie post-operative diet.

Early post-operative visit: This will take place 7-14 days after surgery and will involve the same tests that you underwent during the baseline visit.

Late post-operative visit: This will take place when you have achieved a total of 20% body weight loss from your pre-operative weight and will involve the same tests that you underwent during the baseline visit.

In total you will undergo the clamp and mixed meal tolerance tests 3 times.

Yearly visit: This will take place 1 year after your operation. You will be assessed clinically and the following assessments will take place:

- Body weight and body fat
- Blood pressure and pulse
- Blood tests: routine blood tests like the ones you underwent during screening

- Any complications after surgery
- Number of medications you take
- Psychological/quality of life questionnaires

WHAT ARE WE TESTING?

We are trying to find out if the long-limb gastric bypass is better for diabetes than the standard-limb gastric bypass and if so we want to assess its safety and understand the underlying mechanisms. By understanding how bariatric surgery works we may be able to mimic the mechanisms through less invasive therapies that are safer and hopefully equally effective.

WHAT ARE THE SIDE EFFECTS AND RISKS OF TAKING PART?

Common: discomfort and bruising at the cannulae insertion sites.

Rare: your blood sugar can go low during the clamp test; this will be promptly treated by the research team if it happens.

The long-limb gastric bypass is relatively novel, but the research teams that have studied it so far have not reported any additional risk of complications or side effects compared to the standard-limb gastric bypass. These will be explained to you in great detail by the surgical team. Common risks of the standard-limb gastric bypass include bleeding, any infection, and vitamin deficiencies. Less common risks include a "leak" (i.e. leaking of food from the connections made during surgery), a venous thromboembolism (clot in the legs or lungs), a hernia inside your gut, low blood sugars (hypoglycaemia) and weight regain. Very rare risks include malnutrition and death.

During the study, experienced doctors will be available at any time should you have any concerns. You will be provided with a mobile number that you can call 24 hours a day 7 days a week in case you develop any unusual severe symptoms and want to speak urgently to a member of the team. If you suffer from any ill effects during the study you should report these to the doctors immediately. You may withdraw from the study at any time, without providing any explanation. If there are any unexpected side effects, the study will be stopped.

CAN I TAKE PART IF I AM PREGNANT?

Pregnant women must not take part in this study; neither should women who plan to become pregnant during the study. Women of childbearing age will be asked to have a pregnancy test at the

beginning of each study visit in order to ensure that they are not pregnant before the study visit commences. Women of childbearing age must use an effective contraceptive (e.g. hormonal oral contraceptive pill, hormonal contraceptive depot, barrier methods) during the course of this study and for 18 months after bariatric surgery. These recommendations are given to all female patients undergoing bariatric surgery in our units as part of routine clinical care. This is in order to avoid the less common risks of a preterm delivery (i.e. before 40 weeks) and spontaneous abortion. We expect these risks to be similar between the two procedures; they are not particular to the long-limb gastric bypass. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

You will benefit from frequent direct contact with our specialist team which is not routinely available as part of NHS care. In addition you will learn a lot about your body and diabetes by taking part in the special tests of the trial.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Alternatively, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

WHAT WOULD HAPPEN IF I LOST THE ABILITY TO CONSENT DURING THE COURSE OF THE STUDY?

In the unlikely event that during the course of the study you were no longer able to give your consent because you had lost the capacity to do so, the research team would withdraw you from the study and not perform any further testing on you. However, they would retain body fluid samples and personal data collected previously and would continue to use it for the purposes which you had already consented.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

Once the study has finished, the results of the study will be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided below.

WHAT IF SOMETHING GOES WRONG?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Miras, Dr Tan or Prof Bloom, on 020 8383 3242). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. The NHS and Imperial College London as sponsor may also review records as part of our audit process but all information will be kept strictly confidential. It is a requirement that your GP is informed, with your consent, of your participation, at the start of the study. In order to facilitate payment of travel expenses, your personal details will be required for this purpose only.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results are likely to be published in the 12 months following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results can be made available to you and/or your GP should you wish.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

This study is being organised by the Department of Investigative Medicine, Imperial College London. It is funded by the National Institute of Health Research and the Medical Research Council.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed by the West London & GTAC Research Ethics Committee.

CONTACT FOR FURTHER INFORMATION

If you experience any problems during the study, you may withdraw at any stage. You will also have direct emergency access, 24 hours a day, to one of the doctors involved in the study through mobile number 07958377674. The doctors may also be contacted through Professor Bloom's or Dr Tan's secretary (020 8383 3242) during office hours. The hospital switchboard (020 8383 1000) holds the home and mobile phone numbers for all the doctors involved in the study and can contact them at any time outside normal working hours if necessary.

PAYMENT

You will receive £500 upon completion of the study as a reimbursement for your time and your travel expenses.

Department of Investigative Medicine
Imperial College London

6th Floor Commonwealth Building
Imperial College London at Hammersmith Campus
Du Cane Road, London W12 ONN, UK
Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320

Consent form

Comparison of the effects of the long-limb to the standard-limb gastric bypass on type 2 diabetes mellitus

The participant should complete the whole of this sheet him or herself

(please initial each statement if it applies to you)

I have read the Information Sheet for Research Participants
Version 1.3, dated 12th June 2015 ☐

I have been given the opportunity to ask questions and discuss this study ☐

I have received satisfactory answers to all my questions ☐

I have received enough information about the study ☐

I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting my future medical care ☐

I agree to take part in this study ☐

I agree that my GP will be informed that I am taking part in the study ☐

I understand that the NHS and College as sponsor may also review records as part of audit process ☐

I agree that my blood samples will be kept indefinitely and may be used for further analysis or in future ethically approved research projects ☐

