Identifying factors associated with metabolic and weight-loss success following bariatric surgery

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Declaration

I, Kusuma Chaiyasoot, hereby declare that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Background/Objectives: Bariatric surgery is the most effective treatment for people with severe obesity. In order to maximise the benefit to risk ratio and gain more insights into the pathophysiology of obesity, identifying factors associated with metabolic and weight-loss success after bariatric surgery would be valuable.

Methods: Three studies were performed. 1) Association study of pre- and early post-operative (6 weeks) factors with 1-year weight loss after LRYGB and LSG were examined in a prospective cohort of 85 subjects. 2) 13 subjects with T2D remission were compared with matched percentage weight loss (PWL) 13 subjects without remission. Gut hormones, hepatic, pancreatic and visceral fat, insulin sensitivity, β-cell function and metabolomics were compared between groups. 3) 1,401 patients with severe obesity who were due to undergo primary bariatric surgery were prospectively investigated and genotyped. The GWAS was performed to identify SNPs associated with PWL after surgery. Two genetic risk scores (GRSs) of 941 variants associated with BMI and 49 variants associated with WHR adjusted for BMI (WHRadjBMI) were also constructed to examine the association with PWL.

Results: 6-week post-operative PWL, weight change velocity, and PYY parameters were significantly associated with 52-week PWL. The rise in an AUC of postprandial PYY at 6 weeks from pre-surgery less than 16,000 pgxmin/mL was connected with 8 times greater chance of being poor weight loss (PWL<20%) at 1 year. T2D remitters had significantly greater β-cell function, circulating levels of FGF-19 and acyl ghrelin, but lower visceral fat area and plasma branched-chain amino acids (BCAAs) levels than non-remitters. The GWAS revealed 4 significant SNPs (KLF3-rs1491199, MAMDC2-rs2975907, GSAP-rs740158, CASZ1-rs7555879) associated with 52-week PWL. There is a significantly negative association between GRS of WHRadjBMI and PWL at 2 and 3 years.

Conclusions: Genetics and 6-week weight loss and PYY parameters could predict the weight loss outcome from bariatric surgery. β-cell function, acyl ghrelin, FGF-19, visceral fat and BCAAs related to T2D remission.

Impact statement

Obesity is a complex, progressive and continuing disease, characterised by excessive fat accumulation in the body, that impairs health. Obesity increases the risk of developing several diseases, in particular type 2 diabetes (T2D), leading to disability and death. In 2014, more than 600 million adults worldwide had obesity.

Obesity is driving the global increase in T2D, a serious ongoing disease caused by an impairment in how the body controls and utilises glucose which leads to various complications and premature death. Approximately 422 million people worldwide had T2D in 2014.

Body weight regulation is complex, requiring interaction from many organs; for example, brain, fat tissue, stomach and intestines. Contribution of genetics to body weight ranges from 30 to 70%. Moreover, hedonic hunger, a feeding behaviour that is influenced by palatable food, social elements and mood, and environment are driving obesity pandemic.

A number of gut hormones involve in body weight regulation. Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are secreted from small intestine. They function as satiety hormones and benefit glucose control. Ghrelin is a hunger hormone, secreted by the stomach. Imbalance of hormones involving in satiety and hunger could lead to obesity.

Lifestyle modification is a fundamental treatment of obesity. However, weight-loss maintenance is challenging. Various compensatory mechanisms are activated by weight loss leading to increased hunger, preference over energy-dense food, thus weight regain.

Bariatric surgery is currently the most effective treatment for severe obesity. It also benefits glycaemia, particularly leading to remission of T2D (diabetes patients returning to normal glucose control). Research has revealed that the changes in circulating levels of gut hormones are one of the keys mediating weight loss and T2D remission after surgery.

However, bariatric surgery is not without risks, and access to the surgery is very limited. Only <1% of eligible patients underwent surgery in the UK in 2014. Moreover, the weight

loss achieved is highly variable. In order to maximise the benefit to risk ratio, identifying predictors of weight-loss response to bariatric surgery would be of value. By identifying predictors of weight-loss responses, bariatric surgery can be personalised according to individual genetic variation and biological characteristics.

This work has demonstrated that genetics can help to identify patients who will experience good weight-loss response from bariatric surgery. Thus, the number of patients with poor weight loss will be minimised. Several factors at 6-week post-surgery are also predictive for weight loss at 1 year. These parameters could help to identify patients who need additional support after surgery. The earlier patients gain the additional interventions, the better they achieve long-term weight-loss outcome.

We also discovered some gut hormones associated with weight loss and T2D remission. High circulating levels of branched-chain amino acids and increased fat deposition in the abdomen were found in patients without the remission. These findings add more insight into how obesity and T2D could be cured and leave a room for future research to develop novel treatments. Ultimately, our findings would be beneficial for people who are suffering from obesity and T2D.

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Abbreviations

AEBSF	4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride
AG	Acyl-ghrelin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AUC	Area under the curve
ΔAUC	AUC calculated by subtracting the fasting (0-minute) hormone level from every time-point level during the MMTT
AuROC	Area under ROC curve
BA	Bile acid
BAT	Brown adipose tissue
BCAA	Branched-chain amino acids
BCAT	Branched- chain aminotransferase
BCKD	Branched-chain alpha-ketoacid dehydrogenase
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BMR	Basal metabolic rate
BP	Blood pressure
BPD-DS	Biliopancreatic diversion with duodenal switch
BSA	Bovine serum albumin
BW	Body weight

CI	Confidence interval
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DAG	Desacyl-ghrelin
DBP	Diastolic BP
DHA	Docosahexaenoic acids
diff AUC	The difference in AUC of a hormone between follow-up visits
diff ΔAUC	The difference in ΔAUC of a hormone between follow-up visits
DiRECT	Diabetes Remission Clinical Trial
DJB	Duodenal-jejunal bypass
DPP-4	Dipeptidyl peptidase 4
DXA	Dual-energy X-ray absorptiometry
EBWL	Excess body weight loss
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FDR	False Discovery Rate
FFA	Free fatty acid
FFM	Fat-free mass
FGF	Fibroblast growth factor
FM	Fat mass

fMRI	Functional MRI
FTO	Fat mass and obesity- associated
FXR	Farnesoid X receptor
GHSR	Growth hormone secretagogue receptor
GI	Gastrointestinal
GIBP	Gastrointestinal bypass
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
Gln	Glutamine
GlycA	Glycoprotein acetyls
GOAT	Ghrelin O-acyl-transferase
GP	General practitioner
GRS	Genetic risk score
GSIS	Glucose-stimulated insulin secretion
GWAS	Genome-wide association study
3-HIB	3-hydroxyisobutyrate
HbA_{1c}	Haemoglobin A1c
HCl	Hydrochloric acid
HDL_P	Concentration of high density lipoprotein
HIV	Human immunodeficiency virus
HOMA-β	Homeostatic model assessment of β-cell function

HOMA-IR	Homeostatic model assessment for insulin resistance
HRP	Horseradish peroxidase
HSD	Honestly Significant Difference
HWE	Hardy-Weinberg equilibrium
ICBAS	Institution of Biomedical Science Abel Salazar
IDL_P	Concentration of intermediate density lipoprotein
IL-6	Interleukin 6
Ile	Isoleucine
IMS	Individualized Metabolic Surgery
IT	Ileal transposition
JNK	c-Jun amino-terminal kinase
KLF	Krüppel-like factor
LA	Linoleic acids
LADA	Latent autoimmune diabetes in adults
LC-HRMS	Liquid chromatography coupled to high resolution mass spectrometry
Leu	Leucine
LRYGB	Laparoscopic Roux-en-Y gastric bypass
LS	Latent Structures
LSG	Laparoscopic sleeve gastrectomy
MAF	Minor allele frequency
MAMDC2	MAM domain containing 2

MCT	Monocarboxylate transporter
MDT	Multidisciplinary team
MM	Muscle mass
MMTT	Mixed meal tolerance test
MRAP2	Melanocortin receptor accessory protein 2
MRI	Magnetic resonance imaging
MUP	Methyl umbelliferyl phosphate
NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute for Health and Care Excellence
NIHR UCLH CRF	National Institute for Health Research UCLH Clinical Research Facility
NPV	Negative predictive value
NRY/AgRP	Neuropeptide Y/ agouti-related peptide
OGTT	Oral glucose tolerance test
PCOS	Polycystic ovarian syndrome
PEWL	Percentage of excess weight loss
Phe	Phenylalanine
PI3K	Phosphatidylinositol-3-kinase
PLS-DA	Project of Latent Structures
POMC/CART	Pro-opiomelanocortin/ cocaine and amphetamine-regulated transcript
PPV	positive predictive value
PRS	Polygenic risk scores

PUFA	Polyunsaturated fatty acids
PWL	Percentage weight loss
PYY	Peptide YY
PYYKO	PYY-knockout
QUICKI	Qualitative insulin-sensitivity check index
RPM	Revolutions per minute
RYGB	Roux-en-Y gastric bypass
SAT	Subcutaneous adipose tissue
SBP	Systolic BP
SG	Sleeve gastrectomy
S_HDL_C	Cholesterol in small high density lipoprotein
S_HDL_CE	Cholesteryl esters in small high density lipoprotein
S_HDL_P	Concentration of small high density lipoprotein
SM	skeletal muscle
SMPDB	Small Molecule Pathway Database
SNP	Single nucleotide polymorphism
SOS	Swedish Obese Subjects
sPLS-DA	sparse PLS-DA
T_4	Thyroxine
T2D	Type 2 diabetes
TBW	Total body water

TCA	Tricarboxylic acid
TG	Triglyceride
TGR5	Takeda G-protein receptor 5
TNF-α	Tumour necrosis factor α
TSH	Thyroid-stimulating hormone
Tyr	Tyrosine
UCLH	University College London Hospitals
UHP	University Hospital of Pisa
UMIB	Unit for Multidisciplinary Research in Biomedicine
Val	Valine
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WCV	Weight change velocity
WHR	Waist-hip ratio
WHRadjBMI	WHR adjusted for BMI
YR	Y-receptors

Chapter 1

Introduction

Chapter 1 Introduction

Obesity is a prevalent, complex, progressive and relapsing chronic disease, characterised by abnormal or excessive body fat (adiposity), that impairs health (Wharton et al., 2020). Obesity increases the risk of developing several diseases; for example, type 2 diabetes (T2D), cardiovascular disease (CVD), certain types of cancer and psychological problems, leading to impaired quality of life, morbidity and mortality (Table 1.1) (Klein and Romijn, 2016, Sharma, 2010). Since we cannot measure body fat directly, body mass index (BMI) has been used as a proxy to define overweight and obesity. BMI is determined by body weight (kg)/ (height, m)^2, see Table 1.2. BMI strongly correlates to overall mortality, mainly due to increasing incidence of CVD. A person's median survival is decreased by 2-4 years at BMI 30-35 kg/m^2 and reduced by 8-10 years at 40-45 kg/m^2 (Prospective Studies et al., 2009). Conversely, BMI reduction improves obesity associated comorbidities and increases life expectancy (Abdelaal et al., 2017).

In 2014, more than half of world population were overweight or were living with obesity. Of these, more than 600 million adults had obesity (World Health Organization (WHO), 2016). More importantly, this is driving the global increase in T2D, a serious chronic disease leading to complications in various body systems and increasing in the overall risk of premature death. Myocardial infarction, stroke, renal failure, leg amputation, visual loss and neuropathy are among the potential complications. The worldwide prevalence of T2D almost doubled from 4.7% of adults in 1980 to 8.5% in 2014, thus approximately 422 million people had T2D in 2014 with a projection of 650 million people by 2040. More worryingly T2D, previously considered a disease of older adults, is now presenting in children (WHO, 2016). Hence, obesity and T2D are currently significant global issues.

1.1 Energy homeostasis and body weight control

Energy homeostasis is defined as the balance between energy intake and energy expenditure. The hypothalamus plays a key role in maintaining energy homeostasis and integrates a panoply of signals from the periphery that reflect adipose stores and acute nutrient intake. The rewarding aspects of eating and higher cognitive function also impact upon body weight (BW) regulation. Hedonic hunger occurs when the feeding behaviour is

Table 1.1 Obesity associated comorbidities (Sharma, 2010, Guh et al., 2009, Luppino et al., 2010, Church et al., 2006)

Metabolic complications	Cardiovascular diseases - Stroke - Dyslipidaemia - Hypertension - Coronary artery disease - Heart failure - Pulmonary embolism
	Type 2 diabetes Prediabetes
	Gout
	Asthma
	Non-alcoholic fatty liver disease (NAFLD)
	Gallstones
	Thrombosis
	Infertility
	Cancers - Breast - Colorectal - Endometrial - Oesophageal - Kidney - Ovarian - Pancreatic - Prostate
Mechanical complications	Sleep apnoea
	Chronic back pain
	Arthrosis
	Incontinence
Mental complications	Depression
	Anxiety

predominantly influenced by increase in palatable food availability, sensory cues, social elements, mood, satisfaction and reward. Food choice can be affected by taste and smell (Cummings, 2015). These hedonic hunger and sensory stimuli can overwhelm physiologic satiety, and more importantly diminish the homeostatic mechanisms of controlling normal BW, leading to excessive feeding and ultimately obesity (Berthoud, 2011). Therefore, BW is determined by a complex interaction between homeostatic and hedonic

brain regions, a person's genetics and their environment (Klein and Johannes, 2016). Of these, the environment is a key driver of the obesity epidemic (Yu et al., 2015b).

In brief, the entry of nutrients into the gut brings about numerous biochemical and physiological responses. First of all, the stomach and intestine get distended following the entry of nutrients, triggering the release of bile acids, pancreatic enzymes, gut hormones, and altering enteric and vagus nerve signalling (Pucci and Batterham, 2019). Brainstem and hypothalamic arcuate nuclei are the centre for integrating these gut-derived nutrients, hormonal and neural signals as well as adipokines, proteins produced by adipose tissue such as adiponectin, leptin, tumour necrosis factor α (TNF-α), and interleukin 6 (IL-6). The interplay between first-order neurons in arcuate nuclei (anorexigenic pro-opiomelanocortin/ cocaine and amphetamine-regulated transcript [POMC/CART] and orexigenic neuropeptide Y/ agouti-related peptide [NRY/AgRP]) and second-order neurons in lateral hypothalamic area and paraventricular nucleus physiologically controls homeostatic hunger and satiety (Miras and le Roux, 2013).

Table 1.2 Classification of body mass index (BMI), data from the National Institutes of Health, National Heart, Lung and Blood Institute (1998).

Classification	**Obesity class**	**BMI (kg/m^2)**	**Risk of disease**
Normal weight		18.5 – 24.9	Normal
Overweight		25.0 – 29.9	Increased
Obesity	I	30.0 – 34.9	High
	II	35 – 39.9	Very high
Extreme obesity	III	≥ 40.0	Extremely high

Gut microbiota also impact upon the energy homeostasis via: first, mediating permeability of intestinal membrane thus influence nutrients and energy absorption; second, immunological modulation; and third regulating fat storage in adipose tissue

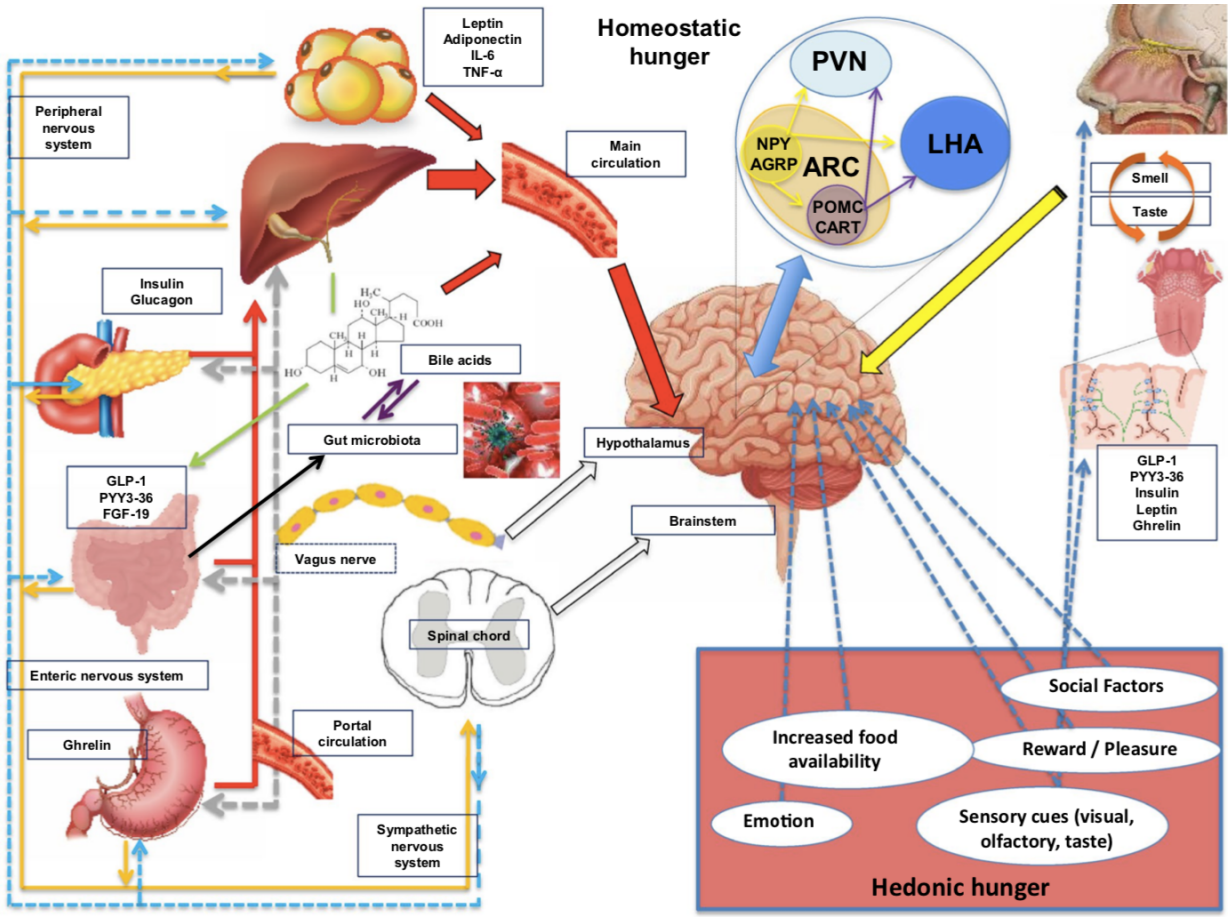


Figure 1.1 Mechanisms involved in regulating energy homeostasis and eating behaviour. AgRP agouti-related peptide, ARC arcuate nucleus, CART cocaine and amphetamine-regulated transcript, FGF-19 fibroblast growth factor-19, GLP-1 glucagon-like peptide 1, IL-6 interleukin-6, LHA lateral hypothalamic area, NPY neuropeptide Y, PNS peripheral nervous system, PVN paraventricular nucleus, PYY peptide YY, POMC pro-opiomelanocortin, SNS sympathetic nervous system. This diagram is taken from Pucci et al. J Endocrinol Invest 2019. (Pucci and Batterham, 2019)

(Martinez et al., 2016, Backhed et al., 2004). Dysbiosis of the gut microbiota, the imbalance of some certain types of bacteria, could impair host energy and nutrient metabolism, bringing about overweight and obesity (Backhed et al., 2004). Figure 1.1 illustrates the mechanisms involved in regulating energy homeostasis and eating behaviour.

1.2 Role of gut hormones and bile acids in determining BW

Multiple gastrointestinal (GI) signals, including hormones secreted by enteroendocrine cells throughout the GI tract (gut hormones), and bile acids play a key role in BW regulation and glucose metabolism.

Figure 1.2 demonstrates a number of gut hormones controlling appetite, food intake and glucose homeostasis ((Monteiro and Batterham, 2017). The scope of this chapter will focus on major gut hormones which have been studied widely and are the key in regulating energy homeostasis and BW.

1.2.1 Glucagon-like peptide-1 (GLP-1)

GLP-1 is a 30-amino-acid peptide hormone encoded by the proglucagon gene. It is expressed within the pancreas, brainstem neurons and GI enteroendocrine cells, particularly L cells of ileum and colon, but also in the stomach, duodenum and jejunum. Its effects are mediated via GLP-1 receptor. In response to the food intake, GLP-1 exhibits a biphasic pattern: early response at 15-30 minutes and late response at 60-90 minutes (Herrmann et al., 1995). It is degraded rapidly after secretion by the dipeptidyl peptidase 4 (DPP-4) enzyme, with less than 10% reaching the systemic circulation. In relation to the beneficial effects on glycaemia, GLP-1 enhances insulin secretion but inhibits glucagon secretion. It also leads to delayed GI motility (Holst, 2007), and appears to suppress appetite, resulting in weight reduction (Monteiro and Batterham, 2017).

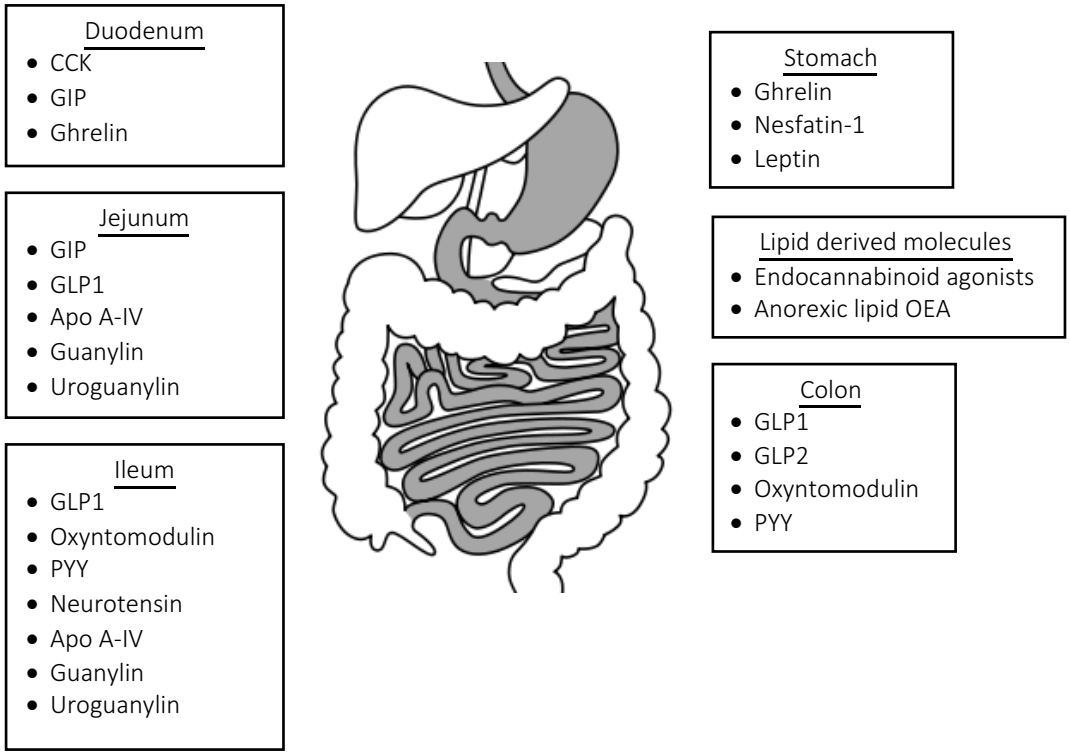


Figure 1.2 Gut hormones produced by different enteroendocrine cells along the entire GI tract play a critical role in regulating BW and glucose homeostasis. Apo A-IV, apolipoprotein A-IV; CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide-1; OEA, oleoyethanolamide; PYY, peptide YY. This figure is adapted from Monteiro et al. Gastroenterology 2017 (Monteiro and Batterham, 2017).

1.2.2 Peptide tyrosine-tyrosine (PYY)

PYY is a peptide consisting of 36 amino acids (PYY1-36). It is a member of the pancreatic polypeptide-fold family and acts via Y-receptors (YR), G-protein-coupled receptors. Similar to GLP-1, PYY is expressed within the brainstem, pancreas and L cells primarily in the ileum and colon but also proximal intestine. DPP-4 cleaves PYY1-36 into PYY3-36 which primarily mediates its effect via Y2R, leading to the reduction in food intake. PYY directly acts upon central appetite-regulating circuits causing its anorexic effect (Monteiro and Batterham, 2017). There is also evidence demonstrating that PYY contributes to blood glucose regulation (Manning and Batterham, 2014). During fasting, PYY levels are low, and they rise rapidly after nutrient ingestion to reach a plateau at 60-90 minutes and remain elevated for several hours after meal.