I am aware that, in the course of the study, if I were to lose the capacity to consent I would be withdrawn from the study. However, the body fluid samples and personal information collected prior to this would continue to be used for the purposes to which I have consented. (This could include further research on the samples after the current project has ended.) ☐

I consent to personal information, related to the study, being kept securely on Imperial College computers. This information will only be accessible to researchers directly involved in the study and Imperial staff processing payment of travel expenses. ☐

Participant's signature…….Date…….
(NAME IN BLOCK CAPITALS)

Investigator's signature……..Date……
(NAME IN BLOCK CAPITALS)

DAY 1

- Welcome the patient and show them around the facility
- Explain the visit protocol
- General health good? ☐Yes ☐No

……

- Happy to proceed? ☐Yes ☐No
- Collect the food diary ☐Yes ☐No
- Any alcohol or strenuous activity in the last 48 hours? ☐Yes ☐No

……

- Did the patient stop their diabetes medications 5 days ago? ☐Yes ☐No

……

- Any new medications prescribed recently? ☐Yes ☐No

……

- BP: …… HR: ……
- Insert 2 intravenous cannula. One in each antecubital fossa
  One cannula to be used for blood sampling, the other for administration of the various infusates
- YSI glucose at admission: ……

- At 5-6 pm provide 2 bottles of Ensure compact as the liquid meal . Meal eaten? ☐Yes ☐No

……

……

……

- Soup from the NHS kitchen allowed up to 9pm
- YSI glucose 2-3 hours after meal: ……
- Investigator to decide on insulin bolus and/or insulin infusion start and rate
- Start fast at 9pm
- Provide faecal collection kit and explain how it should be used (trial + NHS-H.pylori if not done before)
- Start questionnaires
- Document in medical notes

Name: ……Signature ……

Investigations from PREOP visit (date ……)

FBC

WBC ……10^{-9}/L Hb ……g/dL

Haematocrit …… MCV ……fl Platelets……

U&Es DAY 2

Na ……mmol/L K ……mmol/L

Urea ……mmol/L Creatinine ……µmolL eGFR…… ml/min/1.73m^2

U&Es DAY 3

Na ……mmol/L K ……mmol/L

Urea ……mmol/L Creatinine ……µmolL eGFR…… ml/min/1.73m^2

Urine Albumin…….g/l Urine Cr..... Albumin: Cr ratio……..

Metabolic

CORTISOL .....

CRP .....

Metabolic

GLUCOSE .....mmol/L Insulin ……IU/L C-peptide …… nmol/L

HbA1c .....mmol/L HOMA-IR: ……. ([Glucose] * [Insulin] / 22.5)

BLOODS Fasting ☐ Non-fasting ☐

BLOOD RESULTS NORMAL YES ☐ NO ☐

FOLLOW UP NEEDED FOR BLOODS:

Start of variable insulin infusion

- Participants should be commenced Nil by Mouth at 9 pm. No intravenous fluids will be prescribed overnight, but the participant will be allowed to drink water and have their usual medications (but not their glucose-lowering medications).

- The insulin infusion will normally be commenced at 6 am on the morning of Day 2 of the admission. If the patient's blood glucose is ≥15 mmol/L, it will be up to the discretion of the investigator to administer a bolus of subcutaneous Actrapid and/or commence the insulin infusion in the evening of Day 1 of the admission. When Actrapid is administered blood glucose will be checked at 2 and 4 hours after administration.

- Preparation of the insulin infusion syringe: Add 50 units of Actrapid (soluble insulin) in 48 mL Sodium Chloride 0.9% Solution and 2 mL of gelofusine in a 50 mL syringe. Prime the syringe and lines with gelofusine first (insulin in a peptide that "sticks" to plastic, so gelofusine is used as a carrier protein; gelofusine is used in all peptide infusion studies by our Group).

- Infuse using a syringe driver at a rate according to the modified Trust variable rate intravenous insulin infusion in Table 1. The investigator should specify whether insulin infusion rate A or B should be used, depending on the patient's insulin resistance.

- 0.5 mL blood samples should be taken from the blood sampling cannula for YSI blood glucose analysis. YSI Blood Glucose monitoring is covered in a separate SOP (SOP ref).

- If the insulin infusion is started in the evening of Day 1 of the admission, please monitor blood glucose levels 1 hourly until stable (blood glucose 4.0 - 7.9 mmol/L for 3 consecutive hours) and 2 hourly subsequently. If hypoglycaemia occurs or blood glucose greater than 15 mmol/L, revert to 1 hourly monitoring.

- If the insulin infusion is started at 6 am of Day 2 of the admission, please monitor blood glucose levels every 15 - 30 minutes until the investigator arrives.

- Document infusion changes and rate adjustments on the Imperial Clinical Research Facility Insulin Administration details / patient observations Chart.

- Contact the study doctor if you have any questions or concerns. Contact details are in the Contacts Folder at the ICRF Nurses station.

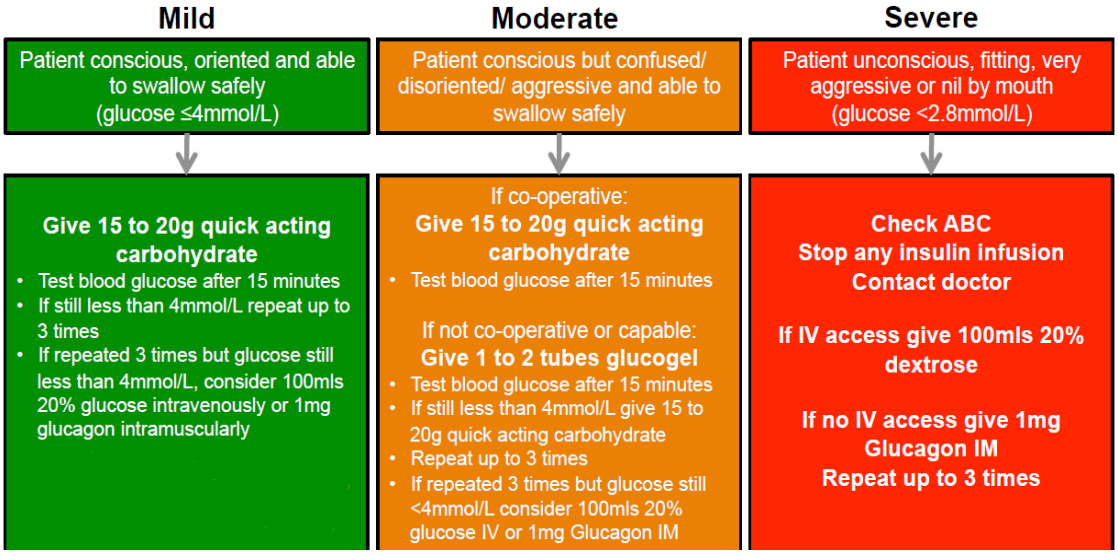
Table 1

Blood glucose (mmol / L)	**Insulin infusion rate A** (mL/hour)	**Insulin infusion rate B** (mL/hour)
0 - 3.9	0	0
4.0 - 7.9	0.5	1.0
8.0 - 11.9	1.0	2.0
12.0 - 15.9	2.0	3.0
16.0 – 19.9	3.0	4.0
≥ 20.0	4.0 - 6.0	6.0 - 8.0

Hypoglycaemia – Blood glucose level <4mmol/L

If blood glucose level falls below <4mmol/L treat as per ICHNT Trust policy specifically as per the table 2 below.

Table 2



- Contact the study doctor immediately. If study doctor is not available or does not respond contact Principal Investigator. If no response contact ICRF Head of Clinical Studies.

- The ICRF Doctors will provide medical cover during working hours: Monday to Friday, 9am to 5pm.

Discontinuation of Infusion

The insulin infusion will take place for approximately 9 hours and will be discontinued by the investigator at the end of the insulin clamp procedure which takes place on Day 2 of the admission. Patients will be provided a meal at the end of the clamp procedure. Patients will be restarted on their usual glucose-lowering medications upon discharge from the clinical research facility on Day 3 of their study visit.

Imperial Clinical Research Facility Insulin Administration details / patient observations Chart

Date (DD/MM/YY)	Time (HH:MM)	Blood Glucose (mmol/l)	Insulin Infusion Rate (ml/hr)	Initials	Comments

DAY 2

Time (min)	**Actual time**		**Glucose** Grey 3ml	**Insulin** Yellow 4ml	**Glucagon** Green 2ml	**C-peptide** from purple 3ml	**NEFA** Purple 3ml	**Total volume of blood (ml)**
-120		• BP= HR= • % Body fat ……% Fat mass:…… Kg FFM:……..Kg Estimated RMR:…… kCal/24hrs • Weight= …… • Explain what will be done to patient. Advise to drink plenty of water • Send yellow (in ice) and grey to NHS lab for FBC, renal, CRP, cortisol, HbA1c, glucose • Collect urine for ACR and metabolomics, and faeces for metabolomics + H.pylori antigen • VAS. Day of menstrual cycle for women…….. • Order NHS lunch • Document in medical notes						
-120			X	X	X	X	X	Plus clinical, gut hormones and metabolomics 30
		START INFUSION WHEN GLUCOSE LEVEL IS IN NORMAL RANGE (4-6 mmol/L)						
-120 to 0		• Inject **1.7mls** priming dose 6,6 $^{2}H_2$ glucose • Start 6,6 $^{2}H_2$ glucose infusion at **5.9 mls/h** • Zero timer • Print 20% glucose infusion excel spreadsheet						
-20			X	X				7
-15			X	X				7
-10			X	X				7
-5			X	X				7
0		Zero timer Toilet break	X	X	X	X	X	15
0-120		• Change insulin rate to 0.5 mU/kg/min=…….. • Infuse spiked 20% dextrose as per excel spreadsheet. • 10 minute YSI glucose measurements, keep level ± 0.5 mmol/l around Time 0 glucose value						
+30			X	X	X	X	X	15
+60			X	X				7
+90			X	X	X	X	X	15
+100			X	X				7
+110			X	X				7
		Toilet break						
+120		BP= HR=	X	X	X	X	X	15
		• Change insulin rate to 1.5 mU/kg/min=…… • **Spike 20% glucose bag with 2.0 mls of 6,6 $^{2}H_2$ glucose, mix it and take 2 cryotubes from bag** • Infuse spiked 20% glucose as per excel spreadsheet • 10 minute YSI glucose measurements, keep level ± 0.5 mmol/l around Time 0 value						
+150			X	X	X	X	X	15
+180			X	X				7
+210			X	X	X	X	X	15
+220			X	X				7

+230			X	X				7
+240			X	X	X	X	X	15
+240		• Stop insulin and isotope infusion and continue 20% dextrose infusion for 30 minutes, give lunch						
+270		• BP= HR= • Check blood glucose (after the meal)= if >4.0 remove infusates • Document in the notes • Patient can have soup from NHS kitchen in the evening and re-start the fast at 9pm						
Comments Name: ……Signature ……								

Data Entry Sheet

Study time	Actual time	YSI glucose	Insulin infusion (mL/h)	Comment		Clamp time	Actual time	YSI Glucose	Spiked infusion rate(mg/Kg/min)	Spiked infusion rate(ml/h)
						0				

Clamp time	**Actual time**	**YSI Glucose**	**Spiked infusion rate(mg/Kg/min)**	**Spiked infusion rate(ml/h)**		**Clamp time**	**Actual time**	**YSI Glucose**	**Spiked infusion rate(mg/Kg/min)**	**Spiked infusion rate(ml/h)**

Visual Analogue Scale

T= -120

How hungry do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——————————————— EXTREMELY

How much do you think you could eat right now?