1.2.3 Ghrelin

Ghrelin, the predominant orexigenic hormone, is comprised of 28 amino acids and secreted by P/D1-type cells in humans, predominantly located within the stomach and duodenum. The only known receptor is the growth hormone secretagogue receptor type 1a (GHSR1a), with an essential partner receptor (the melanocortin receptor accessory protein 2 [MRAP2]) (Yin et al., 2020). Prior to binding with its receptor, a fatty acid side chain attached to the serine-3 residue of ghrelin is needed in order to produce an active form, acyl-ghrelin (AG) and this is mediated by the enzyme ghrelin O-acyl-transferase (GOAT) (Yang et al., 2008). Ghrelin acts directly on arcuate hypothalamic neurons but also modulates neural activity within brain reward regions, leading to an increased energy intake and thus BW. Desacyl-ghrelin (DAG) was previously viewed purely as a degraded form of AG. However, recent evidence has demonstrated that it acts in an opposite manner to AG and the ratio of AG to DAG is significant (Kuppens et al., 2015). The circulating levels of ghrelin rise before the initiation of a meal and subsequently decline after nutrient ingestion (Callahan et al., 2004).

1.2.4 Bile acids (BAs)

BAs are synthesised within hepatocytes by cytochrome P450-mediated oxidation of cholesterol. The metabolism of BAs is closely link to intestinal bacteria, which can modulate the molecular structure of BAs. In addition to their primary well-known role in facilitating dietary fat and oils absorption by micelle formation, the BAs also affects energy, glucose and lipid homeostasis via the farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR5) in the liver and intestines (Huang et al., 2019). By binding to the FXR, pancreatic β-cells are triggered to secrete insulin (Dufer et al., 2012), and by binding to the TGR5, GLP-1 is secreted to enhance glycaemic control (Brighton et al., 2015). Furthermore, circulating BAs increase energy expenditure in skeletal muscles by enhancing thyroid hormone action through TGR5, thus BW regulation (Watanabe et al., 2006). Following bariatric surgery, circulating BAs levels substantially increased, correlating with weight loss and improved glucose homeostasis.

1.3 The obese state and weight loss maintenance

Once obesity has developed, various pathophysiologic alterations arise such as development of insulin and leptin resistance, decreased circulating post-prandial GLP-1, PYY and BA levels, and diminished postprandial ghrelin suppression (Pucci and Batterham, 2019, Glicksman et al., 2010). Furthermore, individuals with obesity' reaction to food cues is distorted from physiological hunger and satiety. In functional magnetic resonance imaging (fMRI) studies, there was an increase in reward response to food, diminished self-control to food stimuli, and a rise in motivation to eat (Makaronidis and Batterham, 2018).

Weight-loss maintenance is a significant problematic issue for the BW reduction induced by lifestyle intervention. Throughout human evolution, weight loss would have been a life threatening, therefore, various compensatory biological mechanisms are activated by reduced energy intake and/or weight loss leading to increased hunger, preference over energy-dense food, and increased neural response to hedonic food cues and sensory stimuli. Together these changes drive weight regain (Pucci and Batterham, 2019).

1.4 Tiers of weight management services in the UK (OEN, NICE, 2014c)

Tier 1: Universal services including health promotion and primary care. Primary health care professionals including general practitioners (GPs), nurses, health visitors, pharmacists, etc. generally take responsibility at this level for prevention, identifying people with BW issues, giving advice, reinforcement of healthy diet and physical activity.

Tier 2: Lifestyle modification. The tier 2 is also usually carried out by local authorities, delivering community based healthy eating and lifestyle as well as providing advice for behavioural modification. These services are basically carried out for less than 3 months and are usually conducted in a group setting.

Tier 3: Specialist weight management services that provide non-surgical intensive medical weight management. These services are normally delivered by specialist weight management clinics with a clinician led multidisciplinary team (MDT) approach. The team

often includes a consultant or a GP with a special interest in obesity, specialist nurses, specialist dietitians, psychologists, psychiatrists, and exercise specialists/physiotherapists.

Tier 4: Bariatric surgery, together with pre-operative assessment, post-operative care and follow-up supported by an MDT in a secondary healthcare setting.

1.5 Bariatric surgery

Bariatric surgery is currently the most effective treatment for severe obesity (Angrisani et al., 2015), and it is indicated for patients with BMI ≥40 kg/m^2 or ≥35 kg/m^2 with obesity associated co-morbidities, when all appropriate non-surgical measures have been attempted (NICE, 2014b). Not only does bariatric surgery lead to significant weight reduction, but it also benefits glycaemia, particularly leading to remission of T2D (Schauer et al., 2016). Bariatric surgery has now been integrated into treatment algorithms for T2D and expedited assessment for surgery is recommended for individuals with a BMI ≥35 kg/m^2 and a recent diagnosis of diabetes (Rubino et al., 2017).

Currently, the most popular procedures worldwide are the Roux-en-Y gastric bypass (RYGB) (39.6%) and sleeve gastrectomy (SG) (45.9%), with those purely restrictive procedures (e.g. adjustable gastric banding) to a lesser extent at 7.4% (Angrisani et al., 2017), thus only SG and RYGB will be in the scope of this thesis. On average, a comprehensive systematic review and meta-analysis of 23 articles with 7,443 patients by Hu and colleagues (Hu et al., 2020) showed that percentage of excess weight loss after LRYGB and LSG at 1 year were 73.4% and 69.1%, at 3 years were 77.8% and 71.6%, and at 5 years were 69.2% and 63.1%, respectively. They also demonstrated that both LRYGB and LSG significantly improved obesity-associated comorbidities, particularly T2D, HT and dyslipidaemia, in short-term, midterm and long-term with LRYGB superior to LSG in short-term and no significant difference between procedures at midterm and long-term.

In LRYGB, the stomach is divided, making a 30-mL gastric pouch, and then the mid-jejunum is transected and anastomosed to the gastric pouch, bypassing the proximal part of the small intestine (biliary-pancreatic limb). Ingested nutrients pass straight from the gastric pouch through the bypassed limb (alimentary limb) to distal small bowel or ileum (the length of alimentary limb is approximately 100 – 120 cm). In LSG, approximately 80% of the stomach is removed, producing a narrow, tubular stomach leading to rapid gastric emptying and nutrients pass rapidly into the duodenum and proximal part of small intestine (Figure 1.3).

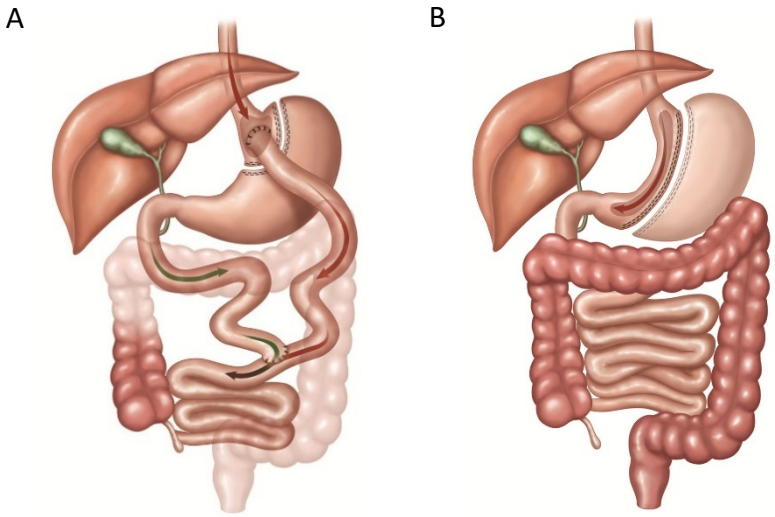


Figure 1.3 The anatomy of bariatric surgery: (A) laparoscopic Roux-en-Y gastric bypass (LRYGB); (B) laparoscopic sleeve gastrectomy (LSG). This figure is adapted from le Roux et al. BMC Med 2013 (Neff et al., 2013).

1.6 Remission/improvement of type 2 diabetes after bariatric surgery

More than 80% of individuals with T2D are either overweight or have obesity, showing a remarkably close relationship between excess adipose tissue and T2D (Lawrence et al., 2009). The prevalence of obesity and T2D cases have been consistently rising globally, leading to significant health and financial burden (Ding et al., 2015). Recent evidence has revealed that reducing 10 – 15 kg of BW in patients with short duration of T2D by either lifestyle intervention, dieting or bariatric surgery can reverse T2D to normoglycaemia in

the absence of any anti-diabetic medications (Henry et al., 1985, Sjostrom et al., 2014, Schauer et al., 2003, Sjostrom, 2013). The Diabetes Remission Clinical Trial (DiRECT) has shown that primary care-led weight management consisting of weight-loss induction and weight-loss maintenance brought about a half of participants achieving diabetes remission at 1 year (Lean et al., 2018). However, at 2 years the remission rate dropped to 36% due to weight regain (Lean et al., 2019).

T2D remission is construed as an accomplishment of non-diabetic measures of glucose metabolism without pharmacological or surgical treatment for at least 1 year, and can be divided into partial and complete remission. Partial remission is defined as sub-diabetic glycaemic levels including HbA_{1c} <48 mmol/mol and fasting plasma glucose 5.6 – 6.9 mmol/L, and complete remission is a restoration of normal glycaemia determining by fasting plasma glucose <5.6 mmol/L and HbA_{1c} in the normal range (Buse et al., 2009). Bariatric surgery is hitherto the most effective intervention in reversing diabetes. At 1 year, 69.5 – 89% of patients underwent RYGB experienced complete diabetes remission (Chikunguwo et al., 2010, Dixon et al., 2013), with low incidence of mortality (1%) , GI side effects, and malnutrition (Bult et al., 2008, Kalarchian et al., 2014).

1.7 Mechanisms of weight loss and T2D remission after bariatric surgery

The Key underpinning weight-loss success and the remission of T2D after bariatric surgery are not well understood, despite substantial efforts. It is undeniable that limited caloric intake by malabsorption and restriction from the surgery is a major mechanism, but it is not the only reason. Manning and colleagues have observed that patients exhibited less hunger, less responsive to food cues, and their food choices became more healthier (Manning et al., 2015a). These findings explain why weight reduction and weight-loss maintenance following bariatric surgery are more successful than dieting and lifestyle interventions. Furthermore, weight loss induced by bariatric surgery is mediated by the substantial changes in circulating gut hormone levels: suppression of ghrelin, a hunger hormone and increase in anorexic hormones (PYY and GLP-1) (Chandarana and Batterham, 2012).

Studying extreme responders (excess and poor weight loss) provides insight into the mechanisms of bariatric surgery. Although there has been no clear consensus of good and poor weight loss definitions (Mann et al., 2015), a percentage weight loss (PWL) of 20% is widely accepted as a cut-off point to distinguish between suboptimal weight reduction (Corcelles et al., 2016) and good weight loss after bariatric surgery (Manning et al., 2015b). For example, post-surgery excessive weight loss and anorexia were shown to be associated with excessive meal-stimulated PYY responses, which was reversed by administration of octreotide, a somatostatin analogue with an inhibitive effect on gut hormones, resulting in increased appetite and weight gain (Pucci et al., 2015). On the contrary, poor responders to bariatric surgery demonstrated attenuation in meal-stimulated PYY and GLP-1 responses following surgery compared to good responders (le Roux et al., 2007, Dirksen et al., 2013).

Two main hypotheses that have been postulated to be key mechanisms mediating weight loss and T2D remission after bariatric surgery are summarised as well as the current evidence of energy restriction, gut hormones, BA, gut microbiome, and intestinal adaptation.

1.7.1 Energy restriction

Studies show that bariatric surgery leads to an improvement in glycaemic indices within a few days post-operatively (Tsilingiris et al., 2019). This highlights that the benefits of bariatric surgery on glucose homeostasis is independent from weight loss. Jackness and colleagues studied 11 participants undergoing Roux-en-Y gastric bypass and 14 participants mean-matched for diabetes duration, HbA_{1c}, and BMI whose energy intake was restricted to 500 kcal/day. After 21 days a frequently sampled intravenous glucose tolerance test was conducted. These results of which found that the improvement in insulin sensitivity, acute phase insulin secretion, and β-cell function were similar between groups, suggesting that energy restriction could primarily contribute to acute glycaemic benefits following the surgery (Jackness et al., 2013). In addition, Lingvay et al. demonstrated no significant differences in the concentrations of fasting plasma glucose, maximal plasma glucose and AUC of plasma glucose during mixed meal challenge tests

between iso-caloric restriction period before and after RYGB in 10 subjects with obesity and T2D which they served as their own controls (Lingvay et al., 2013).

As the weight loss becomes more apparent, it appears that the weight loss plays a key role in T2D remission. A study in subjects with obesity and T2D by Yoshino and colleagues has recently compared matched (~18%) weight loss induced by RYGB and energy restriction. They found that there were no significant differences in β-cell function, insulin-stimulated glucose disposal, and suppression of glucose production during a 3-stage hyperinsulinaemic euglycaemic pancreatic clamp between groups. Furthermore, no significant differences were observed in AUC for 24-hour plasma glucose and insulin levels. Additionally, any other potential clinical mechanisms were not identified. They therefore concluded that glycaemic advantages of RYGB were related to weight loss (Yoshino et al., 2020).

Until now, there has been no evidence strongly suggesting that the glycaemic benefits result from weight loss-independent effects of bariatric surgery (Chondronikola et al., 2016). All in all, the energy restriction and weight loss are most likely play the major role in mediating T2D remission/improvement. Nevertheless, not all patients with obesity and T2D who exhibit marked weight loss experience T2D remission. This finding suggests that other factors such as baseline β-cell function, genetics, gut hormones, etc. also play a role.

1.7.2 The hindgut hypothesis

The key concept of the hindgut hypothesis is that the increase in incomplete digested nutrients and/ or BA delivered to distal GI tract enhances the release of gut peptides such as GLP-1, glucose-dependent insulinotropic peptide (GIP) and PYY from enteroendocrine cells (L-cells), predominantly in the ileum. This occurs as a result of the alteration of gut anatomy, rapid gastric emptying, and/ or shortening the GI tract. Increased circulating levels of gut hormones are thought to lead to weight loss and improved glycaemic control (Karra et al., 2010).

This hypothesis was proven by ileal transposition (IT) studies, by which the intact vascular and neural supplied ileal segment was moved to the proximal intestine. IT resulted in the augmentation of post-prandial levels of PYY and GLP-1, which is related to the decrease

in food intake, body weight, and the improvement in insulin sensitivity and glucose homeostasis (Koopmans et al., 1984, Strader et al., 2005, Patriti et al., 2005, Wang et al., 2008).

1.7.3 The foregut exclusion hypothesis

The foregut exclusion hypothesis proposes that an anti-incretin factor is secreted from the proximal gut in response to nutrient contact. This unidentified factor is suggested to be increased in people with T2D. Thus bypassing the duodenum and proximal jejunum would reduce circulating anti-incretin levels leading to improvement in glycaemic control (Rubino and Marescaux, 2004, Rubino et al., 2006). The proof supporting this hypothesis came from a series of experiments in rats undergoing duodenal-jejunal bypass (DJB) and studies of endoluminal duodenal-jejunal sleeve insertion, which shown to enhance glucose homeostasis and reduce food intake and BW (Rubino and Marescaux, 2004, Rubino et al., 2005). The key common concept of these two procedures is diverting food directly from the stomach to the proximal jejunum.

However, this hypothesis has been opposed by alternative explanations that these foregut exclusion procedures actually accelerate the contact between incomplete digested nutrients and the hindgut L-cells, which eventually stimulate the release of GLP-1 and PYY. Furthermore, the loss of feedback from duodenal osmoreceptors to the pylorus by these interventions could be another reason. Generally, the entry of high concentrated nutrients into the duodenum triggers the osmoreceptors, which in turn caused pyloric contraction, and results in delayed gastric emptying. The bypass of the duodenum and/or the disruption of the pylorus therefore lead to rapid gastric emptying, increased gut peristalsis, and swift delivery of the incomplete digested nutrients to the hindgut (Mason, 2005).

1.7.4 GLP-1 and PYY

With regard to the hindgut hypothesis, the expedited nutrient delivery to the hindgut L-cells by bariatric surgery leads to exaggerated PYY and GLP-1 release. A number of studies have demonstrated that the postprandial concentrations of GLP-1 and PYY substantially rose after RYGB and SG (Karra et al., 2010, Yousseif et al., 2014). In light of the incretin

effects (oral glucose produces larger insulin secretion than iso-glycaemic intravenous glucose) of GLP-1 and its anti-apoptotic effects on β-cell (Lee and Jun, 2014), it has been suggested that GLP-1 is a key mediating glycaemic benefits after bariatric surgery. Furthermore, Dutia and colleagues have shown that in patients who experience T2D remission after RYGB, the improvement in glycaemic control occurred at only 1 month after surgery. However, the normalised β-cell function was only observed from an oral glucose tolerance test (OGTT) with a minimal improvement from an iso-glycaemic intravenous glucose clamp up to 3 years (Dutia et al., 2014). This indicates that GI factors derived from RYGB are critical for enhanced β-cell function after the surgery. Nonetheless, this notion is questioned by studies in GLP-1 receptor knock-out mice and studies in mice with functional deletion of GLP-1, showing that neither GLP-1 nor its receptor is essential to improve glycaemic control (Mokadem et al., 2014). The role of GLP-1 as a principal mechanism of glycaemic benefits following bariatric surgery hence remains inconclusive.

Chandarana and colleagues have shed light on how PYY benefits on glycaemia (Chandarana et al., 2013). They have found that PYY3-36 enhances nutrient-stimulated insulin secretion by promoting local effects of GLP-1. A study in an animal model by Ramracheya et al. also showed that the normalisation of glycaemia in diabetes mice undergoing RYGB was resulted from the restoration of insulin and glucagon secretion in diabetic islets by PYY. In vitro, this effect persisted in the presence of a GLP-1 receptor antagonist but reversed by neutralisation of the PYY (Ramracheya et al., 2016).

Furthermore, they were able to replicate the benefits of chronic PYY exposure on diabetic rat islets' secretory function in vitro. Thus, Guida et al. have proposed that the PYY's beneficial effects on glycaemic control and its function in the pancreas are underrated (Guida et al., 2017). They suggested that the glycaemic benefits of PYY are perhaps caused by local effect of PYY1-36 that is secreted from α-cells and acts upon NPY1R on β-cell surface. Additionally, PYY could regulate glucagon release from α-cells, although the underlying mechanisms remain unknown. In addition, since the DPP-4 cleaves PYY1-36 into PYY3-36, DPP-4 inhibitors can be used to uncover the PYY1-36's benefits on glycaemia. Chronic stimulation of GLP-1 receptor knockout mice's islets with sitagliptin led to improvement in glucose-stimulated insulin secretion (GSIS) and by blockade of

NY1R, it diminished the enhanced insulin secretion induced by the DPP-4 inhibitor (Guida et al., 2017).

1.7.5 Ghrelin

The evidence of the beneficial effects of ghrelin on glucose homeostasis is rather heterogeneous. Previous studies showed that ghrelin can increase the secretion of insulin counter-regulatory hormones, inhibit adiponectin (an insulin-enhancing hormone), suppress phosphatidylinositol-3-kinase (PI3K) (a step in hepatic insulin signalling cascade) which leads to increased hepatic glucose production, and diminished insulin secretion (Thaler and Cummings, 2009). In vivo and in vitro evidence have demonstrated that AG inhibited insulin secretion (Benso et al., 2012, Gasco et al., 2010) via; first, an association with a rise in circulating free fatty acid (FFA) levels (Huda et al., 2009) which diminished insulin sensitivity; second, enlarged fat laden adipocytes, and indirect immuno-modulatory response which ultimately led to insulin resistance (Churm et al., 2017). Overall, most studies have pointed the way that ghrelin, in particular AG has an insulinostatic property.

Recently Yin and colleagues have shown that the action of ghrelin upon β-cells took place indirectly through δ-cell stimulation, since the GHSR1a is mainly expressed in δ-cell. They have identified the melanocortin receptor accessory protein 2 (MRAP2), a single transmembrane protein, as a necessary partner of ghrelin receptors (GHSR1a). The MRAP2 strongly potentiates ghrelin-stimulated signalling from the GHSR1a on δ-cells, which subsequently release somatostatins to bind somatostatin receptors on β-cells, and therefore inhibit insulin secretion (Yin et al., 2020).

Nonetheless, some evidence demonstrated that the rise in plasma AG and DAG was associated with diabetes remission after RYGB, and that ghrelin gene products namely AG, DAG, and obestatin maintained intracellular calcium homeostasis, leading to β-cells protection (Yang et al., 2014). Yang et al. highlighted that AG has a cytoprotective property for regulating cell proliferation and survival. They demonstrated that ghrelin gene products protect β-cells from apoptosis that was induced by a high-glucose condition. These ghrelin gene products, in particular AG, inhibit intracellular Ca^{2+} influx

which leads to cell injury and apoptosis (Yang et al., 2014). This beneficial effect was allegedly mediated via unknown receptors other than the GHSR1a. Hence, Dezaki, Yin and their colleagues described that the AG's attenuated glucose-induced insulin release and promoting hepatic glucose production are to determine physiological secretion of insulin and to prevent hypoglycaemia during fasting (Dezaki et al., 2008, Yin et al., 2020).

1.7.6 Bile acids (BAs) and fibroblast growth factor 19 (FGF-19)

As mentioned above, BAs have a positive impact on glucose control by enhancing insulin secretion through FXR and stimulating GLP-1 secretion through TGR5. In addition, BAs interact with FXR in enterocytes in the terminal ileum to release fibroblast growth factor 19 (FGF-19), which in turn inhibits the BA synthesis enzyme (cholesterol 7 alpha-hydroxylase) through FGF receptor 4 on hepatocyte surface as a negative feedback (Inagaki et al., 2005). The levels of FGF-19 surge at 90 – 120 minutes following the post-prandial rise of BAs (Lundasen et al., 2006). Emerging evidence reveals that FGF-19 benefits insulin sensitivity and glycaemic control by reduced hepatic glucose production, increased glucose uptake by skeletal muscles and adipose tissue, decreased food intake, and increased energy expenditure (Batterham and Cummings, 2016). Individuals with T2D had lower levels of circulating FGF-19 than people with normal glycaemia, and this finding was not dependent on BMI (Batterham and Cummings, 2016).

1.7.7 Hepatic, pancreatic and visceral adipose tissue

The state of chronic energy excess leading to raised hepatic, pancreatic, and visceral fat contents has been postulated to be a part of the pathogenesis of T2D (Shibata et al., 2007, Steven et al., 2016, Taylor, 2013, McGarry, 2002). An increased intracellular diacylglycerol (DAG) in the liver stimulates the action of a protein kinase C isoform PKCepsilon (an inhibitor of the insulin signalling pathway), thus developing hepatic insulin resistance and increased hepatic glucose production (Samuel et al., 2010, Samuel et al., 2004). Moreover, the chronic exposure of β-cells to excess fatty acids or triglyceride (TG) diminishes the β-cell function (McGarry, 2002, Unger, 1995), and brings about the loss of complete β-cell differentiation (Bensellam et al., 2018, Brereton et al., 2014), hence triggering T2D.

In the Diabetes Remission Clinical Trial (DiRECT), Taylor and colleagues have discovered that the hepatic and pancreatic fat contents were significantly reduced in a weight-loss dependent manner and was equally observed in both remitters and non-remitters. However, the improvement in β-cell function was only observed in the remitters (Taylor et al., 2018). This finding suggests that the remission of human T2D requires a reduction in hepatic and pancreatic fat but is essentially dependent on the intrinsic capacity for β-cell recovery.

In addition, it has been widely accepted that visceral adipose tissue (VAT) contributes to cardiometabolic diseases, in particular T2D. The mechanisms underlying this include: first, a hyperlytic property of VAT releases excessive amount of free fatty acids (FFAs) and glycerol to liver via the portal vein, leading to decreased hepatic extraction of insulin (aggravating hyperinsulinemia), increased hepatic gluconeogenesis and increased TG-rich lipoprotein production; second, a release of inflammatory cytokines and a reduction in adiponectin (an anti-inflammatory, anti-atherogenic, and anti-diabetic protein) production; third, the accompanied ectopic fat deposition (liver, pancreas, heart, and skeletal muscles) impairs the respective organ function (Neeland et al., 2018, Neeland et al., 2019).

1.7.8 Gut microbiota

The diversity of gut microbiome has been reported to be associated with BW and T2D (Karlsson et al., 2013, Ley et al., 2006, Turnbaugh et al., 2009). The alterations in composition and the ratio of certain kinds of bacteria from an obese bacterial profile (high ratio of Firmicutes to Bacteroidetes) to a leaner one possibly resulted from a combination of various factors including RYGB, SG, bile diversion, energy restriction, gut motility and intraluminal pH (Batterham and Cummings, 2016, Chondronikola et al., 2016).