NOTHING ——————————————— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

PREPARATION

Supplies needed

- Aprotinin (Trasylol)
- 0.8M HCl
- Timer
- YSI Glucose analyser
- Bag infusion pump
- 2 syringe drivers
- 3 Infusion lines (1 meter)
- 50 ml syringe infusion pumps x2
- 3 way taps x4
- Cannula x2
- Ice for blood samples
- Syringes 1 ml (plenty), 2 ml, 5 ml, 10 ml, 20 ml, 50 ml
- N. Saline bag 500ml
- 20% Glucose bag x1 500ml
- [6,6-$2H^2$] glucose 5ml ampoules

Cryotubes and caps

- 122 Cryotubes 1.8mL
  - 44 Grey caps
  - 38 Yellow caps
  - 8 Orange caps
  - 15 Green caps
  - 8 Violet (Purple) caps
  - 4 White caps
  - 4 tan (brown) caps
  - 1 blue cap

Sample collection tubes/containers

19 Grey 2mL

19 Yellow 4mL

8 Purple 4mL

2 Green 6mL

8 Green 4mL

2 urine universal containers

2 Faecal Collection Kits

Arrangement of blood collection tubes

(Label the clinical ones fully and the rest with the time points only)

Baseline	Glucose (NHS lab)	Chemistry (NHS lab)	Chemistry (ice, NHS lab)	6ml Gut Hormones	6ml Metabonom
-120	Glucose	Insulin	Glucagon	NEFA+ C-peptide	
-20	Glucose	Insulin			
-15	Glucose	Insulin			
-10	Glucose	Insulin			
-5	Glucose	Insulin			
0	Glucose	Insulin	Glucagon	NEFA +C-peptide	
+30	Glucose	Insulin	Glucagon	NEFA + C-peptide	
+60	Glucose	Insulin			
+90	Glucose	Insulin	Glucagon	NEFA +C-peptide	
+100	Glucose	Insulin			
+110	Glucose	Insulin			
+120	Glucose	Insulin	Glucagon	NEFA + C-peptide	
+150	Glucose	Insulin	Glucagon	NEFA +C-peptide	
+180	Glucose	Insulin			

+210	 Glucose	 Insulin	 Glucagon	 NEFA + C-peptide	
+220	 Glucose	 Insulin			
+230	 Glucose	 Insulin			
+240	 Glucose	 Insulin	 Glucagon	 NEFA + C-peptide	