Several studies have supported the relationship of gut microbiota with weight loss and metabolic benefits: first, faecal transplantation from mice undergoing RYGB to germ-free mice led to weight reduction, whilst the transplantation from weight-matched sham-operated mice to the same recipients resulted in weight gain (Liou et al., 2013); second, faecal transplantation from patients undergoing RYGB and SG caused decreased fat mass

in recipient rodents (Tremaroli et al., 2015); third, to examine the interaction of BAs-FXR-microbiome axis, Ryan et al. studied in global FXR knockout mice following vertical SG. They found that there was a decrease in changes in gut microbiome profile, circulating BAs, weight loss, and glycaemic advantages in the global FXR knockout mice post vertical SG (Ryan et al., 2014).

1.7.9 Intestinal glucose metabolism

In rodents, RYGB induces hyperplasia, hypertrophy of intestinal mucosa in the Roux (alimentary) limb and increases expression of glucose transporters, leading to increased glucose uptake, retention and metabolism (Saeidi et al., 2013). However, a study in human could not replicate this finding. Magkos et al., demonstrated that following RYGB, the magnitude of intestinal glucose uptake and retention was small and did not significantly lower postprandial glucose levels (Magkos et al., 2016). A study using ([18]F) fluoro-2-deoxyglucose positron emission tomography-computed tomography found increased jejunal glucose uptake at 6 months after RYGB (Makinen et al., 2015). Nevertheless, there was no comparison with a matched diet-induced weight loss group, thus whether or not the result caused by the surgery or weight loss is still uncertain. Regarding SG, the evidence is scarce with only in rodents showing increased number of GLP-1 containing cells and reduced intestinal glucose absorption (Cavin et al., 2016).

1.7.10 Browning of white adipose tissue (WAT)

It is generally known that brown adipose tissue (BAT) plays an important role in regulation of body temperature, BW, and glucose homeostasis. In the activated state, BAT could uptake glucose up to 12 times that seen in the basal state (Orava et al., 2011, Lavender et al., 1989). A number of studies have revealed that weight loss induced by bariatric surgery resulted in an increased activity of BAT (Kurylowicz and Puzianowska-Kuznicka, 2020, Hankir and Seyfried, 2020). Also, a rise in circulating plasma BAs and GLP-1 levels following bariatric surgery could lead to browning of WAT (Chondronikola et al., 2016).

Interestingly, a recent evidence showed that the effects of bariatric surgery on BAT could be dependent on the type of surgery in humans and rodents: vertical sleeve gastrectomy enhanced classical BAT activity, whilst RYGB primarily induced browning adipose tissue

(Hankir and Seyfried, 2020). Nevertheless, to date, few studies of bariatric surgery-induced WAT browning and BAT activation have been conducted in humans, and the significant differences in the mechanisms regulating thermogenesis and its role in the body's energy expenditure in humans and rodents should be considered. It might occur that in humans, the increased thermogenesis is insufficient to reduce body weight since compensatory mechanisms of the weight loss and the fact that the increase in BAT activity and beige adipose tissue recruitment in humans are considerably weaker than in rodents (Lutz and Bueter, 2016).

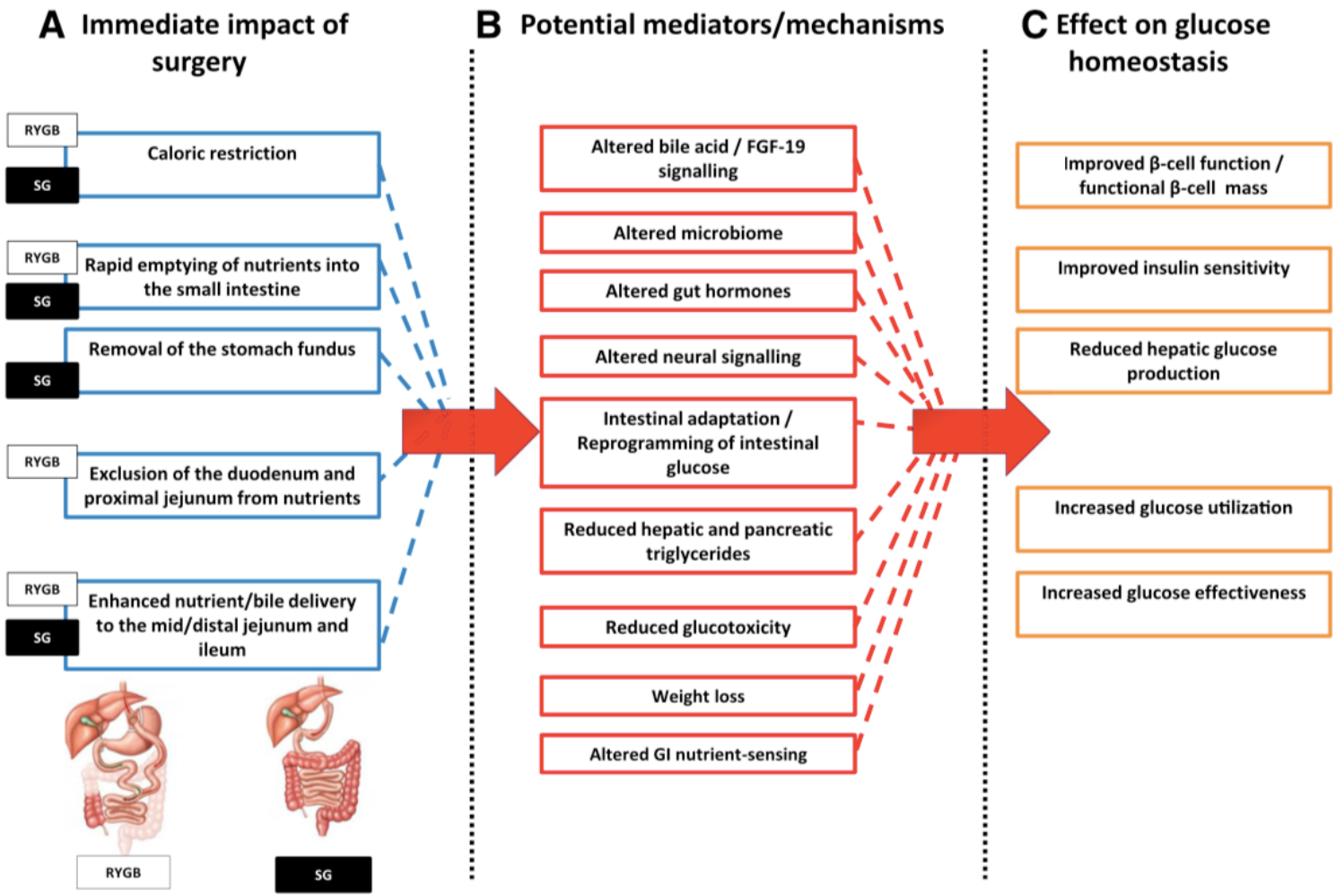


Figure 1.4 Schematic of potential mechanisms contributing to improved glycaemia after LRYGB and LSG. A: immediate effects of RYGB and VSG due to anatomical changes. B: Potential mediators/ mechanisms involved. Cross talk among these factors. C: Effects on glucose homeostasis. This figure is taken from Batterham and Cummings, Diabetes Care 2016 (Batterham and Cummings, 2016).

1.8 Factors associated with weight loss and diabetes remission after bariatric surgery

There is a huge variability in weight loss after surgery, which follows a wide and normal distribution (Figure 1.5) (Manning et al., 2015b). Patients who experienced suboptimal weight loss reported lower quality of life and lower physical activity levels compared to those with good weight loss outcomes after bariatric surgery (Amundsen et al., 2017).

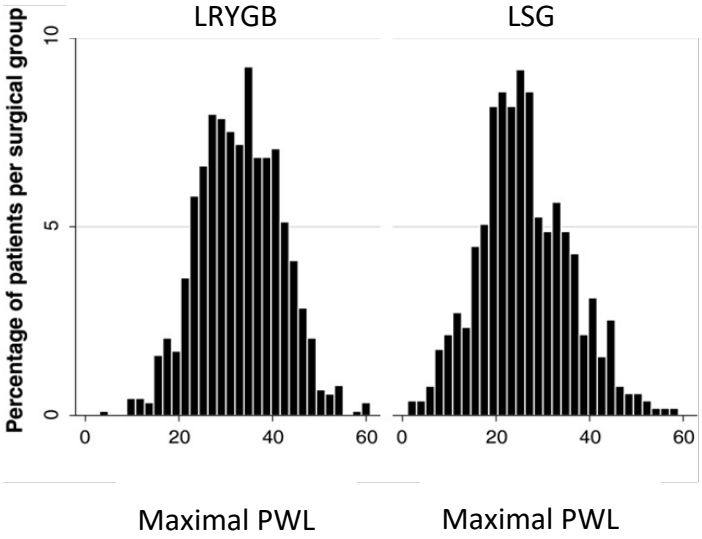


Figure 1.5 Histogram of maximal percentage weight loss (PWL) for patients undergone laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LSG). This figure is adapted from Manning et al. Surgical Endoscopy 2015 (Manning et al., 2015b).

Individuals with poor weight loss also had fewer health benefits, such as the improvement in T2D, hypertension, dyslipidaemia, CVD and OSA compared to those who achieved greater weight loss since these beneficial effects are weight-loss dependent (Laurino Neto et al., 2012).

Of note, bariatric surgery is not without associated risk, albeit very small. The 60-day mortality was approximately less than 1% (Morino et al., 2007). Complications common to any GI surgery may occur during bariatric surgery; such as wound infections,

atelectasis, post-operative infection, deep vein thrombosis, anastomotic leakage and bleeding. Bariatric surgery also carries a long-term risk of nutritional deficiencies hence requiring a life-long follow-up. In addition, dumping syndrome and postprandial hypoglycaemia have been described following bariatric surgery (Klein and Romijn, 2016). Furthermore, access to bariatric surgery is very limited. Only less than 1% of more than two million eligible patients underwent surgery in the UK in 2014 (Ahmad et al., 2014).

Hence, in order to maximise the beneficial effects and to reduce the incidence of unsuccessful weight loss in patients undergoing bariatric surgery, identifying predictors of weight loss response to bariatric surgery would be of value. By identifying predictors of weight loss responses, bariatric surgery can individualise according to individual genomic profile, biological characteristics and health status, that is in line with precision medicine.

1.8.1 Clinical factors

Many clinical factors have been reported to be associated with poor weight loss following bariatric surgery such as higher baseline BMI, female gender, age > 45–50 years, lower early post-operative weight loss velocity, and T2D (Still et al., 2014b, Contreras et al., 2013, Ma et al., 2006, Ochner et al., 2013, Ortega et al., 2012, Scozzari et al., 2012, Manning et al., 2015b). Interestingly, Nielsen and colleagues have shown that 59% of 18-month weight loss variability after RYGB and SG was correlated with pre-operative factors including type of surgery (14%), T2D status (12%), economic resources (9%), sex (7%), binge eating disorder (7%), degree of depression (5%), household type (3%), and physical activity (1%). Moreover, by adding 6-month responses to the pre-operative factors, the power to predict the 18-month weight loss variation rose to 78%. The 6-month responses include early weight loss (47%), changes in energy density of food consumed from a buffet meal (9%), changes in glicentin (5%), degree of depression (5%), gender (5%), type of surgery (2%), economic resources (2%), and changes in drive for thinness (1%) (Nielsen et al., 2020).

In terms of T2D remission, several scores have been developed to predict the remission; for example, DiaRem score (Still et al., 2014a), DiaBetter score (Pucci et al., 2018), ABCD

score (Lee et al., 2013) and Individualized Metabolic Surgery (IMS) score (Aminian et al., 2017).

1.8.2 Gut hormones

Roux et al. demonstrated that the nutrient-stimulated PYY and GLP-1 levels were attenuated in poor weight loss compared to good weight loss individuals at 2 years following LRYGB (le Roux et al., 2007). Dirksen et al. also showed a larger release of GLP-1 and a greater suppression of ghrelin in good weight loss than poor weight loss people whereas PYY did not differ between groups at more than 12 months after LRYGB (Dirksen et al., 2013). Morinigo and colleagues demonstrated that 6-week $AUC_{0\text{-}120}$ of PYY was associated with percentage excess weight loss at 33 months after RYGB (Morinigo et al., 2008), and Faraj et al. showed that 15±6-month PWL after RYGB can be predicted by pre-surgery adiponectin concentrations (Faraj et al., 2003). Nevertheless, Werling et al., concluded that pre-operative gut hormones were not associated with weight loss following RYGB (Werling et al., 2014). Study examining the association between gut hormones and PWL after LSG are currently scarce.

1.8.3 Genetics

Given the strongest contribution on BW of single nucleotide polymorphisms (SNPs) linked to the fat mass and obesity- associated (FTO) gene, there is a great interest in its correlation with weight loss. There is an evidence in 9,563 individuals with obesity showing that individuals carrying the minor allele of the FTO (rs9939609) lost weight by dietary, exercise and medication uniformly well in comparison to those without the minor allele (Livingstone et al., 2016).

The effect of genetic variants in the FTO on weight loss after bariatric surgery is hitherto controversial. Balasar and colleagues have demonstrated that there was no association between the rs9939609 FTO polymorphism and weight loss after LSG (Balasar et al., 2016). In Swedish obese subjects (SOS) intervention study, only the FTO SNP rs16945088 showed a significant correlation with weight loss after adjustable gastric banding, but did not find any association between other SNPs of the FTO and weight loss after gastric bypass (Sarzynski et al., 2011). Kops and colleagues also did not find the association of the

FTO (rs9939609) with weight loss (Kops et al., 2018). In contrast, the association of weight loss and FTO (rs9939609) was observed in several studies; for example, at 3-month weight loss after biliopancreatic diversion surgery (de Luis et al., 2012), at 6-month weight loss after laparoscopic mini-gastric bypass (Liou et al., 2011), and at 2-year weight loss after RYGB (Bandstein et al., 2015).

Two previous groups have examined the genetic influence on weight loss after bariatric surgery using a genome-wide association study (GWAS) approach. GWAS studies of excess body weight loss (EBWL) (Rinella et al., 2013) and PWL (Hatoum et al., 2013) after gastric bypass surgery have identified variants at two loci that associate with EBWL or PWL but not at a GWAS significant threshold level.

Bandstein and colleagues have revealed that genetic risk scores (GRSs) consisting of BMI-associated single nucleotide polymorphisms (SNPs) (*MC4R, TMEM160, PTBP2, NUDT3, TFAP2B, ZNF608, MAP2K5, GNPDA2,* and *MTCH2*) and waist-hip ratio (WHR) associated variants (*HOXC13, LYPLAL1*, and *DNM3-PIGC*) significantly correlated with 2-year weight loss after RYGB (Bandstein et al., 2016). De Toro-Martin et al. showed that 2 polygenic risk scores (PRSs) of 186 and 11 SNPs associated with BMI has a significant impact on 4-year weight reduction following biliopancreatic diversion with duodenal switch (BPD-DS) (de Toro-Martin et al., 2018). Additionally, Katsareli et al. demonstrated that a GRSs of BMI- and WHR-associated variants designated a 4.6% reduction of 12-month percentage of excess weight loss (PEWL), calculated using the following formula: (postoperative weight loss)/ (preoperative excess weight) × 100, per score unit, and a 3% decrease of 24-month PEWL per score unit (Katsareli et al., 2020).

1.9 Research question

As described above, there are a number of factors associated with weight-loss and glycaemic benefits following bariatric surgery. Of these, the relationship of PYY and ghrelin with the weight-loss success and glycaemic benefits is still limited and sometimes controversial. Recent evidence has revealed that the beneficial effects of PYY on weight loss and glycaemic improvement may be underrated (Guida et al., 2017). The post-operative changes of ghrelin circulating levels after LRYGB is inconsistent, which the levels rose in some studies (Holdstock et al., 2003, Sundbom et al., 2007) or did not change (le Roux et al., 2006, Korner et al., 2005, Karamanakos et al., 2008, Leonetti et al., 2003) or decreased (Cummings et al., 2002). Ghrelin's advantages on glycaemia is also currently debatable. Previous studies reported ghrelin's adverse effects on glucose control (Alamri et al., 2016, Dezaki et al., 2006, Tong et al., 2014, Tong et al., 2010); however, some evidence found that the hormone protected pancreatic β-cells from apoptosis (Dezaki et al., 2008) and negatively correlated with insulin resistance (Ikezaki et al., 2002, Poykko et al., 2003).

In addition, it is generally acknowledged that genetic factors play a key role in BW determination. Studies undertaken in twins and close relatives demonstrate that genetic factors explain a significant portion of the variation in weight loss after gastric bypass surgery (e.g. the intra-class correlation coefficient is estimated to be up to 70%) (Rinella et al., 2013). Some genetic variants and genetic risk scores have also been proposed to be able to predict weight loss after bariatric surgery as mentioned earlier in this chapter.

The mechanisms underlying T2D remission after bariatric surgery is still not well understood. The Diabetes UK has prioritised research in the biology of β-cell recovery, the role of gut hormones on remission and novel biomarkers which are predictive for response to remission treatments (Hopkins et al., 2020), since these could lead to additional insights on T2D remission and novel interventions.

Hence, the research questions of this thesis include:

1. Are circulating PYY and/or ghrelin associated with and predictive for weight loss and glycaemic benefits after LRYGB and LSG?

2. Which factors are associated with the remission of T2D after LRYGB and LSG?
3. Which genetic variants are associated with weight loss following LRYGB and LSG?

1.10 Hypotheses

The hypotheses are:

1) A person's pre-operative and/or early post-operative circulating levels of PYY, and ghrelin determine their weight loss and improvement in glycaemia after LRYGB and LSG. (Study 1)
2) In individuals with T2D, a person's gut hormones, hepatic, pancreatic and visceral fat, body composition, insulin sensitivity and β-cell function determine their remission of T2D following LRYGB and LSG. (Study 2)
3) A person's genetics determines their weight loss after LRYGB and LSG. (Study 3)

The pre-operative factors will help to identify patients who will have benefits outweighing risks from bariatric surgery, and the early post-operative factors will help to identify those who will need additional support after bariatric surgery to optimise their weight loss.

In order to test these hypotheses three studies were undertaken.

Study 1: "The role of PYY and ghrelin in predicting weight loss after LRGB and LSG"

In this study, we aimed to examine whether or not the pre-operative circulating PYY, and ghrelin levels are associated with weight loss after bariatric surgery. We also evaluated whether changes in the gut hormones at 6 weeks post-surgery determine the weight loss after LRYGB and LSG. The association of clinical parameters at pre-surgery and at early post-surgery with weight loss after LRYGB and LSG was also examined.

Furthermore, we compared the pre-operative levels and post-operative longitudinal patterns of PYY and ghrelin in:

- Patients undergoing LRYGB versus LSG
- Patients with good weight loss versus poor weight loss
- Patients with T2D versus without T2D
- T2D remitters versus non-remitters

This study will be fully described in **Chapter 3**.

Study 2: "Factors associated with type 2 diabetes remission after LRYGB and LSG"

The objective of this study is to identify factors associated with type 2 diabetes remission. We studied two groups of T2D subjects undergoing either LRYGB or LSG categorised by the remission of T2D status. Since the weight reduction is a key mediator of T2D remission, a group of T2D remitters was compared with another matched percentage weight loss (20%) group of non-remitters. Gut hormones, hepatic, pancreatic and visceral fat, insulin sensitivity, β-cell function and metabolomics were compared between groups. Details of this study will be presented in **Chapter 4**.

Study 3: "The role of genetics in predicting weight loss after LRGB and LSG"

Further, we conducted the genome-wide association study (GWAS) and constructed genetic risk scores (GRSs) in order to identify polymorphisms and/ or clusters of polymorphisms (GRSs) that are associated with the weight loss after LRGB and LSG. The findings of this study are presented in **Chapter 5**

Chapter 2

Materials and Methods

Chapter 2 Materials and Methods

2.1 Subjects

Subjects for all of the studies included in this thesis were recruited under the same ethical approval by the National Health Service Research Ethics Committee (ID#09/H0715/65) with inclusion and exclusion criteria described in each study. Oral and written informed consents were given by all participants.

Patients with obesity were referred from their general practitioners (GPs) to the University College London Hospitals (UCLH) Bariatric Centre for Weight Management and Metabolic Surgery in order to obtain the tier 3 and/or tier 4 weight management services delivered by a multidisciplinary team (MDT). The MDT at UCLH comprised of consultant obesity physicians, consultant bariatric surgeons, bariatric anaesthetists, psychologists, clinical nurse specialists, bariatric dieticians, exercise therapists and pathway co-ordinators, providing comprehensive assessments pre- and post-operatively, education about options of bariatric surgery, evaluation and management of co-morbidities, psychological support, short- and long-term follow-up care.

First of all, patients were invited to attend an educational session delivered by bariatric dietitians and clinical nurse specialists that aim to provide information about medical weight management options and different bariatric procedures, the risk, benefits and the need for long term follow-up. Next, the patients attended one-stop clinic where their BW, medical and psychological history were assessed by a clinical nurse specialist, a bariatric dietician and a bariatric surgeon. All patients were then reviewed, and their treatment plan discussed by the MDT. They were then assigned to either receive an intensive lifestyle management in a tier 3 service or proceed to bariatric surgery (tier 4 service for weight management).

According to the National Institute for Health and Care Excellence (NICE) guideline (NICE, 2014a), those who met the following eligibility criteria would be shortlisted for bariatric surgery. The criteria include:

i. BMI ≥40 or ≥35 kg/m^2 with obesity associated co-morbidities e.g. T2D or hypertension
ii. All non-surgical interventions have been attempted but failed to attain and sustain weight loss
iii. The patient satisfactorily attended the intensive lifestyle modification in tier 3 weight management scheme
iv. The patient is overall fit for the surgery and anaesthesia
v. The patient is aware and accept the long-term follow-up

Patients who were approved by the MDT for surgery attended the pre-assessment clinic for anaesthetic assessment and scheduled for either laparoscopic Roux-en-Y gastric bypass (LRYGB) or laparoscopic sleeve gastrectomy (LSG).

2.1.1 Subjects' recruitment

An electronic bariatric database of patients at the UCLH Bariatric Centre for Weight Management and Metabolic Surgery has been generated for research purposes. All potential subjects were recruited from the UCLH bariatric outpatient clinic. Patients with hepatitis B, C and human immunodeficiency virus (HIV) and incapacity to consent were excluded. Potential subjects were approached, and the details of the bariatric database was explained by a research investigator and a copy of participant information sheet given (see Appendix 1). They were allowed to consider whether or not to participate in the study and were encouraged to ask any questions to the research team before signing the consent form (Appendix 2). Eligible subjects for subsidiary studies were chosen from the database according to the selection criteria of each study.

2.1.1.1 ***Study 1*** *"The role of PYY and ghrelin in predicting weight loss after LRGB and LSG"*

Subjects who consented for the bariatric database were approached at the pre-assessment clinic by a study investigator. They were given an information sheet and verbal explanation detailed specifically for a mixed meal tolerance test (MMTT) (Appendix 3), inviting them to take part in this study. The second informed consent was sought (Appendix 2). Subjects who consented to this subsidiary study were then scheduled to attend the MMTT within a 2-week period pre-surgery and at 6 weeks, 24 weeks and 52 weeks post-operatively.

2.1.1.2 ***Study 2*** *"Factors associated with type 2 diabetes (T2D) remission after LRYGB and LSG"*

The electronic bariatric database was reviewed. Patients who had T2D at surgery and were greater than 1 year post-surgery at the time of study were identified. They were approached by a research investigator by phone, asking about T2D history, for example, their T2D status, medications intake and their current diabetes management if any. MRI safety check list was carried out verbally. The details of the study was explained to subjects and those who agreed to take part in the study were scheduled for an appointment. The appointment letter, participant information sheet for the MMTT (Appendix 3), patient information sheet for the MRI scan (Appendix 4) and MRI safety check list (Appendix 5) were mailed to the patients by post. Patients were encouraged to contact the research investigator by email and phone, should they required more information or wanted to ask any questions. On the test day, the second informed consent form was signed by subjects (Appendix 2).

2.1.1.3 ***Study 3*** *"The role of genetics in predicting weight loss after LRGB and LSG"*

Patients aged 18-65 years old, having BMI ≥40 or ≥35 kg/m^2 with obesity associated co-morbidities and scheduled to undergo primary bariatric surgery either LRYGB or LSG have been recruited since April 2005 from five bariatric centres in three European countries as follows:

1. the University College London Hospitals (UCLH) Bariatric Centre for Weight Management and Metabolic Surgery, London, England
2. the University Hospital of Pisa (UHP), Pisa, Italy
3. the Western Sussex Hospitals NHS Foundation Trust, West Sussex, England
4. the University of Oporto, Porto, Portugal
5. the Imperial College London, London, England

Patients were approached by the respective research team and given a copy of the participant information sheet at the one-stop clinic. They were asked to read the information sheet and to enquire a researcher in case they had any questions. The consent form was then singed by subjects voluntarily. Either peripheral blood or saliva samples were collected from subjects for subsequent DNA extraction and genetic analysis.