Cryotube arrangement

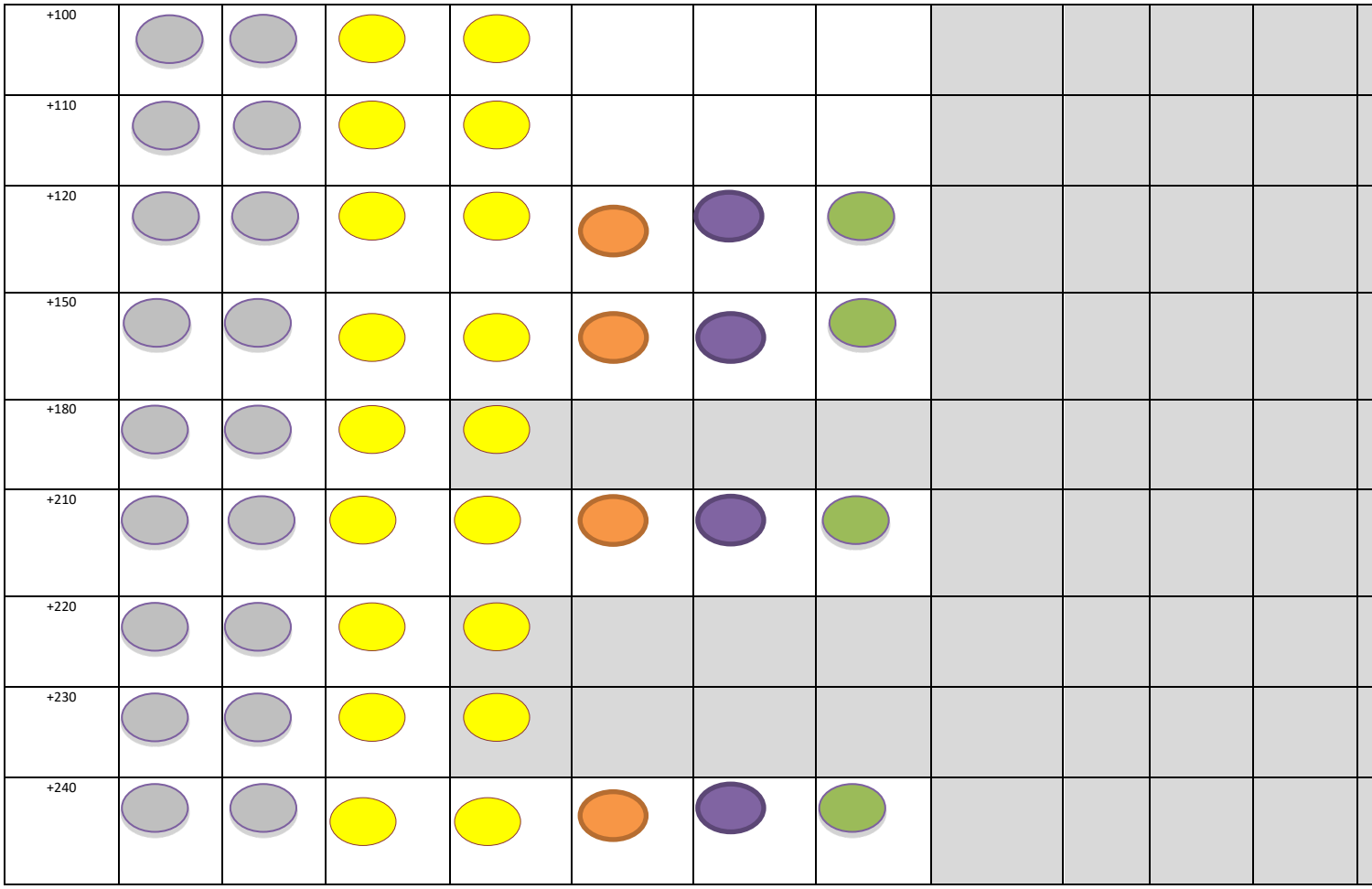
- 122 cryotubes for Insulin Clamps

(36 with Grey caps for Glucose; 36 with Yellow caps for Insulin; 8 with Orange caps for c-peptide;

8 with Green caps for Glucagon; 8 with Violet (Purple) caps for NEFA)

- 2 grey cap cryotubes for the measurement of isotope concentration in syringe
- 6 grey cap cryotubes for the measurement of isotope concentration in 20% glucose bag
- 2 yellow cap cryotubes for the measurement of insulin concentration in insulin syringe
- 3 green cap cryotubes for gut hormone analysis and 1 blue cap for ghrelin
- 4 white cryotubes for urine metabonomics
- 4 tan (brown) cryotubes for stool samples
- 4 green cryotubes for plasma metabonomics

								Gut Hormones				
Insulin concentration in syringe			isotope concentration in syringe				Isotope in 20% dextrose bag before spiking			Isotope in 20% dextrose bag after spiking		
	Glucose c	**Glucose e**	**Insulin**	**Insulin 2**	**C-Peptide**	**NEFA**	**Glucagon**			Isotope in 20% dextrose bag after re-spiking		
-120												
-20								Urine Metabonomics (any time point)				
-15								Stool Metabonomics (any time point)				
-10								Plasma Metabonomics (baseline sample)				
-5												
0												
+30												
+60												
+90												


+100
+110
+120
+150
+180
+210
+220
+230
+240

Preparation of infusates

Preparation of [6, 6-$^2H_2$] glucose tracer

[6, 6-$^2H_2$] glucose stock 100mg/ml 5ml/ampoule

Priming bolus: 170mg **1.7ml** of [6, 6-$^2H_2$] glucose to be given IV over 0.5 min

Continuous infusion (not weight related; make 1.5 hour extra for the line):

Infusion rate 1.7mg/min

For 8.5h need 1.7mg/min×60min×8.5h=867mg i.e. 8.7ml of [6, 6-$^2H_2$] glucose

Mix 8.7ml stock [6, 6-$^2H_2$] glucose with 41.3ml of saline to give total volume of 50ml

50ml/8.5h=5.9ml/h **so infuse at 5.9 ml/h**

Take two samples of 0.5mls in cryotubes (iso syringe) for the measurement of [6, 6-2H2] glucose concentration in the infusate at the start of the study.

Preparation of insulin infusion

Insulin infusate is made up in 48 ml saline and 2ml of gelofusine in a 50ml syringe. Add 50 units of Actrapid insulin to the syringe. Insulin concentration will therefore be 1 unit/ml.

Example: If the weight of the person is 100 kg the insulin infusion rates will be:

Insulin clamp 1st step for 2 hours: **0.5mU/kg/min** to be infused at 3.0 ml/h

Insulin clamp 2nd step for 2 hours**: 1.5mU/kg/min** to be infused at 9.0 ml/h

Take two samples of 0.5mls in cryotubes (ins syringe) for the measurement of insulin concentration .

Preparation of the 20% dextrose infusion spiked with isotope

*Take two samples of 0.5mls in cryotubes **before** (Neat)*

Insulin clamp 1st step for 2 hours: The 500ml bag of 20% dextrose should be "spiked" with [6, 6-$^2H_2$] glucose. Therefore inject 8ml of [6, 6-$^2H_2$] glucose in the 500ml bag of 20% dextrose. Mix well. Store the remaining 2mls for 2nd step.

*Take two samples of 0.5mls in cryotubes **after** spiking (-120 Enrch spk) the dextrose bag for GCMS*

analysis.

Insulin clamp 2nd step for 2 hours: Add 2.0 mls of [6, 6-2H2] glucose to the 20% dextrose bag. Mix well. If all the 20% dextrose is used during the 1st step, make up a new bag of 250ml 20% dextrose with 5ml [6, 6-$^2H_2$] glucose.

Take 2 cryotubes from bag for GCMS analysis (+120 Enrch spk)

The infusion rate of the 20% dextrose is calculated based on the GLUINF excel file.

Preparation of gut hormone tubes

100 µl Aprotinin (Trasylol) should be added to the three gut hormone collection tube

Acidify Ghrelin Cryotube

Pipette 20mcl 0.8M HCl into the ghrelin cryotube

Insulin Clamp Procedure

Time -120: baseline samples

1. Check the patient has been well overnight
2. Explain what will happen today (clamp 6-7 hours, followed by meal)
3. Patient should drink plenty of water, can walk around bed and visit the toilet escorted
4. One cannula to be used for blood sampling, the other for administration of the various infusates (insulin, [6, 6-$^2H_2$] glucose and dextrose with 1x three way tap)
5. Patient should lie supine or semi supine and not allowed to fall asleep
6. Glucose measurements are performed by taking 0.5-1.0 ml of the patient's blood from the venesection cannula and running it through the bedside glucose analyser.
7. Send one yellow (in ice), another yellow (no ice) and grey top to NHS lab for U&Es, glucose, insulin, CRP and cortisol. Send a urine sample in a universal container to the NHS lab.
8. **This is very important**: Before you infuse the isotope, take blood samples for determination of baseline glucose enrichment.
9. Leave insulin sample to clot for 10 minutes at room temperature.
10. Place all other samples on ice and then centrifuge them at 4°C for 10 minutes at 4,000 rpm.
11. Separate the plasma in cryotubes, store in -20°C freezer. Then do the same for the insulin blood sample.
12. Separate:
    a. The first baseline green blood tube to the 3 gut hormone and 1 blue plasma cryotubes
    b. The second baseline green blood tube to the 4 Plasma cryotubes for metabolomics: Collect whole blood into 6ml green sodium heparinized vacutainers. Invert tubes ~10 times immediately after collection, to gently mix the blood and prevent coagulation. Centrifuge to generate plasma. Draw up the plasma supernatant above the white blood

cell layer, transfer to 3 aliquots of 200 µl (for MS) and 1 aliquot of 350-400 µl, and store at - 20 °C ASAP.

c. the grey blood tubes to the 2 grey plasma cryotubes
d. the yellow blood tubes to the yellow plasma cryotube
e. the purple blood tubes to the orange and purple plasma cryotubes
f. the green blood tubes to the green cryotubes

Urine: Collect an early morning urine sample.

Aliquot the urine into 4 cryotubes and deep-freeze them at - 20 °C ASAP. Transfer to - 80 °C when possible.

Transfer to - 80 °C when possible.

Faeces:

Collect the samples, aliquot into 4 cryotubes and freeze at - 20 °C ASAP. Transfer to - 80 °C when possible.

Remaining sample in the container and freeze at - 20 °C ASAP. Transfer to - 80 °C when possible.

Time: -120 to 0. This is the [6, 6-$^2H_2$] glucose equilibration stage.

13. Inject the priming bolus of 1.7ml of [6, 6-$^2H_2$] glucose and flush with 10mls saline.
14. Start infusion of prepared infusate of [6, 6-$^2H_2$] glucose. Run initially at 70mls/h for a few minutes so that it reaches the vein fast and then run at the fixed rate of 5.9 ml/hr.
15. Equilibrium with [6, 6-$^2H_2$] glucose will be achieved within 100 min.
16. During this stage the patient can complete the questionnaires.
17. Take blood samples at time points -20, -15, -10, -5 and 0. Process them as above.
18. Note the blood glucose levels at these time points in the data entry sheet.

Time: 0 to +120. This is the FIRST step of the insulin clamp

19. Measure BP, HR, and ask if the participant wants a toilet break
20. Note the blood glucose at time 0 in the data entry sheet. This is your "clamped" glucose. The aim is to keep the blood glucose at ± 0.5 mmol/l around the clamped glucose by adjusting the infusion rate of the 20% spiked dextrose.
21. Connect the 500ml 20% spiked dextrose bag to the infusion cannula. By now there should be 3 infusions connected to the patient: insulin, [6, 6-$^2H_2$] glucose and the 20% spiked dextrose.
22. Start the low-dose infusion of insulin at 0 minutes. Run at the fixed rate of 0.5mU/Kg/min.
23. Check blood glucose every 5-10 minutes and adjust 20% spiked dextrose infusion based on the rates provided by the GLUINF excel file. Note the blood glucose readings in the data entry sheet.
24. Take blood samples at time points +30, +60, +90, +100, +110, +120 and process them as above.

Time: +120 TO +270. This is the SECOND step of the insulin clamp

25. BP, HR, and ask if the participant wants a toilet break
26. Inject 2.0 of [6, 6-$^2H_2$] glucose to the 20% spiked dextrose bag. Mix well and *take 2 cryotubes for GCMS analysis of the newly spiked bag.*
27. Change the insulin infusion rate to 1.5 mU/Kg/min. The aim is to keep the blood glucose at ± 0.5 mmol/l around the clamped glucose by adjusting the infusion rate of the 20% spiked dextrose.
28. [Increase the 20% spiked dextrose infusion at +125 at double the infusion rate at time point +120 (prophylactically)]. Check blood glucose every 5-10 minutes and adjust 20% spiked dextrose

infusion based on the rates provided by the GLUINF excel file. Note the blood glucose readings in the data entry sheet.

29. Take blood samples at time points +150, +180, +210, +220, +230, +240 and process them as above.
30. At +240 stop insulin infusion, but continue 20% spiked dextrose infusion for 30 minutes to prevent hypoglycaemia.
31. At +270 take check blood glucose. If this is over 4.0 remove all infusions. Check BP, HR.
32. At 6pm the patient can have dinner of their choice from the NHS kitchen
33. Restart the fast at 9pm.
34. Document in the notes

PREPARATION

Supplies needed

- Aprotinin (Trasylol)
- 0.8M HCl
- 1 bottle of Ensure compact
- Timer
- Syringes
- N. Saline bag 250ml for flushing

Cryotubes and caps

- 78 cryotubes
  - 8 yellow cryotubes for insulin
  - 8 orange cryotubes for c-peptide
  - 24 green cryotubes for gut hormones
  - 8 blue cryotubes for Ghrelin
  - 14 red cryotubes for bile acids
  - 8 pink cryotubes for FGFs
  - 8 Purple (violet) cryotubes for Free Fatty Acids