Demographic, anthropometric and detailed clinical data were also electronically collected for each participant. Longitudinal BW and clinical data were recorded after the surgery.

Figure 2.1 summarised subject recruitment in all studies.

2.1.2 Sample size calculation

Since there is a well-established bariatric cohort at the Centre for Obesity Research, UCL with a longitudinal data of patients with severe obesity at pre-surgery, 6-week, 6-month and 1-year post-surgery, in the Study 1 detailed in Chapter 3, all samples of the cohort were used. In the Study 2 detailed in Chapter 4 all eligible patients with T2D at pre-surgery from the bariatric cohort were also recruited. Lastly, in the Study 3 detailed in the Chapter 5 all samples were already recruited from 5 bariatric centres in 3 countries, and thus we analysed all available samples.

The sample size calculation was not performed and this could be one of limitations of all studies described in this thesis. However, all results can be used as a preliminary report and can be used for sample size calculation for further relevant projects.

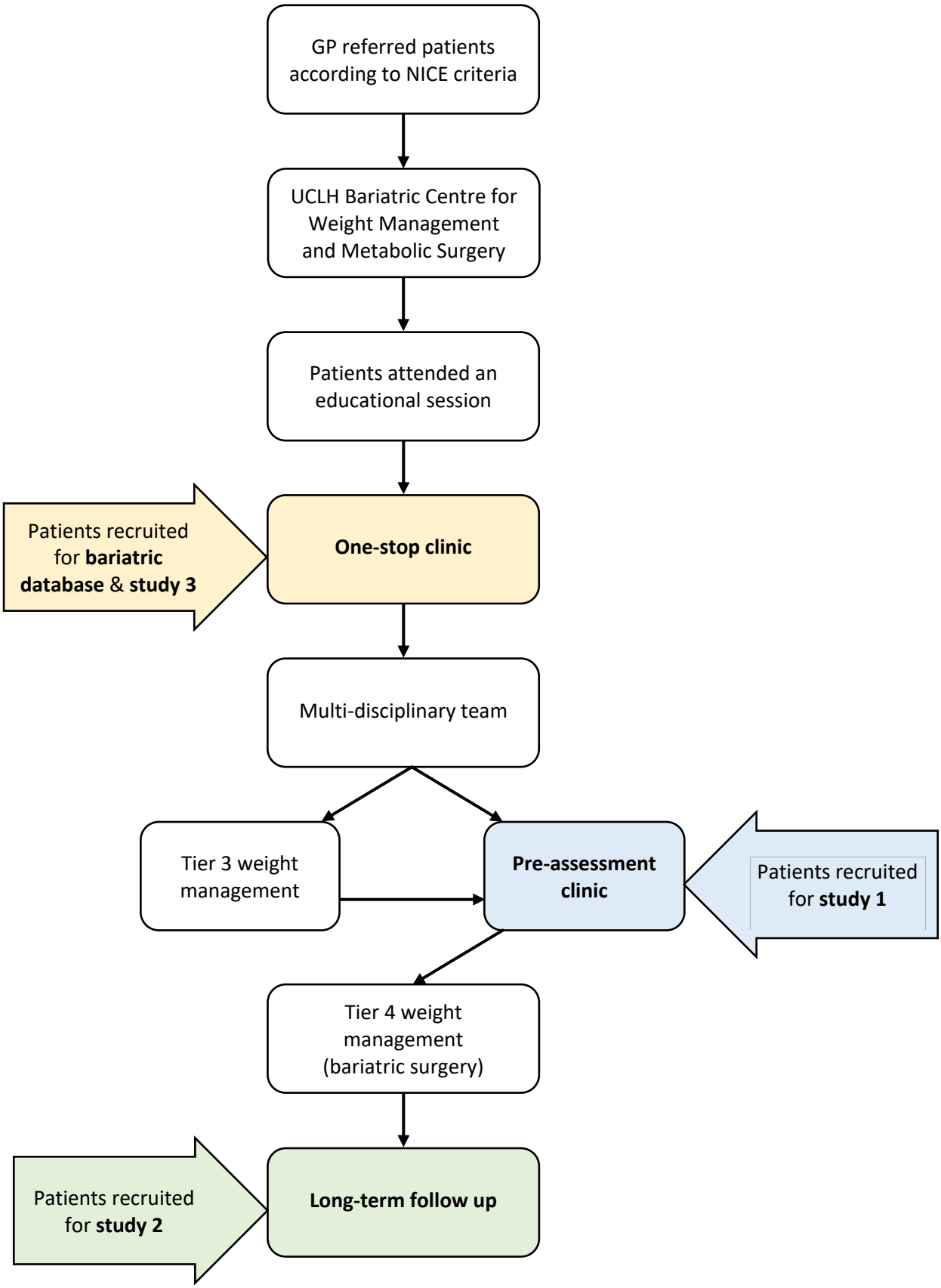


Figure 2.1 Subjects recruitment at the UCLH Bariatric Centre for Weight Management and Metabolic Surgery; NICE, National Institute for Health and Care Excellence; UCLH, University College London Hospitals

2.2 Surgical procedures

At the UCLH Bariatric Centre for Weight Management and Metabolic Surgery, both RYGB and SG were performed laparoscopically with uniform surgical procedures.

In LRYGB, the stomach is divided, making a 30-mL gastric pouch, and then the mid-jejunum is transected and anastomosed to the gastric pouch, bypassing the proximal part of the small intestine (bilio-pancreatic limb). Ingested nutrients pass straight from the gastric pouch through the bypassed limb (alimentary limb) to distal small bowel or ileum (the length of alimentary limb is approximately 100 – 120 cm).

In LSG, approximately 80% of the stomach is removed, producing a narrow, tubular stomach leading to rapid gastric emptying and nutrients pass rapidly into the duodenum and proximal part of small intestine according to Gagner's description (Gagner et al., 2009).

2.3 Anthropometric measurement and clinical data

Patients were weighed in light indoor clothing without shoes and heavy accessories, using a calibrated weighing scale (Seca 877, Seca, UK). Height was determined by a wall-mounted stadiometer (242 Measuring Rod, Seca, UK). BMI was calculated by BW (kg) divided by the square of height in metres. Percentage weight loss (PWL) was used as the outcome of interest as it is less influenced by the baseline BMI than percentage excess weight loss (PEWL). It was calculated by using the following formula: PWL = ([baseline BW – BW at each visit]/ baseline BW) x 100.

Patients' demographic, clinical and biochemical data; for example, age, gender, co-morbidities, fasting plasma glucose, Haemoglobin A1c (HbA_{1c}), thyroid, liver and kidney function, full blood count and lipid and bone profiles were collected from UCLH electronic medical record (Epic).

2.4 Blood pressure and pulse rate measurement

Vital signs including pulse rate, systolic and diastolic blood pressure were measured at one arm during every study visit for a MMTT by using an electronic sphygmomanometer (CARESCAPE™ V100, GE Healthcare, Buckinghamshire, UK). All participants were in a comfortable sitting position for at least 15-minute before the measurement.

2.5 Bioelectrical impedance analysis (BIA)

BIA (Tanita DC-430 MA S, Manchester, UK) is a device used to measure body composition: body fat percentage, fat mass (FM), fat-free mass (FFM), muscle mass (MM) and total body water (TBW), by measuring electrical impedance in human body. It has 4 electrodes on a platform and requires subjects to step on them (2 electrodes under each foot for the toes and the heel) (Figure 2.2). The Tanita DC-430 MA S adopts advanced dual BIA technology which utilises 2 different frequencies of electric current to analyse body

composition. The company claimed that this technique provides higher accuracy for the measurement of body composition.

Based on the fact that electricity barely passes through fat tissue but easily passes through water, which is abundant in muscles, the degree of difference in which the electric current penetrates through a substance called electrical resistance. The machine releases a safe, low and constant electrical current (90 μA) with 2 different frequencies at 6.25 and 50 kHz from the electrodes on the tips of the toes of both feet through the body and the different voltage (electrical resistance) is recorded on the heels of both feet. The BIA calculates the body composition by inputting the electrical resistance of each tissue into scientifically validated Tanita equations.

Compared to dual-energy X-ray absorptiometry (DXA) which is currently a standard reference for body composition measurement, the BIA is more simple and less expensive. Faria et al has demonstrated a significant correlation of FM and FFM between multi-frequency BIA and DXA (intraclass correlation coefficient = 0.832 and 0.899, respectively) in people with BMI greater than 34 kg/m^2 (Faria et al., 2014).

In addition to the FM, FFM and TBW, Tanita DC-430 MA S is able to estimate visceral adipose tissue (VAT) which is known to associate with high blood pressure (BP), metabolic syndrome and T2D (Lopes et al., 2016). The company showed a great correlation between VAT area by Tanita's BIA and MRI (R^2 = 0.71, p <0.0001 in males and R^2 = 0.78, p <0.0001 in females) (Tanita, 2013). The device represents the VAT in rating from 1 to 59. Rating from 1 to 12 indicates a healthy level of visceral fat, whereas the rating from 13 to 59 represents excess level of visceral fat according to the instruction manual.

The machine can also estimate basal metabolic rate (BMR) which is the energy spent by the body at rest for the normal physiological function of organs such as respiratory, circulatory organs, etc. Generally, BMR is calculated by the Harris-Benedict equation, using individual age, weight and height. The company has used body composition to calculate the BMR rather than relying on BW since the BMR is a greatly affected by skeletal muscles composition. This has been validated with indirect calorimetry (R^2 = 0.808, p < 0.0001) (Tanita).

Exercise, alcohol, dehydration and meal could affect BIA measurement. In order to get an accurate measurement, participants were instructed to refrain from strenuous exercise and alcohol for at least 12 hours to avoid dehydration. All measurements were performed after 12-hour fasting with participants wearing indoor light clothing. They were then asked to step on the 4 electrodes with bared feet and arms straight down during measurement. Participants with pacemaker or other mechanical implants were excluded since these devices could be interfered by the electric current generated by the BIA.

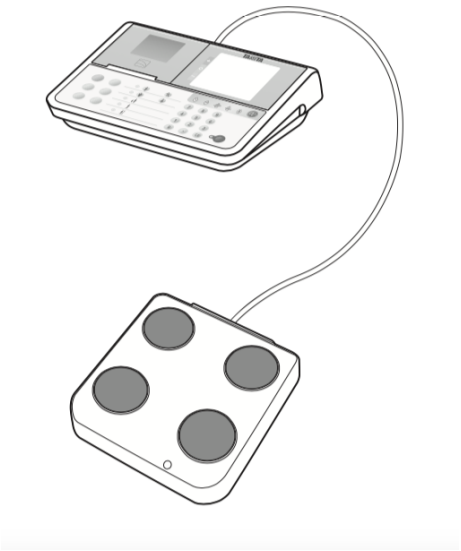


Figure 2.2 Bioelectrical impedance analysis (BIA)

2.6 DNA extraction

Either peripheral blood or saliva samples were collected from subjects for subsequent DNA extraction. Five millilitres of peripheral blood were taken from an antecubital vein by a phlebotomist under aseptic techniques, using a 21 Gauge needle (Kendall, Tyco Healthcare UK, Hampshire, UK). The blood was preserved in an ethylenediamine tetraacetic acid (EDTA) containing BD vacutainer® (Becton Dickinson, Plymouth, UK). Saliva sample was collected in Oragene®•DNA|OG-500 (DNA Genotek, Ontario, Canada) by asking subjects to spit into the funnel of the kits according to the manufacturer's instructions (Figure 2.3). All samples were then stored in a –20℃ freezer immediately for subsequent genomic DNA extraction, using the QIAamp DNA Blood Midi Kit (Qiagen, Manchester, United Kingdom) as per the manufacturer's protocol.

In general, there are 3 main steps of DNA extraction.

1. Cells lysis
2. Separation of DNA from other cell components
3. Isolation and purification of DNA

Table 2.1 shows contents in the QIAamp DNA Blood Midi Kit for DNA extraction. First of all, 100 µl of protease, Subtilisin, was added into the bottom of a 15ml tube. The protease was used for degrading DNA associated proteins and other cellular debris. One millilitre of sample was added into the tube, and then 1.2 ml of buffer AL, guanidine hydrochloride/ maleic acid was also added for cell lysis. The tube was inverted for 15 times and vortexed for 15 seconds. Following the mixing step, the lysate was incubated in a warm water tub at 70℃ for 10 minutes in order to denature some proteins such as nuclease. Since DNA is not soluble in alcohol, 1 ml of absolute ethanol was subsequently added into the lysate for DNA precipitation and preparing the DNA to be able to bind with the QIAamp column. Next, the mixture was applied into the QIAamp mini spin column, which employed silica-based bead extraction method to capture the DNA precipitates. The tube was centrifuged for 3 minutes at 4500 revolutions per minute (RPM). This step was for facilitating the QIAamp silica membrane to capture the DNA precipitates.

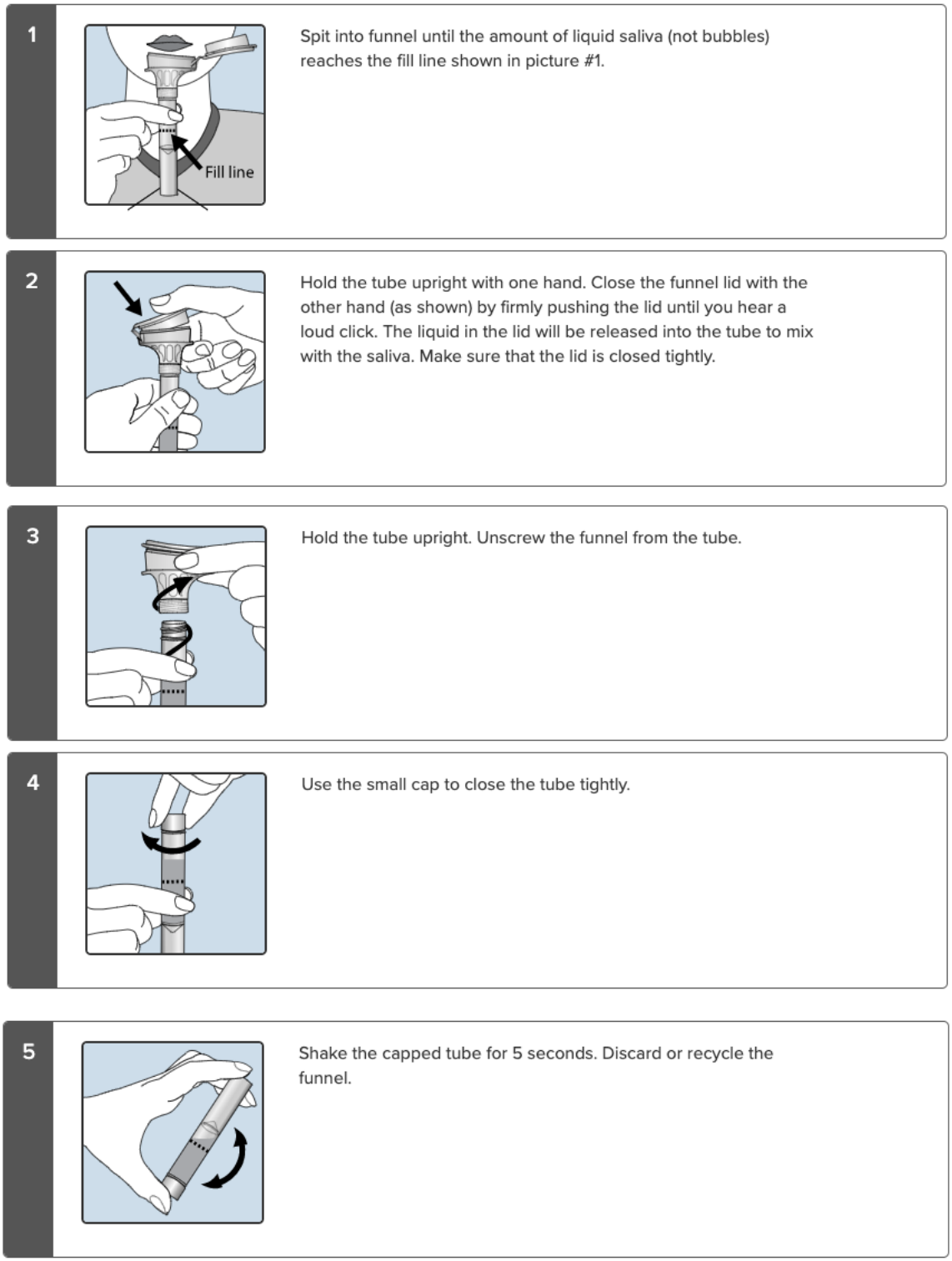


Figure 2.3 Salivary sample collection (DNAgenotek, 2020)

Table 2.1 Contents in a QIAamp DNA Blood Midi Kit for DNA extraction

QIAamp DNA Blood Midi Kit **Catalogue number** **Number of preps**	 51185 100
QIAamp Midi spin columns	100
Collection tubes (15 ml)	100
Buffer AL	265 ml
Buffer AW1 (concentrate)	95 ml
Buffer AW2 (concentrate)	66 ml
Buffer AE	60 ml
QIAGEN® protease	4 vials
Handbook	1

After finishing the centrifuge, the mini spin column was placed in a clean 15ml collection tube and the tube containing the filtrate was discarded. Two millilitres of wash buffer AW1 (guanidine HCl solution) was added to the column, followed by centrifuge for 3 minutes at 4500 RPM. The mini spin column was transferred in a clean collection tube and the tube containing the filtrate was disposed of. Two millilitres of another wash buffer AW2 (guanidine HCl solution) was then added into the column, followed by 15 minutes of centrifuge at 4500 RPM. These wash buffers (AW1 and AW2) enhanced the purification of eluted DNA by removing residual contaminants. The mini spin column was transferred to a new collection tube and 200 µl of elution buffer AE was then added, followed by 5 minutes of centrifuge at 4500 RPM. In order for enhancing the yield of DNA extraction, the previous step was repeated. Finally, the eluted DNA was collected in a 1.5ml Eppendorf tubes.

2.6.1 DNA quantitation and determination of purity

Spectrophotometric analysis by a spectrophotometer, Nanodrop8000 (Thermo Scientific, Epsom, UK), was used to determine the average concentration of DNA present in the eluate, as well as their purity. The principle of the analysis is that DNA absorbs ultraviolet light specifically at the wavelength of 260 nanometres (nm), whereas contaminated protein absorbs the wavelength of 280 nm.

One to two µl of the eluate were placed onto the pedestal. After the arms closed, the sample column was formed. The ultraviolet light passed through the column to strike the photodetector. The amount of light absorbed was then calculated using the Beer-Lambert law for the amount of DNA in ng/µl.

To assess the purity of DNA, the ratio of absorbance of ultraviolet at 260 and 280 nm was utilised. The ratio of 1.7 -2.0 indicates the purity, so samples with outside this range were disposed of and the DNA extraction was repeated. All DNA samples were stored in a -20 ℃ freezer.

2.6.2 Preparation of DNA samples for genotyping

All DNA samples in 1.5ml Eppendorf tubes were thawed at room temperature, and they were then vortexed and quickly centrifuged. The concentration of each DNA sample required for the microarray was approximately 100 ng/µl for 11 µl in 96-well microplates (ThermoFisher Scientific, Epsom, UK). Thus, the amount of DNA needed to add into the plate was calculated from: (100/ DNA concentration in ng/µl) x 11. Nuclease free water was subsequently added into a well to reach the total volume of 11 µl. The plates were sent to the Oxford Genomics Centre (Headington, UK) for genotyping.

2.6.3 Genotyping

Genotyping was performed using Illumina HumanCoreExome-24 BeadChip genotyping arrays (Illumina Inc., San Diego, CA) at the Wellcome Trust Sanger Institute (Hinxton, UK) and the Oxford Genomics Centre (Headington, UK).

2.7 Mixed-meal tolerance test (MMTT)

2.7.1 Schedule for a MMTT

A MMTT was conducted in study 1 and 2. In study 1, participants underwent the test for 4 time-points: at pre-surgery, 6-week, 6-month and 1-year after bariatric surgery. At the first visit (pre-surgery), all participants were on liver shrinkage diet which is a low-calorie diet shown in Table 2.2 whereas at post-operative visits, a regular well-balanced diet was resumed. Participants in the study 2 underwent the MMTT only once throughout the study which was on the study day when they consumed a regular diet.

2.7.2 Standard protocol for mixed-meal tolerance test

The MMTT allows us to understand the physiological changes by a meal such as gut hormones, appetite, etc. All participants were asked to refrain alcohol for at least 24 hours and to continue their habitual physical activity and diet. On the day prior to the study, participants were asked to fast and to drink only water for a total of 12 hours.

On the study day, the participants were asked to arrive at the National Institute for Health Research UCLH Clinical Research Facility (the NIHR UCLH CRF) at approximately 08:00 – 09:00 am. In total, the study took around 3 hours. After the informed consent was given both verbally and in writing by participants, a 20-gauge plastic cannula (BD Nexiva™ closed iv cannula system with BD Vialon™ biomaterial, dual port, BD, Oxford, UK) was inserted into an antecubital vein in order to obtain blood samples during the study.

Forty-five minutes of acclimatisation was allowed prior to assessing participants' gut hormone profile and appetite, since the stress from cannulation could suppress participants' hunger and affect the levels of some gut hormones (i.e. PYY) (Chandarana et al., 2009). during this acclimatisation period, vital signs (GE Carescape V100, GE healthcare, Buckinghamshire, UK), BW and height were measured. Next, baseline blood was taken.

Table 2.2 Pre-operative liver shrinkage diet followed by all patients for 14 days before bariatric surgery (copied from the UCLH dietetic patient leaflet/hand-out).

Per day you should take **only**:

- 4 cans Weight Watchers soup
- 4 Mullerlight yogurts (200g)
  or:
  4 supermarket own brand e.g. "Be Good to Yourself" (200g)
- 1 pint semi-skimmed milk
- Multi-vitamin table e.g. 1 a day of Sanatogen A-Z, Forceval.

You may divide this up during the day however suits you. You may take as much water, black tea and coffee as you wish. Likewise, diet cola, diet lemonade or diet squashes are permitted. Avoid other drinks such as fruit juice, or other sugar-containing drinks.

Milk in tea and coffee must come out of the above allowance.

In warm weather please make sure that you drink **a minimum** of 4 pints of fluid per day in total.

At time 0 minute (t = 0), participants consumed the test meal (Resource 2.0 Fibre, Nestle Nutrition, Croydon, UK) within 15 minutes. The test meal consisted of 18% of protein (22.5g), 40% carbohydrate (50g) and 39% fat (21.8g). Blood samples were taken repeatedly at 15, 30, 60, 90, 120, 150 and 180 minutes (Figure 2.4).

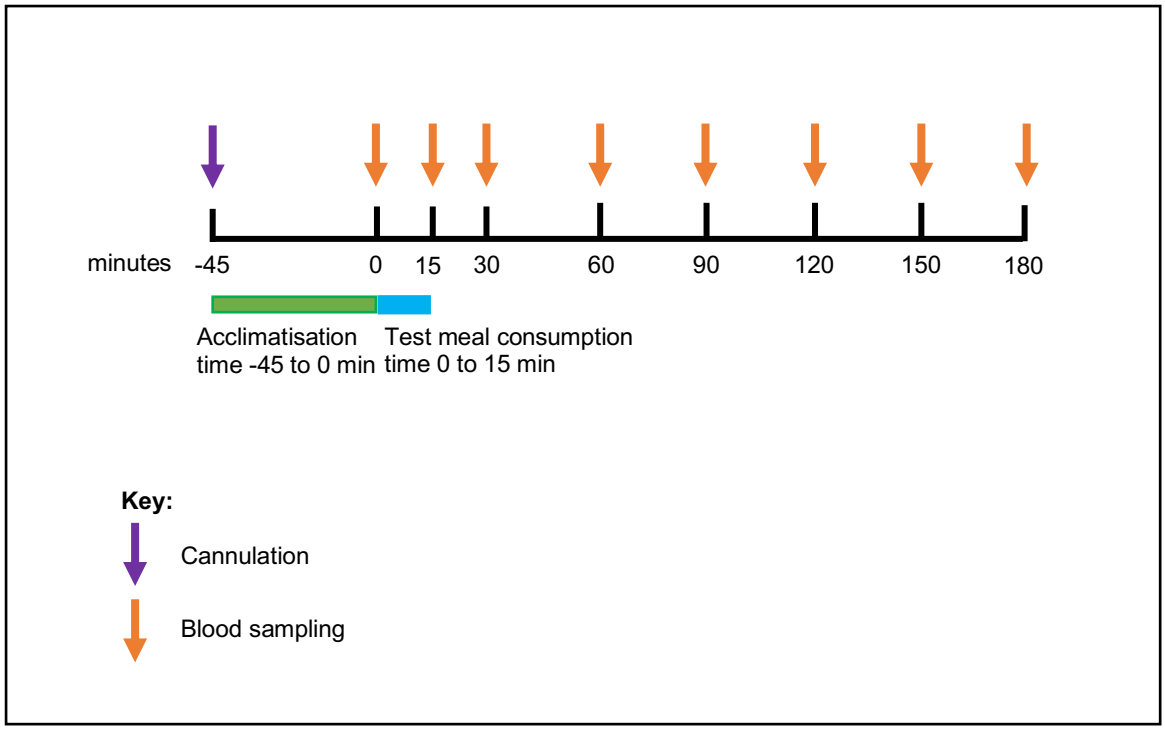


Figure 2.4 Diagram illustrating mixed-meal tolerance test protocol, participants were asked to refrain from alcohol and to maintain regular physical activity and diet for 24 hours. They were also asked to fast and to drink only water for 12 hours. On the test day, after obtaining informed consent, cannulation was performed with subsequent 45-minute acclimatisation. At time 0 minute, the test meal was consumed within 15 minutes and baseline blood sampling was collected. Blood sampling were done at time 15, 13, 60, 90, 120, 150 and 180 minutes.

An alternative design for the MMTT is to relate the energy content of the meal to energy expenditure; for example, 30% of patient's total energy expenditure for a meal. This method is tailor-made for each individual which is more physiological than a fixed-calorie meal. Nonetheless, it requires manpower to operate and is rather time-consuming for the calculation and preparing the meal. Hence, we opted to use the fixed-calorie meal since it is also generally used and widely accepted (Shankar et al., 2016).

2.7.3 Sample collection and processing

The majority of commercial gut hormone kits require the addition of 5000 units of aprotinin, a kallikrein inhibitor, (Trasylol, Bayer, UK) to 1 mL of blood sample in order to inhibit some proteases; for example, chymotrypsin, trypsin, kallikrein and plasmin.

In terms of PYY, there are two forms of circulating PYY; PYY1-36 and PYY3-36. DPP-4 is the enzyme responsible for the cleavage of the first 2 amino acids, producing PYY3-36 from PYY1-36. Likewise, active GLP-1 is rapidly degraded by the DPP-4 enzyme. Thus, to prevent ex-vivo conversion of the PYY and GLP-1, blood samples were collected into syringes containing DPP-4 inhibitor (10-µL DPP-4 inhibitor [Millipore, Watford, UK] per mL of blood).

In terms of ghrelin, acidification is necessary for the measurement. The active form of ghrelin, AG, is inactivated by endogenous esterase, generating DAG; consequently, an esterase inhibitor, 10µL per mL of blood of 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF) (Fluka, UK), are required for the accurate measurement.

Six mL of blood were drawn into a plain 10-mL syringe (BD, Oxford, UK), and 5 mL of blood were taken into another 10-mL syringe. Two ml of blood were collected into a 3-mL syringe (BD, Oxford, UK) containing 20 µL of DPP-4 inhibitor. Each 2 mL of blood in the first syringe were transferred to 3 chilled ethylene diamine tetra-acetic acid (EDTA) tubes containing 100 µL of aprotinin. To one of these, 20µL of AEBSF was added. All 5 mL of blood from the second syringe were transferred to an EDTA tube without aprotinin for further metabolomics study. The 2 mL of blood from the 3-mL syringe containing DPP-4 inhibitor were transferred to an EDTA tube with 100 µL of aprotinin for the measurement of GLP-1 and PYY.

Blood tubes were stored on ice and immediately centrifuged at 1800 rpm for 10 minutes at 4℃. Plasma samples were then aliquoted in 1-mL plastic tubes. The plasma samples for AG and DAG were acidified by addition of 50 µL of 1M hydrochloric acid (HCl) per mL of plasma. All samples were subsequently stored at -80℃ until assayed. **Figure 2.5** summarised the sample collection and processing.

2.8 Gut hormone assays

All gut hormones studied in this thesis, namely AG, DAG, PYY, GLP-1, insulin and FGF-19 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits.

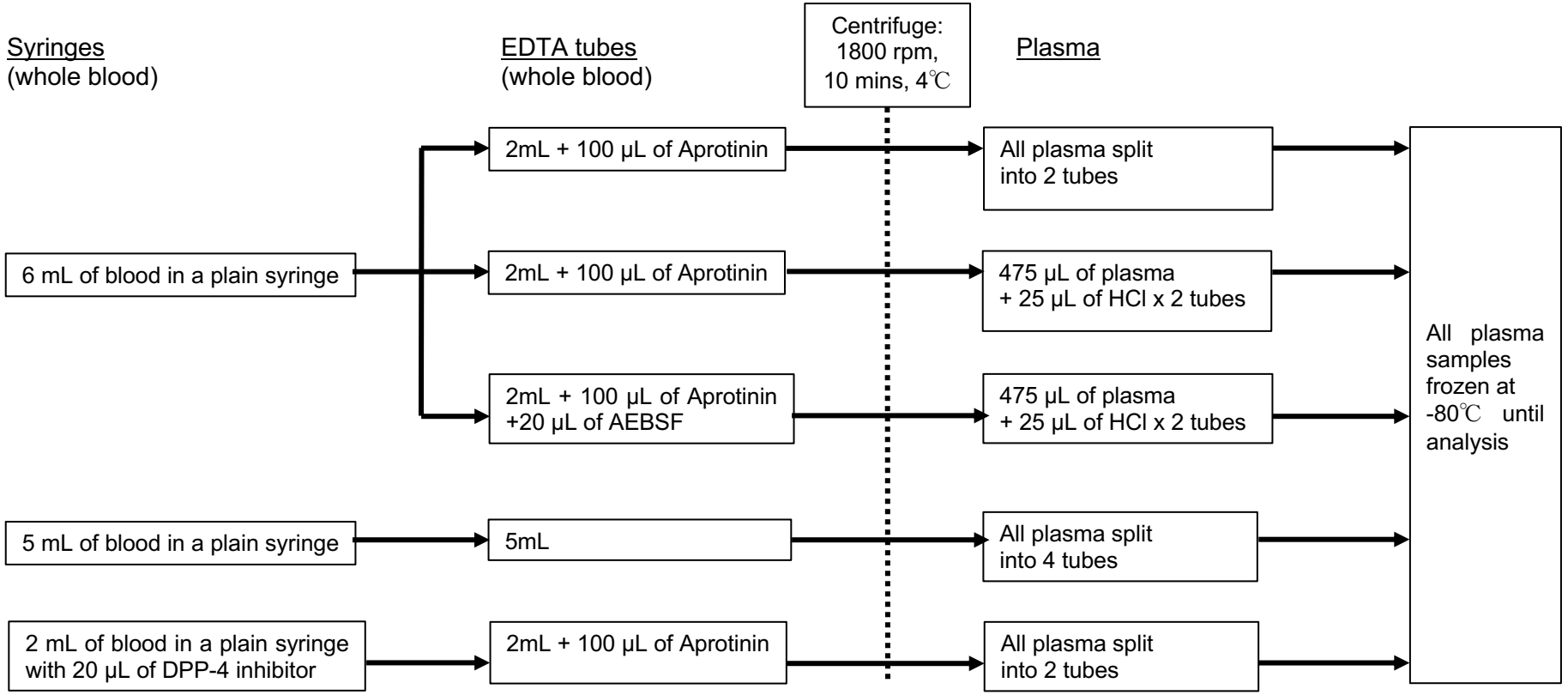


Figure 2.5 Scheme of samples collection and processing; EDTA, Ethylene diamine tetraacetic acid; DPP-4, dipeptidyl-peptidase 4; AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride; HCl, 1 N hydrochloric acid, adapted from Chandarana et al. Gastroenterology 2009

2.8.1 General principle of enzyme-linked immunosorbent assay (ELISA)

ELISA is an assay widely used for detection and quantification of an analyte of interest; for example, proteins, peptides, hormones and antibodies. The key component of the assay is a plate (usually 12 strips of 8 wells, thus 96 wells in total) which can bind antibodies and antigens. Some plates designed to capture analytes directly whilst some have a specific antibody coated on the plates to capture an analyte. The analyte is subsequently detected by either primary detecting antibody which is called “direct assay” or secondary detecting antibody which is called “indirect assay”. The detecting antibody is conjugated with an enzyme (usually alkaline phosphatase [AP] or horseradish peroxidase [HRP]). A substrate is then added to the plate and altered into a measurable product by the enzyme; as a result, the detection is completed by assessing the end product spectrophotometrically, fluorometrically or luminometrically.

Currently, sandwich technique of ELISA has been the most popular and powerful in detecting analytes due to its high sensitivity and robustness. The sandwich format is basically composed of a capture antibody coated on the plate; thus, the analyte is bound between the capture and detecting antibodies. Most commercial ELISA kits have adopted this technique.

The kits used in this thesis were ordered in batched. We tried to use the same lot number of kits for the assay of each hormone in order to limit variability.

2.8.2 The general ELISA method

Before setting up the assay, all reagents were warmed at room temperature and diluted or prepared as per manufacturer’s instruction. First of all, the plate for PYY, GLP-1 and insulin was washed by 300 µL of an assay-specific wash buffer for 3 times, using a multi-channel pipette (the assays for FGF-19, AG and DAG skipped this step). Following each wash, the buffer was discarded, and the plate was smartly tapped on paper towels in order to remove remnant completely. A suggested amount of assay buffer was then poured into each well using a repeater pipette (Eppendorf, Stevenage, UK). In assays for PYY and insulin, a matrix solution (treated human serum for insulin and serum matrix containing DPP4 inhibitor for PYY) was added into blanks, standards and controls wells.

Next, standards with known concentration, controls with known range of concentration and unknown samples were pipetted into each well following a predetermined plate plan. Assay buffer was added to the blank wells at the same amount as standards, controls and samples. The plate was covered with a transparent plastic film and then incubated on an orbital microtitre plate shaker set to 400 – 500 rpm for approximately 2-4 hours (up to 24 hours for GLP-1 assay). For PYY assay, a blocking solution (proprietary reagents to block false positive signals in assay sample) was added into each well before a 30-minute incubation, followed by the addition of 1:1 mixture of capture and detection antibodies and a 1.5-hour incubation. In insulin assay, the detection antibody was added before a 1-hour incubation.

Following the incubation, the seal was removed, and the solutions were decanted and discarded. The plate was then washed 3 – 5 times according to each specific assay manufacturer's instruction to eliminate unbound material. In AG, DAG, FGF-19 and GLP-1 assays, a recommended volume of specific detection antibody to each hormone was added into each well using the repeater pipette. In insulin and PYY assays, a standard volume of enzyme solution was added to the wells. The plate was then sealed with a plastic film and incubated on the plate shaker set to 400 – 500 rpm for 1-2 hours.

After the incubation, the plastic seal was removed and the contents in the wells were decanted and discarded. The plate was then washed 3 – 6 times. A standard amount of substrate solution was subsequently poured into the wells. The plate was sealed with a plastic film and covered with a piece of foil to protect light-sensitive substrates from the light. After 15-20 minutes, the reaction was terminated by the addition of a recommended volume of acidic stop solution. The absorbance of each well which represents substrate concentration was measured at 450 nm immediately, using a spectrophotometry (SpectraMax M2, Molecular Devices, Wokingham, UK) and the SoftMax Pro Software (Molecular Devices, Wokingham, UK).

2.8.3 AG and DAG ELISA

The kits for measuring AG and DAG are also based on the principle of 2-site sandwich ELISA. Both types of kits manufactured using high specific antibody pairs and the use of

horseradish peroxidase (HRP) as an enzyme conjugated to the detection antibody. The enzyme activity was measured spectrophotometrically by absorbency at 450 nm. They can detect not only human ghrelin but also rat/mouse ghrelin. The kits were purchased from SCETI K.K., Tokyo, Japan. The lot number of AG kits was R955 and the expiry date was June 2020. The lot number of DAG kits was T951 and the expiry date was May 2020.

Components

1. Standard (lyophilized)	1 vial
2. Assay buffer	22 mL
3. Antibody coated plate	96 wells
4. HRP conjugated antibody	250 µL
5. HRP dilution buffer	22 mL
6. Substrate solution	22 mL
7. Stop reagent (0.5 mol/L H_2SO_4)	6 mL
8. Washing buffer concentrate	40 mL

2.8.4 PYY ELISA

This assay is a sandwich ELISA. The microtiter plate is pre-coated by a pre-titered amount of anti-rabbit IgG antibodies. Human PYY molecules (both 1-36 and 3-36) in the samples are bound with rabbit anti-human PYY IgG, and the complexes are then captured to the plate by the anti-rabbit IgG antibodies. Next, a second biotinylated antibody to the PYY is added. After wash, HRP was conjugated to the immobilised biotinylated antibodies. Quantification of immobilised antibody-enzyme conjugates is completed by monitoring HRP activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm.

The sensitivity of the assay is 6.5 pg/mL with the specificity of 100% for 1-36 and 3-36 PYY. The intra- and inter-assay variations are 0.9 – 5.78% and 3.7 – 16.5% respectively. The catalogue number was EZHPYYT66K, lot number was 3248677 and the expiry date was 31 January 2020, and they were purchased from Millipore, Watford, UK.

Components

1. Microtiter plate coated with pretitered antibodies 96 wells
2. 10x Concentrate of 50mM tris buffered saline containing tween-20 100mL
3. Human PYY 3-36 standard (lyophilised) 1 vial
4. Human PYY quality controls 1 and 2 (lyophilised) 0.5mL/vial
5. Matrix solution (serum matrix containing DPP-4 inhibitor) 1.5 mL
6. Assay buffer (0.05 M Borate saline, pH8.5, containing 0.025 M EDTA, 0.08% sodium azide, 0.1% bovine serum albumin [BSA]) 10 mL
7. PYY capture antibody (Pre-titered rabbit anti-human PYY antibody) 3 mL
8. PYY detection antibody (Pre-titered biotinylated anti-human PYY antibody complementary to capture antibody) 3 mL
9. Blocking solution (Proprietary reagents to block false positive signals) 3 mL
10. Enzyme solution (Pre-titered streptavidin-HRP conjugate in buffer) 12 mL
11. Substrate (3,3',5,5'-tetramethylbenzidine in buffer) 12 mL
12. Stop solution (0.3 M HCl) 12 mL

2.8.5 GLP-1 ELISA

These kits were purchased from Millipore, Watford, UK with catalogue number: EP35, lot number: 3031996 and expiry date: Feb 2020. The kits are fluorophore based assays. The sensitivity is 2 pM (100 µL plasma sample size) with the intra-assay and inter-assay variations of 6 – 9% and <1 – 13%, respectively.

The plate is pre-coated by a monoclonal antibody which specifically binds to the N-terminal region of active GLP-1 molecule. The detection antibody used in this assay is an anti GLP-1 AP. Quantification of bound detection conjugate was done by adding methyl umbelliferyl phosphate (MUP) which in the presence of AP forms the fluorescent product, umbelliferone. Since the amount of fluorescent generated is directly proportional to the concentration of active GLP-1 in the unknown samples, the latter can be derived by interpolation from a reference curve calculated from reference standards. The plate was read by a fluorescence plate with an emission wavelength of 355 nm/ 460 nm.

Components

1. ELISA plate coated with anti-GLP-1 monoclonal antibody 96 wells
2. 10x wash buffer concentrate (10x Concentration of 10 mM PBS buffer containing tween 20 and sodium azide) 50 mL
3. GLP-1 (7-36) amide ELISA standards (GLP-1 [7-36 amide] in assay buffer: 2, 5, 10, 20, 50 and 100 pM) 1 mL/vial
4. ELISA GLP-1 (active) quality controls 1 and 2 1 mL/vial
5. GLP-1 (active) assay buffer (0.05 M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween 20, 0.08% sodium azide and 1% BSA) 25 mL
6. GLP-1 (active) detection conjugate (Anti GLP-1 AP conjugate) 21 mL
7. Substrate (MUP) 10 mg
8. Substrate diluent 21 mL
9. Stop solution 6 mL

2.8.6 Insulin ELISA

The plate is pre-coated by a pre-titered amount of monoclonal mouse anti-human insulin antibodies, and the detection antibody used is a biotinylated monoclonal mouse anti-human antibody to the captured insulin. HRP was then conjugated to the immobilised biotinylated antibodies. Quantification of immobilised antibody-enzyme conjugates is done by monitoring HRP activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm after acidification of formed product.

These kits were purchased from Millipore, Watford, UK with catalogue number: EZHI-14K, lot number: 3439773 and expiry date: 30 June 2020. The sensitivity of the assay is 1 µU/mL when using a 20 µL sample size with the intra-assay and inter-assay variations of 4.6 – 7% and 9.1 – 11.4%, respectively.

Components

1. ELISA plate coated with mouse monoclonal anti-human insulin antibodies 96 wells
2. 10x HRP wash buffer concentrate (10x Concentrate of 50 mM tris buffered saline containing Tween-20) 50 mL
3. ELISA human insulin standards (Human insulin in buffer: 2, 5, 10, 20, 50, 100 and 200 µU/mL) 0.5 mL/bottle
4. ELISA quality controls 1 and 2 (Purified recombinant human insulin in assay buffer) 0.5 mL/bottle
5. Matrix solution (treated human serum) 1 mL
6. Assay buffer (0.05 M PBS, pH 7.4, containing 0.025 M EDTA, 0.08% sodium azide, 1% BSA) 8 mL
7. Human insulin detection antibody (Pre-titered biotinylated monoclonal mouse anti-human insulin antibody) 3 mL
8. Enzyme solution (Pre-titered streptavidin-HRP conjugate in buffer) 12 mL
9. Substrate (3,3',5,5'-tetramethylbenzidine in buffer) 12 mL
10. Stop solution (0.3 M HCl) 12 mL

2.8.7 FGF-19 ELISA

The plate is pre-coated by a monoclonal antibody specific for human FGF-19. Thus, the FGF-19 is captured by this immobilised antibody. The detection antibody used in this assay is an enzyme-linked polyclonal antibody specific for human FGF-19. Quantification of immobilised antibody-enzyme conjugates is done by monitoring HRP activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm after acidification of formed product.

These kits were purchased from Bio-techne, Abingdon, UK with catalogue number: DF 1900, lot number: P227112 and expiry date: 29 August 2020. The sensitivity of the assay

is 1.17 pg/mL with the intra-assay and inter-assay variations of 3.6 – 6.4% and 4.5 – 5.5% respectively.

Components

1. Human FGF-19 microplate (Coated with a monoclonal antibody specific for human FGF-19) 96 wells
2. Human FGF-19 conjugate polyclonal antibody specific for human FGF-19 conjugated to HRP 21 mL
3. Human FGF-19 standard (lyophilised) (Recombinant human FGF-19 in a buffered protein base) 1 vial
4. Assay diluent RD1S (A buffered protein base with preservation) 11 mL
5. Calibrator diluent RD5P concentrate (A concentrated buffered protein base with preservation) 21 mL
6. Wash buffer concentrate 21 mL
7. Colour reagent A (stabilised hydrogen peroxide) 12 mL
8. Colour reagent B (stabilised chromogen tetramethylbenzidine) 12 mL
9. Stop solution (2 N sulfuric acid) 6 mL

2.8.8 ELISA: criteria of result acceptance and calculation

The assay was rejected if one of the two quality controls fails outside of 2 standard deviations of the applicable mean. If the difference between duplicate results of a sample is >15% of the coefficient of variation (CV), the sample must be repeated. All accepted values exceeded the sensitivity limit of each assay.

With regard to the result calculation, a reference curve is plotted with the absorbance unit of 450 nm (except of 355 nm/ 460 nm for GLP-1 assay) on the Y-axis against the concentration of standards on the X-axis. The results of unknown samples can be then extrapolated from the curve, using a 4- or 5-parameter logistic function.

2.9 Surrogate markers for glucose homeostasis

2.9.1 Insulin resistance

The surrogate markers used to represent the insulin resistance in this thesis are:

1. Homeostatic model assessment for insulin resistance (HOMA-IR) calculated by the following formula:
   HOMA-IR = (fasting glucose [mmol/L] x fasting insulin [µIU/L]) /22.5
   Matthews et al. showed that there is a great correlation of the formula with the euglycaemic insulin clamp ($r = 0.81$, $P < 0.001$) (Matthews et al., 1985).
2. Fasting insulin
   The fasting insulin has long been considered as the most convenient and practical surrogate marker for insulin resistance, in particular for healthy people who have not developed diabetes. Therefore, it has been useful to identify insulin-resistant individuals from normal individuals. Nonetheless, the use of fasting insulin in diabetes and glucose-intolerant subjects who might have inappropriately low insulin secretion is limited (Singh and Saxena, 2010).

2.9.2 Insulin sensitivity

1. Qualitative insulin-sensitivity check index (QUICKI) calculated by:
   QUICKI = 1/ (log [fasting plasma insulin] + log [fasting plasma glucose])
   Chen and colleagues has demonstrated that the QUICKI accurately predicted insulin sensitivity as determined by the reference glucose clamp method (Chen et al., 2005).
2. 1/ Fasting insulin
   Similar to the fasting insulin, the 1/ fasting insulin could indicate insulin sensitivity in healthy individuals who become insulin resistance as the fasting insulin is rising. The limitation is the same as the fasting insulin (Singh and Saxena, 2010).

2.9.3 β-cell function

Homeostatic model assessment of β-cell function (HOMA-β) was utilised as an indicator of β-cell function in this thesis. It can be calculated by this formula: HOMA-β = [20 x fasting

plasma insulin] / [fasting plasma glucose – 3.5] in molar units. The estimation of β-cell activity using the HOMA-β has been widely accepted with a great correlation with the reference glucose clamp method (Wallace et al., 2004)

Chapter 3

The role of PYY and ghrelin in predicting weight loss after LRGB and LSG

Chapter 3 The role of PYY and ghrelin in predicting weight loss after LRGB and LSG

Subject recruitment and mixed-meal tolerance tests in this study were conducted by Dr. Jason Cheung and Dr. Andrea Pucci, research associates in the Centre for Obesity Research at the Division of Medicine, University College London. Kusuma Chaiyasoot was responsible for creating research questions, hypotheses, objectives, all laboratory work, data collection, data analysis and critical discussion.

3.1 Introduction

Many clinical factors have been reported to be associated with weight-loss success following bariatric surgery including baseline BMI, gender, age and early post-operative weight loss velocity (Still et al., 2014b, Contreras et al., 2013, Ma et al., 2006, Ochner et al., 2013, Ortega et al., 2012, Scozzari et al., 2012, Manning et al., 2015b). Nevertheless, the evidence showing that gut hormones are able to predict weight loss after bariatric surgery is scarce, elusive and limited to only RYGB. Previously, le Roux et al., demonstrated that nutrient-stimulated PYY and GLP-1 levels were attenuated in people with poor weight loss compared to people with good weight loss at 2 years following LRYGB (le Roux et al., 2007).

Dirksen et al. also showed a larger release of GLP-1 and a greater suppression of ghrelin in people with good weight loss compared to people with poor weight loss, whereas PYY did not differ between groups at more than 12 months after LRYGB (Dirksen et al., 2013). Morinigo and colleagues demonstrated that 6-week post-operative $AUC_{0\text{-}120}$ of PYY in response to a meal was associated with percentage excess weight loss at 33 months after RYGB (Morinigo et al., 2008), and Faraj et al., showed that 15±6-month PWL after RYGB was predicted by pre-operative adiponectin concentrations (Faraj et al., 2003). However, Werling and colleagues concluded that pre-operative assessment of gut hormones did not correlate to weight loss after RYGB (Werling et al., 2014).

Recent evidence has revealed that the beneficial effects of PYY on weight loss and glycaemic improvement may be underrated. In human and mice model, chronic

treatment of PYY enhanced insulin and glucagon release (Guida et al., 2017). Ramracheya et al. also demonstrated that in rodents with the presence of GLP-1 receptor antagonist, PYY restored β-cells function after RYGB (Ramracheya et al., 2016).

The post-operative change in ghrelin levels contributing to the weight-loss success, particularly after RYGB, has been controversial. A substantial and sustained reduction in nutrient-stimulated ghrelin concentrations post-SG has been reported. Nonetheless, the concentrations post-RYGB rose in some studies (Holdstock et al., 2003, Sundbom et al., 2007) or did not change (le Roux et al., 2006, Korner et al., 2005, Karamanakos et al., 2008, Leonetti et al., 2003) or decreased (Cummings et al., 2002) .

Since there is a relationship of the circulating levels of PYY and ghrelin with body weight (Kim et al., 2008, Cahill et al., 2014), the study and/or comparison of the changes in these hormones should specifically focus in early post-operative period rather than in late post-operative period when the differences in weight could confound the measurements.