Blood collection tubes

8	Grey 2mL
8	Yellow 4mL
13	Green 6mL

Preparation of gut hormone tubes

100 µl Aprotinin (Trasylol) should be added to the gut hormone collection tubes

Acidify Ghrelin Cryotube

Pipette 20mcl 0.8M HCl into the ghrelin cryotube

Arrangement of blood collection tubes

-30	Glucose NHS	Insulin and c-peptide	NHS lab	Gut hormones	
T= 0	Glucose NHS	Insulin c-pep, FGF 19 & 21		Gut hormones	Bile acids and FFA
+15	Glucose NHS	Insulin and c-peptide		Gut hormones	Bile acids
+30	Glucose NHS	Insulin, c-peptide, FGF 19 & 21		Gut hormones	Bile acids and FFA
+60	Glucose NHS	Insulin c-pep		Gut hormones	Bile acids
+120	Glucose NHS	Insulin c-pep, FGF 19 & 21		Gut hormones	Bile acids and FFA
+180	Glucose NHS	Insulin, c-peptide, FGF 19 & 21		Gut hormones	Bile acids and FFA

Cryotube arrangement

	Insulin	C-peptide	Gut hormones	Gut hormones	Gut hormones	Ghrelin	Bile acids	Bile acids	FGFs	FGFs	FFA	FFA
-30												
T= 0												
+15												
+30												
+60												
+120												
+180												

DAY 3

- Check the patient has been well overnight
- Explain what will happen today (mixed meal followed by blood tests) - Order NHS lunch
- Have you eaten anything since dinner last night? ……. - Document in medical notes
- General health today? …….

Time (mins)	BP	Pulse	Bloods	Total volume	VAS (tick)	YSI glucose	Protocol/C	NHS Gluc.
-30 **Actual Time**			1 gold 7mls (NHS electrolytes+insulin, glucose, c-peptide) 1 gold 4ml (Insulin, c-peptide) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	20				
T= 0 **Actual**			1 gold 6mls (Insulin, c-peptide, FGF 19 & 21) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	18			**GIVE 1 ENSU**	
+15 **Actual**			1 gold 4ml (Insulin, c-peptide) 1 grey 3ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	15				
+30 **Actual**			1 gold 4mls (Insulin, C-peptide) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	15				
+60 **Actual**			1 gold 6mls (Insulin, C-peptide, FGF 19 & 21) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	15				
+120 **Actual Time**			1 gold 6mls (Insulin, C-peptide, FGF 19 & 21) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin) 1 green 4 mls (PA+EFA)	18				
+180 **Actual**			1 gold 6 mls (Insulin, C-peptide, FGF 19 & 21) 1 grey 2ml (Glucose) 1 green 6mls (gut hormones, ghrelin)	18				

- Remove cannula
- Give patient instructions about what happens next and prescriptions as required
- Give patient a food diary to complete before the next visit
- Prepare letter for GP
- Document in the medical notes
- Store all samples from whole visit + Input all data in database
- **ENSURE HbA1c, FBC, glucose, insulin, c-peptide, U&E, bone profile, LFT are checked on the day of surgery and patient NOT discharged on OAD or ursodeoxycholic acid.**

Comments

Name: ……Signature ……

VISUAL ANALOGUE SCALE

T=-30

How hungry do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——————————————— EXTREMELY

How much do you think you could eat right now?

NOTHING ——————————————— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

VISUAL ANALOGUE SCALE

T=0

How hungry do you feel right now?

NOT AT ALL ——— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——— EXTREMELY

How much do you think you could eat right now?

NOTHING ——— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——— EXTREMELY

HOW TASTY WAS THE MEAL?

NOT AT ALL ——— EXTREMELY

VISUAL ANALOGUE SCALE

T= +30

How hungry do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——————————————— EXTREMELY

How much do you think you could eat right now?

NOTHING ——————————————— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

T=+60

How hungry do you feel right now?

NOT AT ALL ——— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——— EXTREMELY

How much do you think you could eat right now?

NOTHING ——— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——— EXTREMELY

VISUAL ANALOGUE SCALE

T=+120

How hungry do you feel right now?

NOT AT ALL ——— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——— EXTREMELY

How much do you think you could eat right now?

NOTHING ——— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——— EXTREMELY

VISUAL ANALOGUE SCALE

T=+180

How hungry do you feel right now?

NOT AT ALL ——— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——— EXTREMELY

How much do you think you could eat right now?

NOTHING ——— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——— EXTREMELY

How stressed do you feel right now?

NOT AT ALL ——— EXTREMELY

Patient information for next study visit

- Please restart all of your diabetes medications from today
- Over the next few weeks you should be given a date for surgery
- You will not need to go on a pre-operative diet, but will still see our specialist nurses before surgery
- For the first two days after the surgery you will have a liquid/puree diet and gradually return to a normal diet. You will be given a diet sheet with suggestions of what you can eat.
- Following surgery you will be discharged on no diabetes medications. You should NOT take any medications for gallstones (ursodeoxycholic acid).
- **Please do not allow anyone to change your medications or diet other than the clinical or research teams.**
- 1-2 weeks after surgery we will repeat exactly the same tests that you had in the last 36 hours. Please bring everything you need including clothes and toiletries, your medications and something to keep you busy (e.g. books, laptop, or tablet). Following your visit you will be able to either drive home or take public transport.
- **Start completing your food diary booklet 3-7 days before the study visit**
- Check your blood sugars before breakfast only and write the results in your food diary booklet
    - If the blood sugars are over 10, please contact us
    - If you have any hypos (blood sugar less than 4) treat them as usual and contact us
- Avoid alcohol and strenuous exercise for 48 hours before the study visit
- Arrive at the Clinical Research Facility at 15 pm on the day of your study visit

If you are unable to attend this appointment or if you have any other queries or concerns please contact the study team on **07710067018** (available 24 hours per day) or email b.pevida@imperial.ac.uk/akamocka@nhs.net

Many thanks for taking part in this study.

DIETARY RECORD

NAME:

_____

DATE: _____

3 days before visit

1-2 weeks after surgery

This record is designed to obtain accurate information about the type and quantity of food that you eat.

DIETARY RECORD SHEET – DAY 1

Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.