The relationship between T2D and poor weight-loss following lifestyle modification, pharmacotherapy and bariatric surgery has been reported (Arterburn et al., 2018, Pi-Sunyer, 2005, Wing et al., 1987), and there is evidence showing the discrepancy in PYY circulating levels between individuals with and without T2D (Ukkola et al., 2011, Olivan et al., 2009). Emerging evidence postulated that gut hormone is one of the key mechanisms underlying diabetes remission after bariatric surgery (Batterham and Cummings, 2016), yet the role of PYY and ghrelin on glycaemic benefits and T2D remission following bariatric surgery is still debatable.

Hence, this study aims to examine the hypothesis that PYY and ghrelin are associated with weight-loss and glycaemic benefits after primary LRYGB and LSG. We will investigate:

1. Longitudinal pattern of weight loss and circulating PYY and ghrelin changes in patients undergoing primary LRYGB and LSG, comparing between:
    1.1. Patients undergoing LRGB versus LSG
    1.2. Patients with T2D versus patients without T2D
    1.3. Patients with good versus poor weight loss
    1.4. T2D remitters versus non-remitters

2. Association of pre-operative factors with the following outcomes after primary LRYGB and LSG at 1 year:
   2.1. PWL
   2.2. Weight-loss outcomes (good or poor weight loss)
   2.3. T2D remission
3. Association of early post-operative (6-week) factors with the following outcomes after primary LRYGB and LSG at 1 year:
   3.1. PWL
   3.2. Weight-loss outcomes (good or poor weight loss)
   3.3. T2D remission

3.2 Materials and Methods

3.2.1 Subjects

Patients were recruited from the University College London Hospitals (UCLH) Bariatric Centre for Weight Management and Metabolic Surgery as described in the Chapter 2. The inclusion criteria were:

1. BMI ≥40 or ≥35 kg/m^2 with obesity associated co-morbidities
2. Aged 18-65 years
3. Scheduled to undergo primary bariatric surgery

The exclusion criteria were:

1. Intra-operative or early post-operative complications
2. Patients with T2D who were taking GLP-1 agonist and/ or insulin
3. Smoking
4. Alcohol consumption >20 units per week

All patients were given an information sheet and verbal explanation by a study investigator in person. They then provided a written informed consent. The study was

sponsored by UCL/UCLH Joint Research Office and ethical approval was given by the National Health Service Research Ethics Committee (ID#09/H0715/65).

3.2.2 Study protocol

Patients who were due to undergo bariatric surgery had their meal study within a 2-week period pre-surgery and at 6 $\pm$ 2 weeks, 24 $\pm$ 4 weeks and 52 $\pm$ 4 weeks post-operatively. They were asked to refrain from alcohol for 24 hours and to fast overnight for a minimum of 12 hours prior to the test, usually from 9pm to 9am. They were allowed to drink only water during the fasting period. On arrival, an intravenous cannula was inserted, which was used to obtain blood samples. Following cannulation, a 45-minute acclimatisation period was allowed. At 0 minutes, a baseline blood sample was collected. Subjects then consumed the 500-kcal test meal (250mL Resource 2.0 Fibre, Nestle Nutrition, Croydon, UK) within 15 minutes, with subsequent blood samples drawn at 15, 30, 60, 90, 120, 150 and 180 minutes post-meal. The test meal consisted of 18% of protein (22.5g), 40% carbohydrate (50g) and 39% fat (21.8g).

3.2.3 Sample collection and processing

Fasting blood were collected at 0 minute as a baseline blood sample with subsequent blood samples drawn at 15, 30, 60, 90, 120, 150 and 180 minutes post-meal. The blood samples were subsequently processed as described in the '*Sample collection and processing*' section, Chapter 2.

3.2.4 Hormone assays

Total PYY (PYY1-36 and PYY3-36), AG and DAG were assayed using enzyme-linked immunosorbent assay (ELISA) kits (Millipore, Watford, UK for PYY and LSI Medience Corporation, Tokyo, Japan for AG and DAG) as per the manufacturer's instructions. The sensitivity of PYY assays is 6.5 pg/mL, inter-assay variability is 7.41%, and intra-assay variability is 2.27%. For AG and DAG assays, the sensitivity is 1.5 pM, inter-assay variability is <11 % and intra-assay variability is 1.5 %. Details were described in '*AG and DAG ELISA*' and '*PYY ELISA*' sections, Chapter 2.

3.2.5 Anthropometric measurement

BW was measured while subjects wore indoor clothing without shoes and heavy accessories using a calibrated weighing sale (Seca 877, Seca, UK). Height was determined by a wall-mounted stadiometer (242 Measuring Rod, Seca, UK). Some anthropometric indexes were used in this study as follows:

1. BMI was calculated by dividing BW (kg) by the square of height (in metres).

2. Percentage weight loss (PWL) was used since it is less influenced by baseline BMI than percentage excess weight loss (Hatoum and Kaplan, 2013), and was calculated by the following formula: PWL = ([baseline BW – BW at each study visit]/ baseline BW) x 100.

3. Weight change velocity (WCV) was expressed as BW change (kg) per week between consecutive visits including during 0 – 6 weeks, 6 weeks – 6 months and 6 months – 1 year (Manning et al., 2015b).

4. The definition of good weight loss was PWL ≥ 20% from pre-surgery.

3.2.6 Definition of gut hormone parameters

1. Fasting levels: gut hormone levels measured after a 12-hour fast
2. $AUC_{0\text{-}180}$: an area under the curve (AUC) of a gut hormone measured during a MMTT from time 0 – 180 minutes, calculated by the trapezoid rule
3. $AUC_{0\text{-}60}$: an AUC of a gut hormone measured during a MMTT from time 0 – 60 minutes
4. $\Delta AUC_{0\text{-}180}$: an AUC calculated by subtracting the fasting (0-minute) hormone level from every time-point level during the MMTT. In other words, it is an AUC calculated from t0 – t 0, t15 – t0, t30 – t0, t60 – t0, t90 – t0, t120 – t0, t150 – t0, and t180 – t0 levels, using the trapezoid rule
5. $\Delta AUC_{0\text{-}60}$: a ΔAUC from time 0 – 60 minutes
6. diff $AUC_{0\text{-}180}$: the difference in $AUC_{0\text{-}180}$ of a hormone between follow-up visits
7. diff $\Delta AUC_{0\text{-}180}$: the difference in $\Delta AUC_{0\text{-}180}$ of a hormone between follow-up visits

3.2.7 Statistical analysis

Results from the MMTT are described as AUC and ΔAUC. The ΔAUC PYY represents the increase in nutrient-stimulated PYY from fasting state, and the ΔAUC of AG and DAG indicates the postprandial suppression of AG and DAG from fasting levels by a test-meal.

The Shapiro-Wilk test was used to determine the normality of all variables. Continuous data with normal distribution was expressed as mean ± SD whilst the non-normally distributed data was presented as median (25^{th}, 75^{th} percentiles). Categorical variables were reported as percentages and χ^2 tests were used to compare between groups. In order to compare the variables with normal distribution within groups, paired-sample t-tests were used whereas the unpaired t-tests were used for the comparison between groups. Mann-Whitney tests were used for the comparison of non-normally distributed data between groups. Mixed modelling was utilised to compare quantitative variables over time between groups and to compare the effects of PYY over time on PWL using their baseline values as covariates. Model selection was based on BIC criteria. The assumptions for mixed models (e.g., normality of error terms) were checked thoroughly using the residual plots.

Multiple linear regression was performed to examine whether clinical parameters, the FTO genotype and gut hormones (PYY, AG, DAG and AG:DAG) are predictive for weight-loss outcomes, using 1-year PWL as the dependent variable with adjustment for age, gender, surgical procedures and the presence of T2D. The association of gut hormone changes at 6 weeks with poor weight loss was analysed by using logistic regression. Confounding factors of the weight loss were selected by evaluating the degree of association between factors of interest and the weight loss. Therefore, factors having univariate P-value <0.2 including age, gender, T2D, and type of surgery were entered into the multiple logistic regression. The ROC curve was plotted in order to demonstrate the performance of 6-week PYY changes in association with the poor weight loss, and to define the best cut-off point of area under ROC curve (AuROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence interval.

Statistical analyses were performed by using SPSS for Windows (version 24.0, Chicago, Illinois, USA) and all tests were two-sided with a significance level at P-value < 0.05.

3.2.8 Subgroup analysis

Two subgroup analyses were subsequently performed in patients with T2D:

1. Patients with T2D undergoing LSG versus LRYGB
2. T2D remitters versus non-remitters

Patients with T2D who achieved FPG <6.9 mmol/L and HbA_{1c} <6.5% for at least 1 year were included into the remitters group (Buse et al., 2009), whereas those subjects who did not meet the criteria would be classified as non-remitters.

3.3 Results

3.3.1 Longitudinal pattern of weight loss and circulating PYY and ghrelin changes in patients undergoing primary LRYGB and LSG

3.3.1.1 Baseline characteristics

Table 3.1 shows baseline characteristics of 85 subjects recruited in this cohort, divided by type of bariatric surgery (LRYGB and LSG), the presence of T2D and weight-loss outcomes at 1 year. Of these, 27 subjects (31.8%) underwent LRYGB and 58 subjects (68.2%) had LSG. The majority of subjects were female (90.6%), and 32.9% were diagnosed as T2D preoperatively. Overall, the mean age was 45.9 $\pm$ 11.6 years old, BW was 125.5 ± 22.5 kg, and the median of BMI was 44.5 (41.1, 50.5) kg/m^2. The average systolic BP (SBP), diastolic BP (DBP) and pulse rate were 125 ± 14 mmHg, 67 (62, 76) mmHg and 74 ± 15 rate /minute. The median of HbA_{1c} was 35 (33, 38) mmol/mol. Hormonal profiles of PYY, AG, DAG and AG:DAG were also shown in Table 3.1.

There was no statistically significant difference in baseline characteristics between LRYGB and LSG groups. When categorised subjects using the presence of T2D, patients with T2D were older than patients without diabetes; 51.9 (41.3, 58.1) vs 45.1 (35.7, 51.5) years old

(P = 0.04) (Table 3.1). As expected, the level of HbA_{1c} in T2D group was higher than non-T2D group; 49.2 (43.2, 54.1) vs 36.6 (35.2, 40.2) mmol/mol (P <0.001). Interestingly, in T2D, the levels of fasting PYY and $AUC_{0\text{-}180}$ PYY were greater than non-T2D; 84 (61, 110) vs 53 (36, 64) pg/mL (P <0.001) and 28,508 (22,808, 37,674) vs 22,578 (18,923, 27,640) pg x min/mL (P = 0.04) (Table 3.1). Nonetheless, after subtraction with fasting PYY levels ($\Delta AUC_{0\text{-}180}$ PYY), there was no statistically significant difference between groups (Table 3.1).

In the groups divided by 1-year weight-loss outcomes, there was no statistically significant difference in baseline characteristic between poor and good weight-loss groups except SBP and pulse rate (SBP: 119 [108, 126] mmHg for poor weight loss vs. 127 [116, 135] mmHg for good weight loss [P = 0.02] and pulse rate: 64 [60, 72] bpm for poor weight loss and 76 [68, 84] bpm for good weight loss [P <0.01]). The number of patients with T2D in poor weight loss (53.3%) was marginally significantly greater than the good weight loss group (29.4%) (P = 0.08, Table 3.1). The medians of BW and BMI in poor weight loss group were higher than another group at pre-surgery; however, it did not reach statistical significance (BW: 137 [116, 142.5] vs 121.3 [108.6, 137.9] kg, P = 0.16, BMI: 45.8 [41.7, 51.6] vs 43.9 [41, 50.3] kg/m^2, P = 0.6, Table 3.1). The percentage of patients with good weight-loss outcome undergoing LRYGB was greater than poor weight loss, even though it was not statistically significant difference (35.3% vs 20%, P = 0.25, Table 3.1).

17/28 patients with T2D (61%) were taking metformin at pre-surgery. Of these, 1 patient had metformin + gliclazide, 1 patient had metformin + sitagliptin, and another one patient had metformin + saxagliptin. At 6-week post-operative, 8/28 patients (29%) continued metformin. At 1-year post-operative, only 2/28 patients (7%) were still on metformin.

Table 3.1 Baseline characteristics of all patients and patients divided by type of bariatric surgery, T2D status and weight-loss outcomes at 1 year

	Total (n = 85)	Type of surgery		T2D status		Weight-loss outcomes at 1 year	
		LRYGB (n = 27)	LSG (n = 58)	Non-T2D (n = 57)	T2D (n = 28)	Poor (n = 15)	Good (n = 68)
Age, years	45.9 ± 11.6	48.6 ± 11.7	44.7 ± 11.4	45.1 (35.7, 51.5)	51.9 (41.3, 58.1)	46 ± 12.3	46.2 ± 11.5
n	85	27	58	57	28	15	68
Female, %	90.6	92.6	89.7	94.7	82.1	80	92.6
BW, kg	125.5 ± 22.5	123.1 ± 23.1	126.7 ± 22.3	122.4 (110.1, 142)	123.9 (106.5, 140.8)	137 (116, 142.5)	121.3 (108.6, 137.9)
n	84	27	57	56	28	15	68
BMI, kg/m²	44.5 (41.1, 50.5)	45.5 (42.3, 50.7)	44.4 (40.6, 50.4)	43.7 (41.1, 50.1)	45.8 (41, 51)	45.8 (41.7, 51.6)	43.9 (41, 50.3)
n	84	27	57	56	28	15	68
T2D, %	32.9	37	31	0	100	53.3	29.4
LRYGB, %	31.8	100	0	29.8	35.7	20	35.3
FTO genotype							
- **AA, %**	20.7	11.5	25	20	22.2	13.3	21.5
- **AT, %**	50	50	50	50.9	48.1	66.7	46.2
- **TT, %**	29.3	38.5	25	29.1	29.6	20	32.3
SBP, mmHg	125 ± 14	128 ± 16	123 ± 12	123 (115, 132)	127 (117, 137)	119 (108, 126)	127 (116, 135)
n	85	27	58	57	28	15	68
DBP, mmHg	67 (62, 76)	72 (62, 80)	67 (62, 75)	68 (63, 76)	67 (62, 77)	67 (60, 77)	67 (62, 76)
n	85	27	58	57	28	15	68
PR, bpm	74 ± 15	74 ± 18	74 ± 13	74 (67, 83)	75 (61, 84)	64 (60, 72)	76 (68, 84)
n	84	27	57	56	28	15	67

HbA_{1c},	35	34.5	35	36.6	49.2	42.1	39.9
mmol/mol	(33, 38)	(33, 36.8)	(33, 39)	(35.2, 40.2)	(43.2, 54.1)	(33.3, 50.8)	(35.5, 45.4)
n	82	24	58	54	28	14	66
Fasting PYY,	60	63	59.5	53	84	50	60
pg/mL	(40, 85)	(36, 89)	(43.5, 83.5)	(36, 64)	(61, 110)	(39, 102)	(40, 85)
n	83	27	56	55	28	15	68
$AUC_{0\text{-}180}$ PYY,	24,218	24,653	24,209	22,578	28,508	24,209	24,226
pg x min/mL	(19,578, 30,379)	(19,993, 39,117)	(19,154, 29,048)	(18,923, 27,640)	(22,808, 37,674)	(19,154, 32,743)	(19,713, 29,933)
n	82	27	55	55	27	15	67
$\Delta AUC_{0\text{-}180}$ PYY,	13,039	16,923 ± 11,138	13,753 ± 7,421	13,200	12,878	12,878	13,200
pg x min/mL	(8,768, 19,542)			(9,320, 19,493)	(6,818, 19,688)	(6,994, 20,453)	(9,086, 19,275)
n	82	27	55	55	27	15	67
Fasting AG,	7.8	8	7.6	8	7.6	7	7.9
fmol/mL	(4.9, 11.5)	(5.2, 12.1)	(4.8, 10.4)	(4.5, 11)	(5.2, 12.2)	(4.8, 11.9)	(4.8, 11.4)
n	81	26	55	54	27	14	67
$AUC_{0\text{-}180}$ AG,	1,035	1,142	945	1,062	1,022	1,035	1,035
fmol x min/mL	(679, 1,498)	(636, 1,542)	(689, 1,498)	(689, 1,489)	(606, 1,564)	(756, 1,418)	(669, 1,520)
n	79	26	53	53	26	13	66
$\Delta AUC_{0\text{-}180}$ AG,	-361	-328	-386	-392	-350	-290	-374
fmol x min/mL	(-792, -92)	(-907, -63)	(-722, -93)	(-840, -78)	(-710, -134)	(-932, -193)	(-773, -77)
n	79	26	53	53	26	13	66
Fasting DAG,	102 ± 41	108 ± 48	98 ± 38	97 (67, 131)	106 (75, 118)	105 (64, 124)	98 (74, 131)
fmol/mL							
n	82	26	56	55	27	14	67

$AUC_{0\text{-}180}$ DAG, fmol x min/mL	11,776	12,312 ± 5,206	13,159 ± 5,718	11,650	12,812	12,501 ± 4,877	13,025 ± 5,706
	(8,381, 17,063)			(8,494, 17,304)	(8,235, 15,645)		
n	80	26	54	54	26	13	66
Δ$AUC_{0\text{-}180}$ DAG, fmol x min/mL	-4,925	-6,312	-4,490	-4,441	-5,325	-4,917	-5,321
	(-7,788, -2,623)	(-9,596, -2,740)	(-6,530, -2,457)	(-7,485, -2,546)	(-8,787, -3,625)	(-6,192, -3,034)	(-8,221, -2,546)
n	80	26	54	54	26	13	66
Fasting AG:DAG	0.077	0.09	0.076	0.083	0.076	0.076	0.077
	(0.055, 0.115)	(0.05, 0.117)	(0.057, 0.115)	(0.057, 0.116)	(0.05, 0.102)	(0.05, 0.117)	(0.055, 0.115)
n	81	26	55	54	27	14	67
$AUC_{0\text{-}180}$ AG:DAG	0.088	0.092	0.086	0.089	0.078	0.089	0.087
	(0.062, 0.112)	(0.064, 0.123)	(0.06, 0.108)	(0.062, 0.117)	(0.062, 0.111)	(0.059, 0.117)	(0.062, 0.111)
n	79	26	53	53	26	13	66
Δ $AUC_{0\text{-}180}$ AG:DAG	0.062	0.05	0.063	0.066	0.054	0.099	0.052
	(0.024, 0.14)	(0.025, 0.136)	(0.02, 0.149)	(0.01, 0.149)	(0.026, 0.13)	(0.042, 0.174)	(0.012, 0.14)
n	79	26	53	53	26	13	66

3.3.1.2 Weight-loss outcome

In order to examine the distribution of 26-week and 52-week PWL trajectories, histograms were created, and they exhibited a normally distributed pattern (Figure 3.1).

The PWL in LRYGB was statistically significantly greater than LSG over 52-week period of time (P <0.05, Figure 3.2 A, Appendix 6). The average PWL at 6 weeks in LRYGB vs. LSG was 8.6 (7.1, 9.7)% vs. 9 (7.2, 11.6)%, at 26 weeks was 21.2 ± 5.9% vs. 21.5 ± 5.4% and at 52 weeks was 27.4 ± 7.1% vs 24.5 ± 7.5%, respectively. The BW and BMI at 1 year were significantly lower than pre-surgery in both groups with no statistically significant difference between groups (Figure 3.2 D, Appendix 6).

PWL in patients with T2D was significantly lower than patients without T2D over time (P <0.01, Figure 3.2 B, Appendix 7). The average PWL at 6 weeks in T2D vs non-T2D was 9 (7.1, 10.2)% vs 8.9 (7.1, 11.3)%, at 26 weeks was 19.6 ± 5% vs 22.3 ± 5.6% and at 52 weeks was 22.6 ± 6.6% vs 26.9 ± 7.6%, respectively. The BW and BMI at 1 year were significantly lower than pre-surgery in both groups, and there was a statistically significant difference between groups with the figures of T2D greater than non-T2D (P = 0.001 for BW and P = 0.02 for BMI, Figure 3.2 E, Appendix 7).

The median of PWL in good weight loss subjects was 9.4 (7.8, 11.3)% at 6 weeks, 21.6 (19.3,26.1)% at 26 weeks and 26 (23.5, 31.1)% at 52 weeks. In poor weight loss group, the median of PWL at 6 weeks was 6.9 (6.3, 8.9)%, at 26 weeks was 15.4 (12.2, 18.6)% and at 52 weeks was 15.5 (11, 19)%. As expected, the difference between groups was statistically significant (P <0.001, Figure 3.2 C and Appendix 8). The BW and BMI at 1 year were also significantly lower than pre-surgery in both groups (P <0.001 for both BW and BMI of both groups, Appendix 8) with the figures of BW and BMI in poor weight loss higher than good weight loss (P <0.001, Figure 3.2 F, Appendix 8).

In terms of WCV, the highest rate of weight loss occurred in the first 6-week period. The velocity of weight loss then markedly dropped during 6-week to 6-month period of time until it reached the lowest rate during 6-month to 1-year period. This pattern was seen in every category of subjects (Figure 3.2 G-I, Appendix 6-8). LRYGB showed statistically greater rate of weight loss than LSG over 1 year (P = 0.02, Figure 3.2 G, Appendix 6).

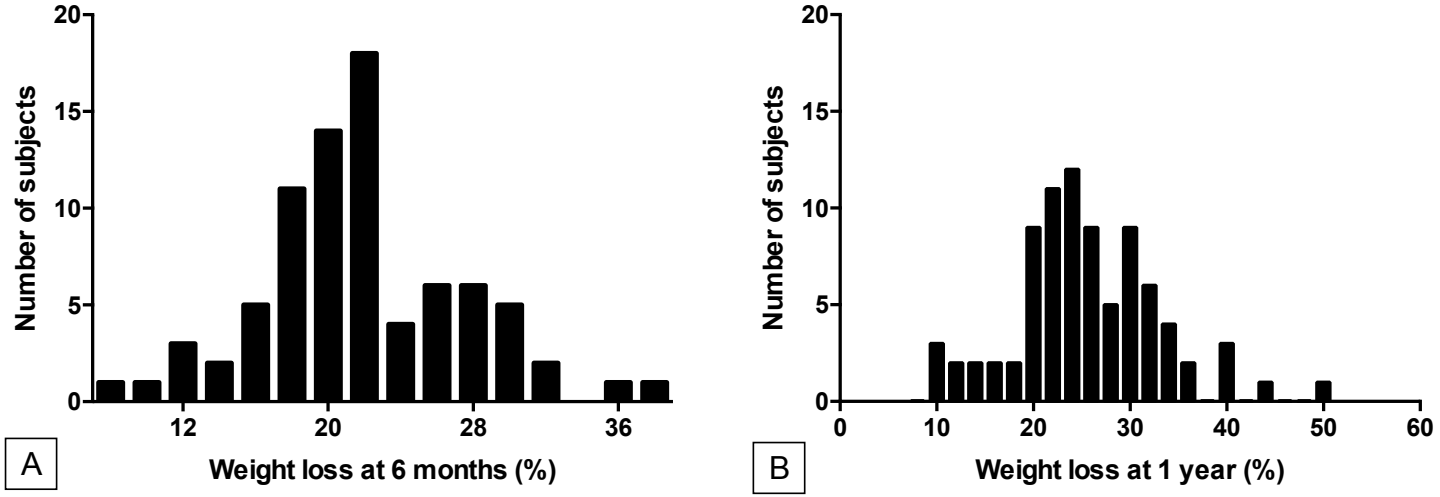


Figure 3.1 Histograms of percentage weight loss at 6 months (A) and 1 year (B)

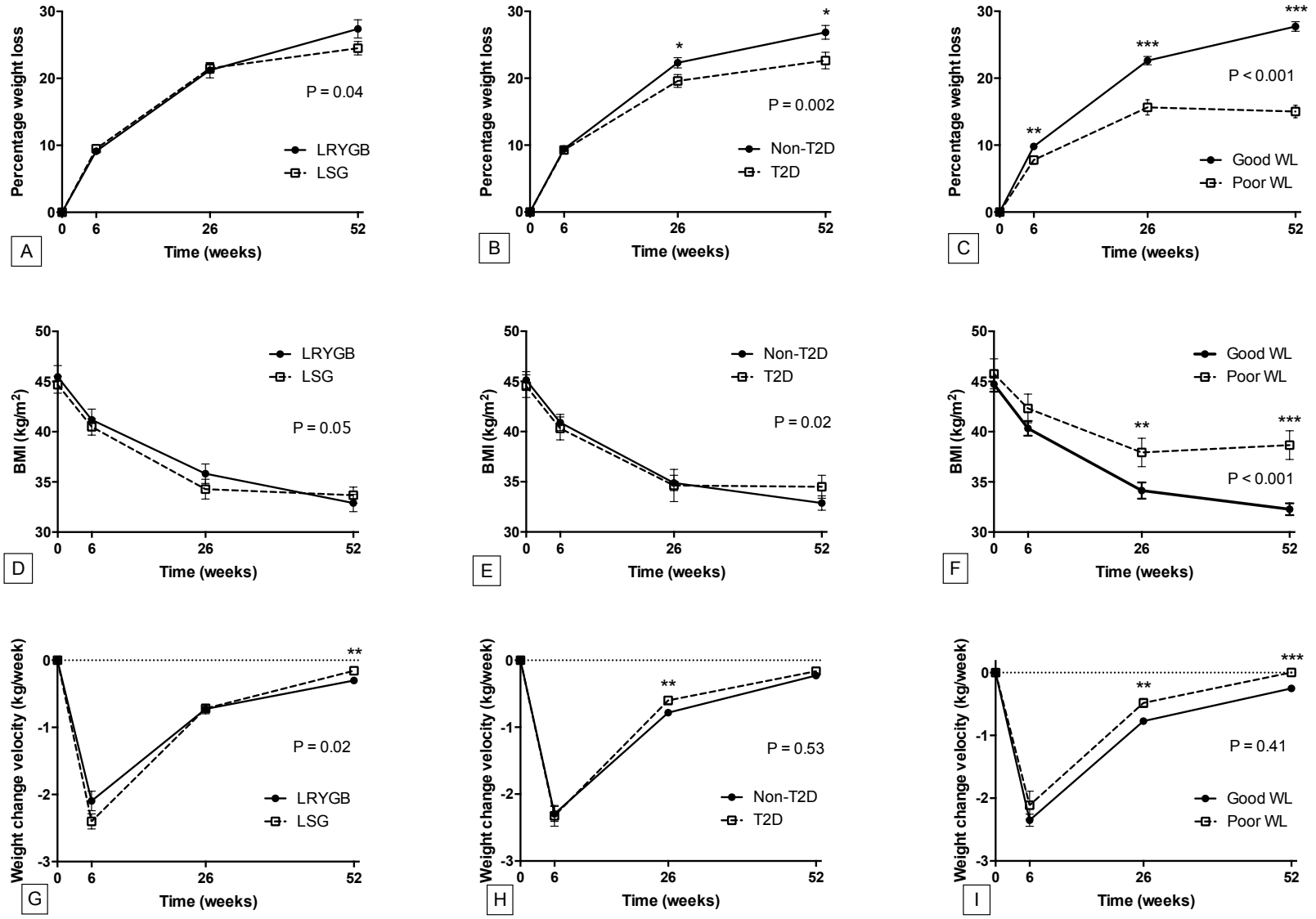


Figure 3.2 Weight loss outcomes. Percentage weight loss (A) patients divided by type of surgery, (B) patients divided by T2D status and (C) patients divided by 1-year weight loss outcomes; BMI (D) patients divided by type of surgery, (E) patients divided by T2D status and (F) patients divided by 1-year weight loss outcomes; weight change velocity (G) patients divided by type of surgery, (H) patients divided by T2D status and (I) patients divided by 1-year weight loss outcomes; * P<0.05, ** P<0.01, *** P<0.001 of the comparison between groups at each visit; P-value from mixed model analysis

3.3.1.3 HbA_{1c} changes after bariatric surgery

Following both types of surgery, the levels of HbA_{1c} statistically significantly dropped from pre-surgery in both groups (LRYGB: from 38.3 [35.5, 44.8] mmol/mol at baseline to 34.5 [33, 36.8] mmol/mol at 1 year; LSG: from 39.9 [35.5, 46.7] mmol/mol at baseline to 35 [33, 39] mmol/mol at 1 year, P <0.001 for both), but there was no statistically significant difference between groups (P >0.05, Appendix 6).

When divided patients by the presence of T2D, the fall of HbA_{1c} from baseline was also statistically significant in both non-T2D and T2D (non-T2D: from 36.6 [35.2, 40.2] mmol/mol at baseline to 34 [32, 35] mmol/mol at 1 year; T2D from 49.2 [43.2, 54.1] mmol/mol at baseline to 39 [36, 42] mmol/mol at 1 year, P <0.001 for both) with the figure of T2D group statistically significantly higher than another group over 1 year (P <0.001, Appendix 7).

After surgery, both poor and good weight loss groups experienced statistically significantly reduced levels of HbA_{1c} from pre-surgery (poor weight loss group: from 42.1 [33.3, 50.8] mmol/mol at baseline to 39 (31.5, 40.5) mmol/mol at 1 year, P <0.01; good weight loss groups: from 39.9 [35.5, 45.4] mmol/mol at baseline to 35 [33, 38] mmol/mol at 1 year, P <0.001, Appendix 8). However, there was no significant difference between groups over time (Appendix 8).

3.3.1.4 PYY and ghrelin changes after bariatric surgery

3.3.1.4.1 LRYGB versus LSG

PYY: Fasting levels were statistically significantly greater after LRYGB than LSG at all visits (P <0.05 at 6 and 26 weeks, P <0.01 at 52 weeks, P <0.05 over 1 year, Figure 3.3A, Appendix 6). There was a dramatic augmentation of meal-stimulated plasma concentrations of PYY ($AUC_{0\text{-}180}$ and $\Delta AUC_{0\text{-}180}$) after the surgery in both groups, compared to baseline (P <0.001 for both $AUC_{0\text{-}180}$ and $\Delta AUC_{0\text{-}180}$ after LRYGB and LSG, Appendix 6). The $AUC_{0\text{-}180}$ and $\Delta AUC_{0\text{-}180}$ in LRYGB were statistically significantly higher than LSG at all visits (P <0.001 at all visits for $AUC_{0\text{-}180}$ and P <0.001 at 6 weeks, P <0.01 at 26 and 52 weeks for $\Delta AUC_{0\text{-}180}$, Figure 3.3 B-C, Appendix 6).

With regards to meal-stimulated plasma concentrations of PYY, in LRYGB, the significant rise in PYY was observed from 15 – 180 minutes at 6 weeks and 26 weeks and from 15 – 90 minutes at 52 weeks (Figure 3.4A). In LSG, there was a marked increase in PYY from 15 – 120 minutes at 6 weeks, from 15 – 90 minutes at 24 weeks and from 15 – 30 minutes at 52 weeks. In addition, at 52 weeks, the levels of postprandial PYY were statistically significantly lower than baseline from 120 – 180 minutes in the LSG group (Figure 3.4B).

AG: In LSG group, there was a considerable suppression of fasting AG and $AUC_{0\text{-}180}$ AG after surgery at every visit; whereas, in LRYGB, the reduction was apparent only at 6 weeks. The fasting AG and $AUC_{0\text{-}180}$ AG levels after LRYGB rose to be equal to or more than baseline at 1 year. The fasting levels and $AUC_{0\text{-}180}$ after LSG were statistically significantly lower than LRYGB at all visits ($P < 0.01$ at 6 weeks and $P < 0.001$ at 26 and 52 weeks for both fasting levels and $AUC_{0\text{-}180}$, Figure 3.3D-E, Appendix 6). However, the suppression of AG after meal represented by $\Delta AUC_{0\text{-}180}$ AG in LSG was significantly less than LRYGB at 26 and 52 weeks ($P < 0.05$ at 26 weeks and $P < 0.001$ at 52 weeks, Figure 3.3F, Appendix 6).

Following LSG, the levels of nutrient-stimulated AG statistically significantly dropped at all visits compared to baseline; from 0 – 180 minutes at 6 weeks, from 0 – 30, 90 – 180 minutes at 26 weeks and from 0 – 30, 90, 150 – 180 minutes at 52 weeks (Figure 3.4D). After LRYGB, the fall of AG levels only reached statistical significance from 30 – 60 minutes at 6 weeks. There was a marked rise from 120 – 150 minutes at 26 weeks and from 90 – 180 minutes at 52 weeks (Figure 3.4C). Greater fluctuation of postprandial hormone was observed in LRYGB than LSG (Figure 3.4C-D).

DAG: The findings of DAG were consistent with AG. The fasting levels and the $AUC_{0\text{-}180}$ in LSG were significantly lower than LRYGB at 6, 26 and 52 weeks ($P < 0.001$ at all visits for both fasting DAG and $AUC_{0\text{-}180}$ DAG, Figure 3.3G-H, Appendix 6). In contrast, the $\Delta AUC_{0\text{-}180}$ in LSG was statistically significantly greater than LRYGB at all post-operative visits ($P < 0.01$ at 6 weeks and $P < 0.001$ at 26 and 52 weeks, Figure 3.3I, Appendix 6), reflecting that the postprandial suppression of DAG in LSG was less than LRYGB.

During the MMTT, in LSG, the DAG levels statistically significantly decreased from baseline; from 0 – 180 minutes at all visits (P <0.001 for all, Figure 3.4F, Appendix 6). In contrast, in the LRYGB group, the levels statistically significantly increased from baseline at 26 weeks from 120 – 180 minutes and at 52 weeks from 120 – 180 minutes (Figure 3.4E). The fluctuation of postprandial hormone in LRYGB was more pronounced than LSG (Figure 3.4E-F).

AG:DAG ratio: In LSG, the $AUC_{0\text{-}180}$ AG:DAG significantly increased after surgery (P = 0.002), and the ratio was statistically significantly greater than LRYGB over 1 year (P = 0.03, Appendix 6, Figure 3.3K). The changes in fasting AG:DAG and $\Delta AUC_{0\text{-}180}$ AG:DAG from pre-surgery within each group and the differences of these parameters between groups were not observed (Appendix 6, Figure 3.3J,L).

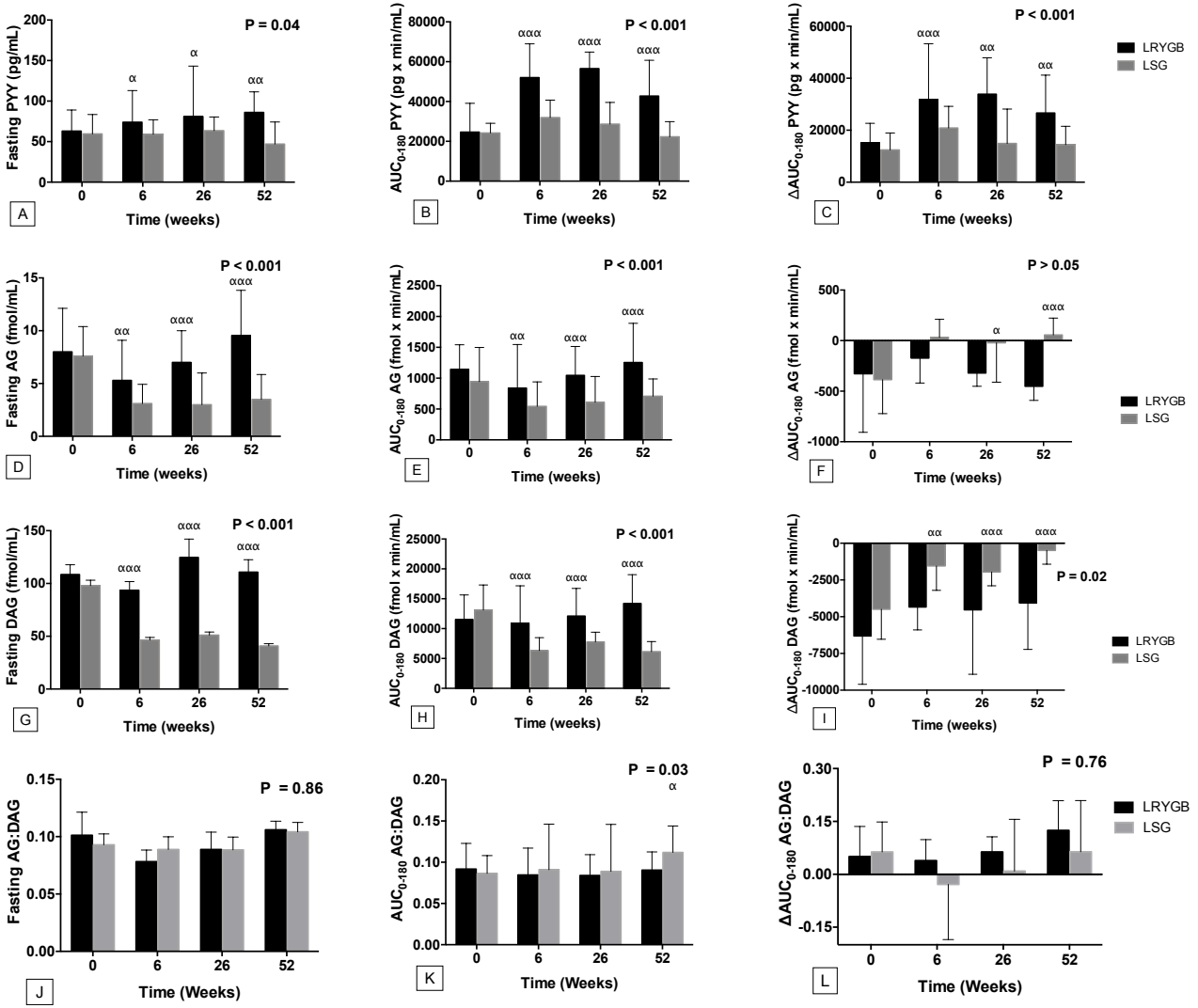


Figure 3.3 Comparison of PYY, AG and DAG parameters between LRYGB vs LSG at each visit (A) fasting PYY (B) $AUC_{0\text{-}180}$ PYY (C) $\Delta AUC_{0\text{-}180}$ PYY (D) fasting AG (E) $AUC_{0\text{-}180}$ AG (F) $\Delta AUC_{0\text{-}180}$ AG (G) fasting DAG (H) $AUC_{0\text{-}180}$ DAG (I) $\Delta AUC_{0\text{-}180}$ DAG (J) fasting AG:DAG (K) $AUC_{0\text{-}180}$ AG:DAG (L) $\Delta AUC_{0\text{-}180}$ AG:DAG. Results were expressed as mean ±SEM or median (interquartile range), according to their distribution. $^{\alpha}P < 0.05$, $^{\alpha\alpha}P < 0.01$, $^{\alpha\alpha\alpha}P < 0.001$ of the comparison between groups at each visit, P from mixed model analysis of the comparison between groups over 1 year.

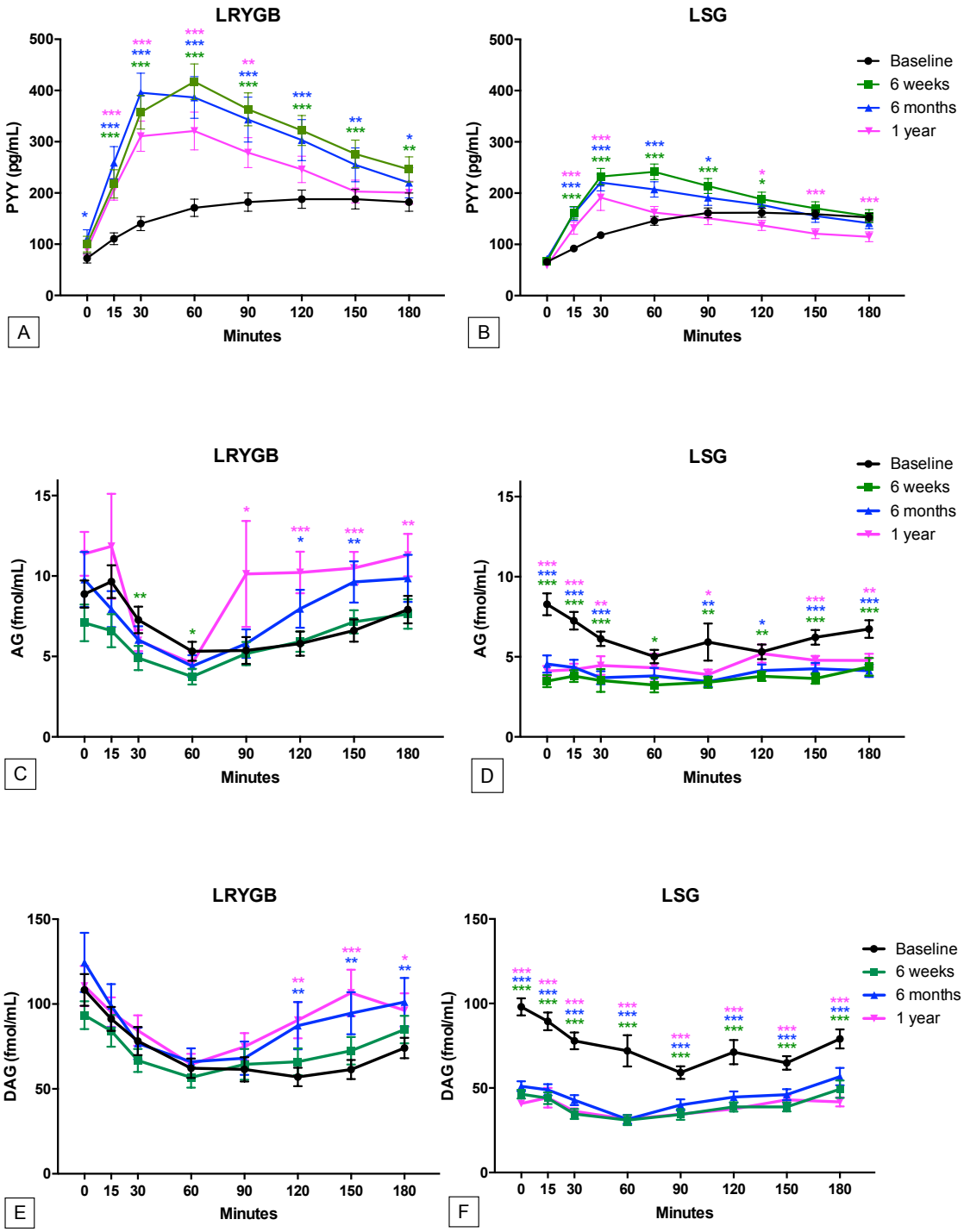


Figure 3.4 Effects of LRYGB and LSG on fasting and nutrient-stimulated PYY, AG and DAG levels at each visit (A) PYY after LRYGB (B) PYY after LSG (C) AG after LRYGB (D) AG after LSG (E) DAG after LRYGB (F) DAG after LSG. Results were expressed as mean ±SEM. $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001 in green represents the comparison between baseline and 6 weeks, in blue represents the comparison between baseline and 6 months and in pink represents the comparison between baseline and 1 year

3.3.1.4.2 Patients without T2D versus patients with T2D

PYY: The fasting PYY levels and $AUC_{0\text{-}180}$ PYY in patients with T2D were statistically significantly greater than patients without T2D at all visits (P <0.05 at 6 weeks, P <0.01 at 26 and 52 weeks for both fasting PYY and $AUC_{0\text{-}180}$ PYY Figure 3.5A-B, Appendix 7). After subtraction with fasting levels, the $\Delta AUC_{0\text{-}180}$ PYY in T2D was statistically significantly higher than non-T2D at 26- and 52-week visits (P <0.05 at both 26 and 52 weeks, Figure 3.5C, Appendix 7).

There was a substantial augmentation of PYY occurring after bariatric surgery in both groups. This was greater in patients with T2D than those without T2D. Patients with T2D exhibited a marked rise from 15 – 150 minutes at 6 weeks, 15 – 150 minutes at 26 weeks and from 15 – 90 minutes at 52 weeks (Figure 3.6B). In patients without T2D, a significant increase was observed from 15 – 150 minutes at 6 weeks, from 0 – 120 minutes at 26 weeks and from 15 – 30 minutes at 52 weeks. Interestingly, at 52 weeks, the PYY levels were statistically significantly lower than baseline from 120 – 180 minutes in the non-T2D group (Figure 3.6A).

AG: The levels of post-operative levels of fasting AG and $AUC_{0\text{-}180}$ AG were lower than pre-surgery in both groups; however, this only reached statistical significance in patients without T2D (Appendix 7). The levels of fasting AG in patients with T2D were non-significantly greater than patients without T2D at all visits (P = 0.28, Figure 3.5D, Appendix 7), and the suppression of AG during the MMTT, $\Delta AUC_{0\text{-}180}$ AG, in patients with T2D seemed to be higher than patients without T2D (P = 0.17,Figure 3.5F, Appendix 7). There was no significant difference in $AUC_{0\text{-}180}$ AG between groups (P = 0.74, Figure 3.5E, Appendix 7).

Overall, the levels of AG in patients with T2D during the MMTT showed more fluctuation than patients without T2D. In patients with T2D, a statistically significant decrease was seen at 0 minute, 30 – 60 minutes, at 120 minutes for 6 weeks; and at 30 – 60 minutes for 26 and 52 weeks (Figure 3.6D). In non-T2D group, the significant fall occurred 0 – 90, 150 – 180 minutes at 6 weeks; 0 – 30 minutes, at 180 minutes for 26-week visit and from 0 – 15 minutes at 52 weeks (Figure 3.6C).

DAG: In both groups, following the surgery, there was a statistically significant reduction of fasting DAG levels and $AUC_{0\text{-}180}$ DAG from pre-surgery (P <0.001 for both, Appendix 7) whereas the postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) statistically significantly declined from pre-surgery (Appendix 7). No statistically significant difference between groups over 1-year was seen in any parameters of this hormone, even though the levels of postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) in T2D seemed to be greater than patients without T2D (Figure 3.5G-I, Appendix 7).

During the MMTT, subjects with T2D exhibited a greater fluctuation of postprandial DAG than subjects without T2D. In T2D, a significant fall was observed from 0 to 180 minutes at 6 weeks, from 30 to 60 minutes at 26 weeks and from 0 to 120 minutes, at 180 minutes for 52-week visit (Figure 3.6F). In non-T2D, at 6 weeks the hormone dropped significantly from 0 to 180 minutes. At 26 and 52 weeks, the fall was statistically significant from 0 to 90 minutes and at 180 minutes (Figure 3.6E).

AG:DAG ratio: There was no statistically significant alteration in fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG, and $\Delta AUC_{0\text{-}180}$ AG:DAG from pre-surgery in both groups. When compared these parameters between groups, patients with T2D exhibited non-statistically significantly greater levels of fasting AG:DAG and postprandial suppression of AG:DAG ($\Delta AUC_{0\text{-}180}$ AG:DAG) than patients without diabetes (Figure 3.5J,L, Appendix 7).

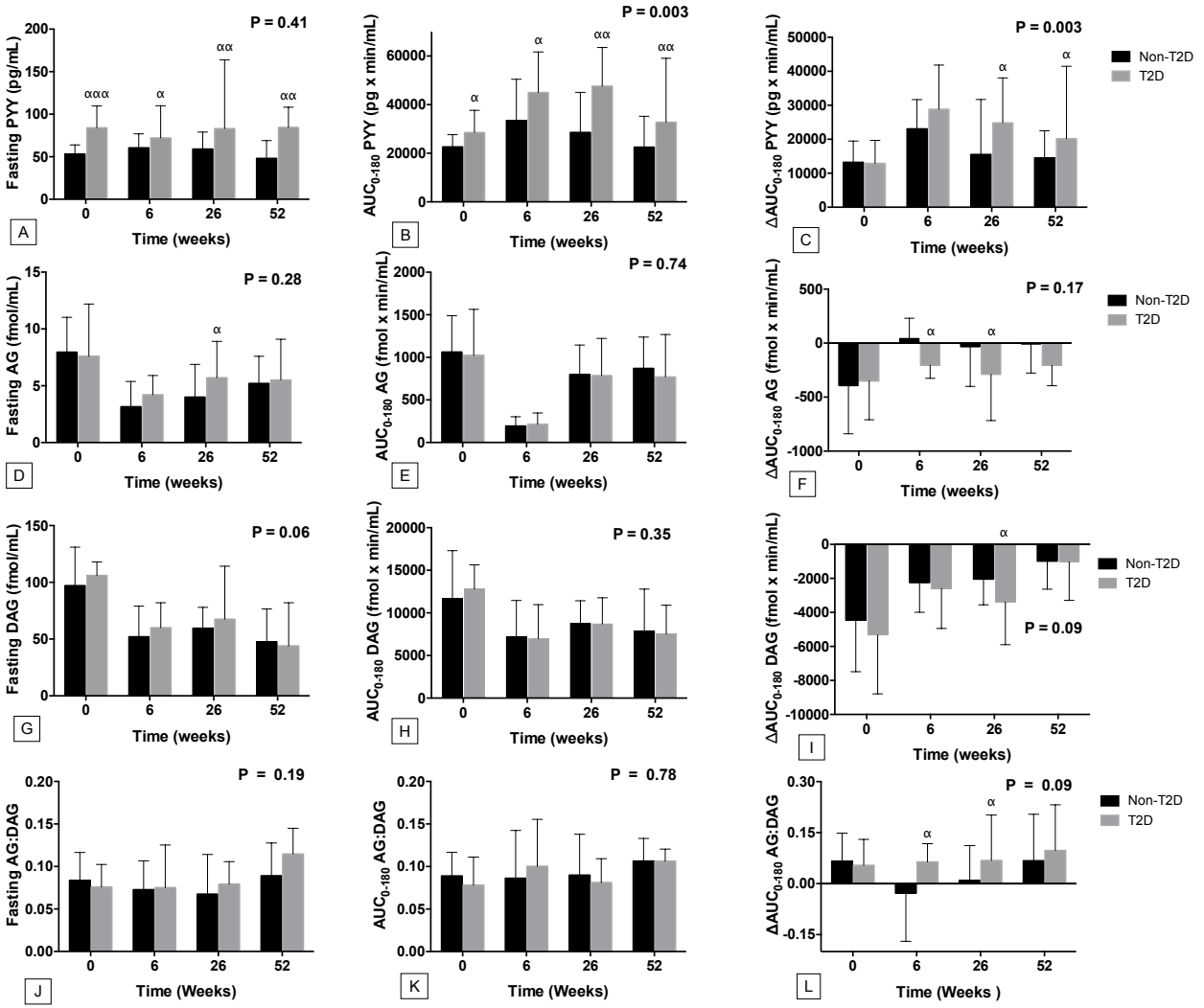


Figure 3.5 Comparison of PYY, AG and DAG parameters between Non-T2D vs T2D at each visit (A) fasting PYY (B) $AUC_{0\text{-}180}$ PYY (C) $\Delta AUC_{0\text{-}180}$ PYY (D) fasting AG (E) $AUC_{0\text{-}180}$ AG (F) $\Delta AUC_{0\text{-}180}$ AG (G) fasting DAG (H) $AUC_{0\text{-}180}$ DAG (I) $\Delta AUC_{0\text{-}180}$ DAG(J) fasting AG:DAG (K) $AUC_{0\text{-}180}$ AG:DAG (L) $\Delta AUC_{0\text{-}180}$ AG:DAG. Results were expressed as mean ±SEM or median (interquartile range), according to their distribution. $^{\alpha}P < 0.05$, $^{\alpha\alpha}P < 0.01$, $^{\alpha\alpha\alpha}P < 0.001$ of the comparison between groups at each visit, P from mixed model analysis of the comparison between groups over 1 year.