Record method of cooking, type and quantity of food

eg: 6 tbsp boiled wholemeal spaghetti

2 egg sized roast potatoes.

WEIGHT _____

DATE:_____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood sugar
Early Morning:			
Breakfast:			
During Morning:			
Midday:			

DAY1:_____

DATE:_____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood Sugar
During Afternoon:			
Evening Meal:			
During Evening:			
Bedtime Snack:			

DIETARY RECORD SHEET – DAY 2

Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.

Record method of cooking, type and quantity of food

eg: 6 tbsp boiled wholemeal spaghetti
2 egg sized roast potatoes.

WEIGHT _____ DATE: _____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood Sugar
Early Morning:			
Breakfast:			
During Morning:			
Midday:			

DAY2:_____

DATE:_____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood Sugar
During Afternoon:			
Evening Meal:			
During Evening:			
Bedtime Snack:			

DIETARY RECORD SHEET – DAY 3

Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.

Record method of cooking, type and quantity of food

eg: 6 tbsp boiled wholemeal spaghetti
2 egg sized roast potatoes.

WEIGHT _____ DATE: _____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood Sugar
Early Morning:			
Breakfast:			
During Morning:			
Midday:			

DAY 3: _____

DATE:_____

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Blood Sugar
During Afternoon:			
Evening Meal:			
During Evening:			
Bedtime Snack:			

Detailed standard operating procedure for the standard and long-limb Roux-en-Y gastric bypass operations

Standard-limb RYGB
1. The procedure is performed by a Consultant surgeon using Covidien instruments.
2. Patient is placed on the operating table. General anaesthesia is administered.
3. The patient's abdomen is prepped and draped in sterile fashion.
4. The abdominal cavity is entered and pneumoperitoneum is established to 15 mmHg pressure carbon dioxide. The procedure is filmed.
5. Laparoscopic bladeless 12-mm trocars are passed obliquely through the abdominal wall, including left upper quadrant, left flank and umbilical midline.
6. The omentum and the transverse colon are then reflected cephalad to expose the ligament of Treitz.
7. From this position, the small intestine (jejunum) is measured with 5 cm marks (steristrip) placed on graspers
8. The small bowel is divided 50-cm from the ligament of Treitz with an endostapler. This proximal segment of intestine defines the biliopancreatic limb.
9. The distal segment of intestine is then further measured to 100 cm and this is the length of the Roux / alimentary limb.
10. A side-to-side entero-enterostomy is performed by stapling the biliopancreatic limb to the 100 cm mark on the alimentary limb making parallel antimesenteric enterotomies and firing the endostapler into the lumen of each. The enterotomy is closed.
11. All mesenteric defects will be closed.
12. A completely isolated proximal gastric pouch 30-40 ml in volume is created using endostaplers. The actual length of the pouch may vary depending on the anatomical conditions seen at the time of surgery, but in general terms the horizontal transection of the pouch will be at the level of the 2nd gastric vein, lesser curve side, below the fat pad.
13. The previously measured alimentary / Roux limb is taken up to the gastric pouch (antecolic) with the 100cm alimentary limb on the patient’s right and a 50 cm biliopancreatic on patients' left. The antecolic antegastric approach will be used unless during the surgery, there is a clinical need to use the retrocolic approach.
14. The alimentary limb is anastomosed with a circular or linear stapler to the gastric pouch and a leak test is performed with the Roux loop occluded.
15. The pneumoperitoneum is allowed to escape.
16. The trocars are withdrawn under laparoscopic vision ensuring there is no bleeding from the port site.
17. The wound is irrigated with normal saline, infiltrated with 0.25% Marcaine and closed with staples.

Long-limb RYGB
1. The procedure is performed by a Consultant surgeon using Covidien instruments.
2. Patient is placed on the operating table. General anaesthesia is administered.
3. The patient's abdomen is prepped and draped in sterile fashion.
4. The abdominal cavity is entered and pneumoperitoneum is established to 15 mmHg pressure carbon dioxide. The procedure is filmed.
5. Laparoscopic bladeless 12-mm trocars are passed obliquely through the abdominal wall, including left upper quadrant, left flank and umbilical midline.
6. The omentum and the transverse colon are then reflected cephalad to expose the ligament of Treitz.
7. From this position, the small intestine (jejunum) is measured with 5 cm marks (steristrip) placed on graspers
8. The small bowel is divided 150-cm from the ligament of Treitz with an endostapler. This proximal segment of intestine defines the biliopancreatic limb.
9. The distal segment of intestine is then further measured to 100 cm and this is the length of the Roux / alimentary limb.
10. A side-to-side entero-enterostomy is performed by stapling the biliopancreatic limb to the 100 cm mark on the alimentary limb making parallel antimesenteric enterotomies and firing the endostapler into the lumen of each. The enterotomy is closed.
11. All mesenteric defects will be closed.
12. A completely isolated proximal gastric pouch 30-40 ml in volume is created using endostaplers. The actual length of the pouch may vary depending on the anatomical conditions seen at the time of surgery, but in general terms the horizontal transection of the pouch will be at the level of the 2nd gastric vein, lesser curve side, below the fat pad.
13. The previously measured alimentary / Roux limb is taken up to the gastric pouch (antecolic) with the 100 cm alimentary limb on the patient’s right and a 150 cm biliopancreatic on patients' left. The antecolic antegastric approach will be used unless during the surgery, there is a clinical need to use the retrocolic approach.
14. The alimentary limb is anastomosed with a circular or linear stapler to the gastric pouch and a leak test is performed with the Roux loop occluded.
15. The pneumoperitoneum is allowed to escape.
16. The trocars are withdrawn under laparoscopic vision ensuring there is no bleeding from the port site.
17. The wound is irrigated with normal saline, infiltrated with 0.25% Marcaine and closed with staples.