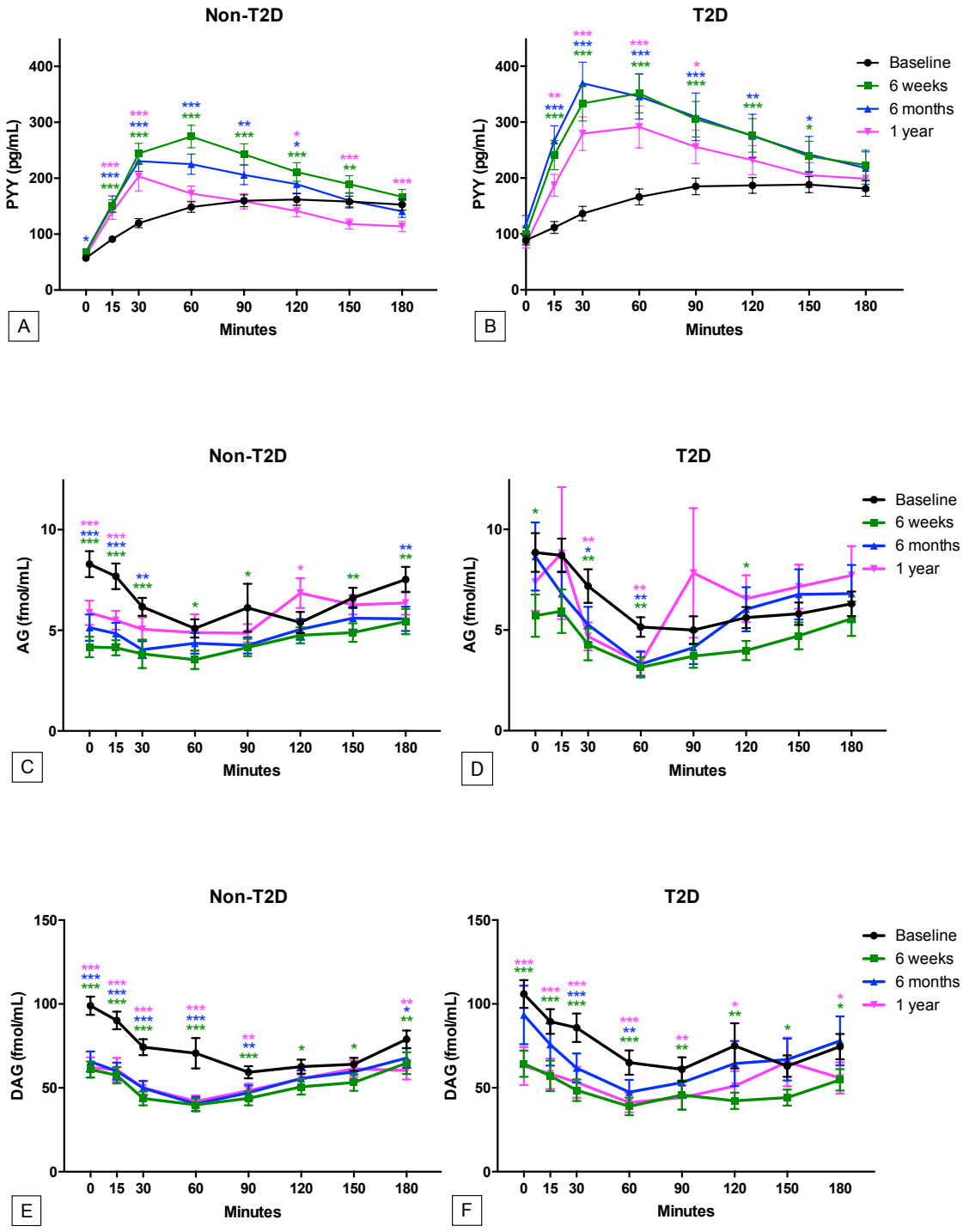


Figure 3.6 Comparison of fasting and nutrient-stimulated PYY, AG and DAG levels between patients with and without T2D (A) PYY in non-T2D (B) PYY in T2D (C) AG non-T2D (D) AG in T2D (E) DAG non-T2D (F) DAG in T2D. Results were expressed as mean ±SEM. *P <0.05, **P <0.01, ***P <0.001 in green represents the comparison between baseline and 6 weeks, in blue represents the comparison between baseline and 6 months and in pink represents the comparison between baseline and 1 year.

Stratification of T2D status (patients without T2D vs with T2D) by type of surgery

Since we observed a substantial impact of the type of bariatric surgery on gut hormone profiles, we then analysed gut hormone parameters stratified by the type of surgery. Table 3.2 shows PYY, AG, DAG and AG:DAG parameters in patients without T2D and patients with T2D after LSG and Table 3.3 represents those parameters after LRYGB.

Following both types of surgery, the levels of fasting PYY, $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in patients with T2D were greater than patients without T2D at all visits (Table 3.2, Table 3.3); however, the statistically significant difference between groups was only observed in $AUC_{0\text{-}180}$ PYY after LRYGB (P = 0.04, Table 3.3).

Following the LSG, the post-operative fasting AG and $AUC_{0\text{-}180}$ AG were significantly lower than pre-surgery in both groups as well as the post-meal suppression of AG ($\Delta AUC_{0\text{-}180}$ AG). The levels of fasting AG and the postprandial suppression of AG in T2D were non-significantly greater than non-T2D, and there was no statistically significant difference in $AUC_{0\text{-}180}$ AG between groups (Table 3.2). In LRYGB, there was neither statistically significant changes of post-operative fasting AG, $AUC_{0\text{-}180}$ AG, and $\Delta AUC_{0\text{-}180}$ AG from pre-surgery in both groups, nor differences between groups (Table 3.3). Similar to LSG, the levels of fasting AG and post-meal suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) in T2D were non-significantly greater than non-T2D (Table 3.3).

The DAG profiles followed the same pattern as AG, following LSG, fasting DAG and $AUC_{0\text{-}180}$ DAG were significantly lower than pre-surgery in both patients with and without T2D as well as the post-meal suppression of AG ($\Delta AUC_{0\text{-}180}$ DAG). There was no significant difference between groups, although the fasting DAG and postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) in T2D seemed to be higher than non-T2D (Table 3.2). After LRYGB, no significant differences from baseline in each group and between groups were observed (Table 3.3).

In terms of AG:DAG ratios, no statistically significantly differences between groups were observed after both LSG and LRYGB (Table 3.2, Table 3.3).

Table 3.2 PYY, AG and DAG changes after laparoscopic sleeve gastrectomy (LSG) in patients without T2D versus patients with T2D

	Patients without T2D (n = 40)					Patients with T2D (n = 18)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	53 (37, 62)	57 (38, 73)	55 (43, 80)	45 (35, 60)	0.14	84 (62, 105)	63 (44, 8)	72 (61, 83)	72 (39, 104)	0.73	0.89
n	38	35	33	33		18	15	15	15		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	22,948 (18,833, 27,103)	31,357 (20,172, 39,721)	23,239 (18,931, 37,073)	20,643 (15,832, 6,358)	<0.001	28,508 (22,823, 30,651)	34,598 (27,705, 45,353)	37,356 (22,673, 43,200)	29,088 (22,297, 42,610)	<0.01	0.28
n	38	34	33	32		17	15	15	15		
Δ $AUC_{0\text{-}180}$ PYY, pg x min/mL	12,582 (9,049, 18,575)	19,917 (11,304, 27,710)	12,608 (5,937, 28,031)	13,131 ± 11,608	<0.001	11,135 (5,939, 19,350)	21,474 (15,143, 31,354)	23,428 (3,633, 8,382)	19,288 ± 2,052	<0.01	0.12
n	38	34	33	32		17	15	15	15		
Fasting AG, fmol/mL	8.1 (4.3, 10.3)	2.6 (0.8, 4.9)	2.8 (1.6, 5.4)	3.5 (2.5, 6)	<0.001	6.4 (5.2, 11.6)	3.5 (2.4, 5)	4.6 (2.6, 8.1)	4.5 (2.1, 5.5)	<0.001	0.45
n	38	35	30	34		17	15	14	15		
$AUC_{0\text{-}180}$ AG, fmol x min/mL	945 (714, 1,489)	614 (383, 973)	582 (434, 1,009)	817 (596, 1,016)	<0.001	965 (581, 1,565)	476 (330, 674)	640 (387, 1,040)	621 (393, 935)	<0.001	0.8
n	37	34	30	34		16	15	14	15		
Δ $AUC_{0\text{-}180}$ AG, fmol x min/mL	-392 (-769, -78)	87 (-107, 252)	43 ± 412	72 (-197, 228)	<0.001	-354 (-674, -144)	-206 (-290, 62)	-355 ± 566	-97 (-239, 210)	0.02	0.37
n	37	34	30	34		16	15	14	15		
Fasting DAG, fmol/mL	98 ± 39	45 ± 16	48 ± 19	38 (28, 51)	<0.001	99 ± 36	49 ± 22	57 ± 23	40 (35, 45)	<0.001	0.58
n	39	35	32	34		17	15	15	15		
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	13,100 ± 5,341	6,391 (4,365, 8,470)	7,853 (4,555, 10,476)	6,760 (4,995, 8,534)	<0.001	13,301 ± 6,720	5,542 (4,180, 8,708)	7,535 (5,036, 8,729)	5,972 (4,907, 7,516)	<0.001	0.65
n	38	34	32	33		16	15	15	15		

Δ $AUC_{0\text{-}180}$ DAG,	-4,111	-1,455	-1,061	-460 ± 1,654		-5,090	-2,297	-2,852	-750 ± 1,012		
fmol x min/mL	(-6,572, -2,149)	(-2,890, 10)	(-2,792, 276)		<0.001	(-6,597, -3,996)	(-3,501, -824)	(-3,643, -1,958)		<0.001	0.81
n	38	34	32	33		16	15	15	15		
Fasting AG:DAG	0.073	0.07	0.063	0.089		0.075	0.068	0.084	0.1		
	(0.058, 0.116)	(0.02, 0.125)	(0.031, 0.116)	(0.052, 0.137)	0.09	(0.046, 0.141)	(0.053, 0.15)	(0.044, 0.119)	(0.06, 0.145)	0.85	0.23
n	38	35	30	34		17	15	14	15		
$AUC_{0\text{-}180}$ AG:DAG	0.089	0.091	0.095	0.116		0.07	0.084	0.075	0.101		
	(0.057, 0.108)	(0.054, 0.148)	(0.054, 0.15)	(0.094, 0.155)	0.01	(0.06, 0.106)	(0.049, 0.135)	(0.051, 0.112)	(0.083, 0.124)	0.36	0.74
n	37	34	30	33		16	15	14	15		
Δ$AUC_{0\text{-}180}$ AG:DAG	0.071	-0.054	-0.028	0.061		0.059	0.073	0.118	0.069		
	(0.013, 0.149)	(-0.242, 0.019)	(-0.342, 0.118)	(-0.161, 0.208)	0.33	(0.018, 0.166)	(-0.08, 0.124)	(-0.007, 0.226)	(-0.206, 0.248)	0.2	0.07
n	37	34	30	33		16	15	14	15		

Table 3.3 PYY, AG and DAG changes after laparoscopic Roux-en-Y gastric bypass (LRYGB) in patients without T2D versus patients with T2D

	Patients without T2D (n = 17)					Patients with T2D (n = 10)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	56 ± 28	65 (46, 100)	65 (55, 80)	65 (48, 97)	0.17	100 ± 66	77 (55, 197)	122 (86, 246)	89 (86, 118)	0.26	0.3
n	17	15	14	14		10	10	10	7		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	22,407 (19,524, 36,163)	50,233 ± 20,961	39,845 (24,459, 61,669)	36,182 ± 14,774	<0.001	30,817 (20,763, 52,007)	67,415 ± 26,633	63,535 (52,652, 69,776)	63,372 ± 18,475	<0.001	0.04
n	17	15	14	14		10	10	10	7		
Δ $AUC_{0\text{-}180}$ PYY, pg x min/mL	17,303 ± 10,816	35,666 ± 17,176	30,551 ± 17,179	21,259 ± 12,346	<0.001	16,278 ± 12,236	44,226 ± 30,303	42,561 ± 34,899	44,701 ± 20,732	<0.01	0.1
n	17	15	14	14		10	10	10	7		
Fasting AG, fmol/mL	8.6 ± 4	4.8 (2.6, 9.6)	6.5 (5.1, 8.7)	10 ± 5	0.02	9.3 ± 5	5.9 (3.6, 9.9)	8.9 (5.2, 20.6)	13.8 ± 8.1	0.31	0.62
n	16	15	14	14		10	10	9	8		
$AUC_{0\text{-}180}$ AG, fmol x min/mL	1,073 (591, 1,577)	844 (563, 1,491)	975 (790, 1,359)	1,236 (845, 1,779)	0.08	1,246 (790, 1,555)	834 (613, 1,648)	1,222 (852, 1,778)	1,445 (980, 3,232)	0.12	0.34
n	16	15	14	14		10	10	9	8		
Δ $AUC_{0\text{-}180}$ AG, fmol x min/mL	-386 ± 719	-164 (-399, 88)	-342 (-440, 106)	-348 (-525, -23)	0.32	-449 ± 618	-188 (-657, 42)	-288 (-756, -130)	-577 (-868, -315)	0.84	0.58
n	16	15	14	14		10	10	9	8		
Fasting DAG, fmol/mL	102 ± 45	97 ± 37	89 (71, 133)	101 (68, 154)	0.52	118 ± 53	88 ± 48	126 (77, 180)	94 (62, 114)	0.06	0.07
n	16	15	14	14		10	10	9	8		

$AUC_{0\text{-}180}$ DAG,	11,810 ± 5,354	13,448 ± 6,496	12,409	15,415		13,115 ± 5,133	11,133 ± 6,601	11,916	12,030		
fmol x min/mL			(9,099, 16,911)	(10,342, 20,350)	0.07			(10,052, 18,064)	(9,834, 16,494)	0.09	0.21
n	16	15	14	14		10	10	9	8		
Δ $AUC_{0\text{-}180}$ DAG,	-6,610 ± 3,940	-4,010 ± 1,543	-3,869	-4,331 ± 3,823		-8,113 ± 8,428	-4,716 ± 4,259	-7,267	-4,022 ± 3,407		
fmol x min/mL			(-5,818, -2,689)		0.05			(-18,386, -3,754)		0.06	0.11
n	16	15	14	14		10	10	9	8		
Fasting AG:DAG	0.094	0.075	0.087	0.091 ± 0.031		0.074	0.076	0.064	0.131 ± 0.027		
	(0.043, 0.121)	(0.044, 0.089)	(0.053, 0.114)		0.27	(0.051, 0.105)	(0.065, 0.097)	(0.047, .093)		0.32	0.19
n	16	15	14	14		10	10	9	8		
$AUC_{0\text{-}180}$ AG:DAG	0.089	0.076	0.089 ± 0.033	0.085		0.093	0.104	0.094 ± 0.033	0.109		
	(0.061, 0.132)	(0.057, 0.09)		(0.072, 0.108)	0.18	(0.064, 0.125)	(0.063, 0.178)		(0.091, 0.117)	0.33	0.13
n	16	15	14	14		10	10	9	8		
Δ$AUC_{0\text{-}180}$ AG:DAG	0.055	0.032 ± 0.132	0.08	0.102		0.05	0.034 ± 0.166	0.04	0.149		
	(0.007, 0.168)		(-0.019, 0.11)	(-0.022, 0.196)	0.62	(0.028, 0.13)		(0.014, 0.145)	(-0.188, 0.23)	0.58	0.77
n	16	15	14	14		10	10	9	8		

3.3.1.4.3 Good versus poor weight loss

PYY: At pre-surgery, the levels of fasting PYY and $\Delta AUC_{0\text{-}180}$ PYY in good weight loss were non-significantly greater than poor weight loss, whilst the levels of $AUC_{0\text{-}180}$ PYY were comparable between groups (Appendix 8, Figure 3.7A-C). At 6-week post-surgery, patients with good weight loss exhibited higher levels of fasting PYY, $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY than poor weight loss patients (Appendix 8, Figure 3.7A-C). The fasting PYY levels in good weight loss were also greater than poor weight loss at 6 months and 1 year (Appendix 8, Figure 3.7A). However, the levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in good weight loss were lower than poor weight loss at 6 months, and then the levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in good weight loss were greater than poor weight loss again at 1 year (Appendix 8, Figure 3.7 B-C).

Figure 3.8A-B shows postprandial response of PYY to the test meal. The graphs show that the augmentation in the poor weight loss group was flatter than the good weight loss group. In good weight loss, a significant rise was seen from 15 to 180 minutes at 6 weeks, from 0 to 150 minutes at 26 weeks and from 15 to 60 minutes at 52 weeks. There was a significant drop from 150 to 180 minutes at 52 weeks (Figure 3.8A). In poor weight loss, a considerable increase was observed from 15 to 60 minutes at all visits (Figure 3.8B).

AG: Following the surgery, the levels of fasting AG and $AUC_{0\text{-}180}$ AG declined substantially at 6 weeks and gradually rose afterwards in both good and poor weight loss groups (Figure 3.7D-E, Appendix 8). The postprandial suppression of AG, $\Delta AUC_{0\text{-}180}$, also significantly decreased after the surgery in both groups (Figure 3.7F, Appendix 8). No statistically significant difference in fasting AG, $AUC_{0\text{-}180}$ AG and $\Delta AUC_{0\text{-}180}$ AG between groups was seen.

During the MMTT, there was a statistically significant fall from 0 – 60, 150 – 180 minutes at 6 weeks, from 0 – 30 minutes at 26 weeks, and at 0, 30, 120 minutes for 1-year visit in good weight loss subjects (Figure 3.8C). In poor weight loss group, the considerable drop was seen from 0 – 90 minutes at 6 weeks, at 0 minute and from 30 – 60 minutes for 26-week visit and at 0, 30 minutes for 1-year visit (Figure 3.8D).

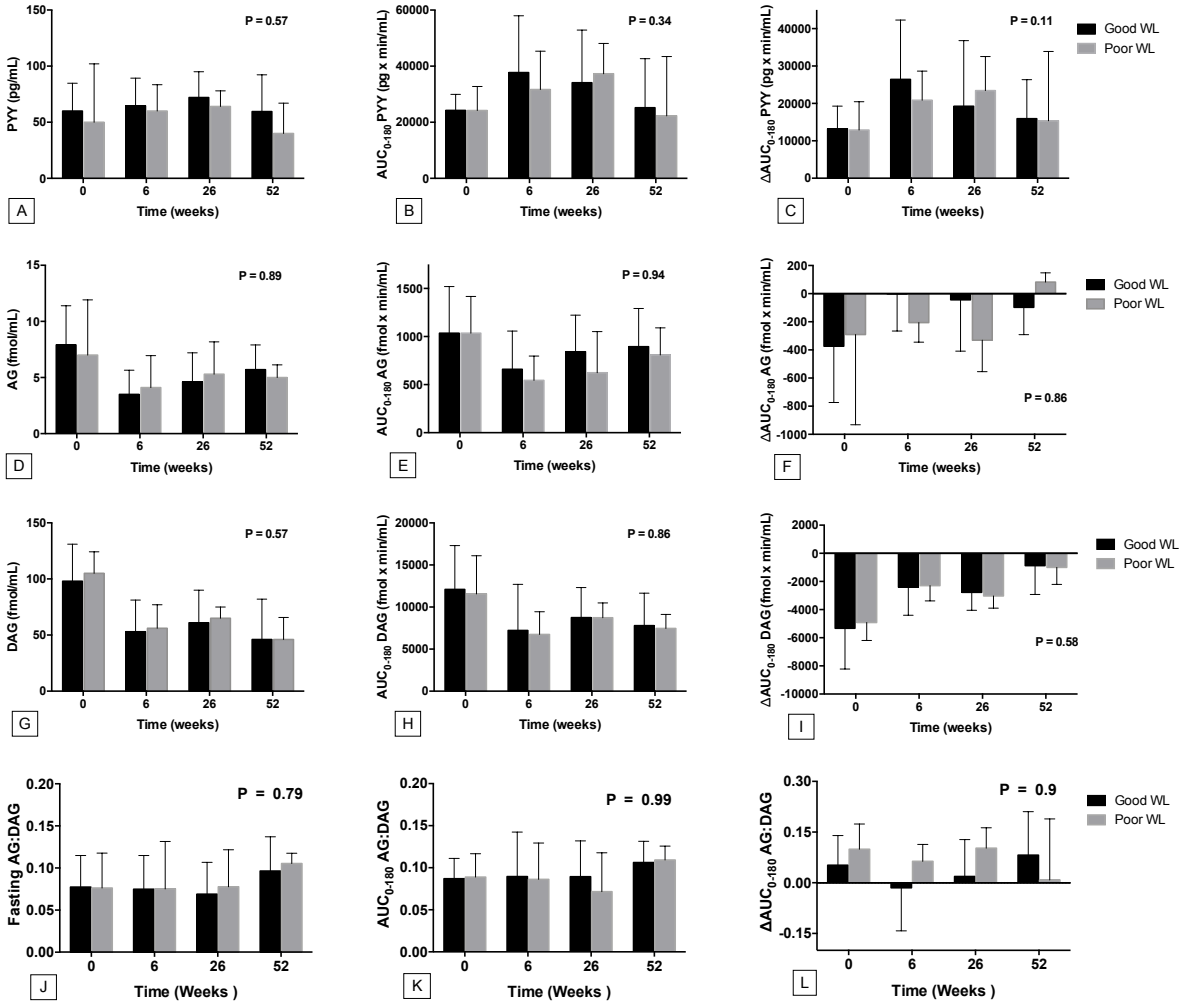


Figure 3.7 Comparison of PYY, AG and DAG parameters between good and poor weight loss at each visit (A) fasting PYY (B) $AUC_{0\text{-}180}$ PYY (C) $\Delta AUC_{0\text{-}180}$ PYY (D) fasting AG (E) $AUC_{0\text{-}180}$ AG (F) $\Delta AUC_{0\text{-}180}$ AG (G) fasting DAG (H) $AUC_{0\text{-}180}$ DAG (I) $\Delta AUC_{0\text{-}180}$ DAG (J) fasting AG:DAG (K) $AUC_{0\text{-}180}$ AG:DAG (L) $\Delta AUC_{0\text{-}180}$ AG:DAG. Results were expressed as mean ±SEM or median (interquartile range), according to their distribution. $^{\alpha}P < 0.05$, $^{\alpha\alpha}P < 0.01$, $^{\alpha\alpha\alpha}P < 0.001$ of the comparison between groups at each visit, P from mixed model analysis of the comparison between groups over 1 year.

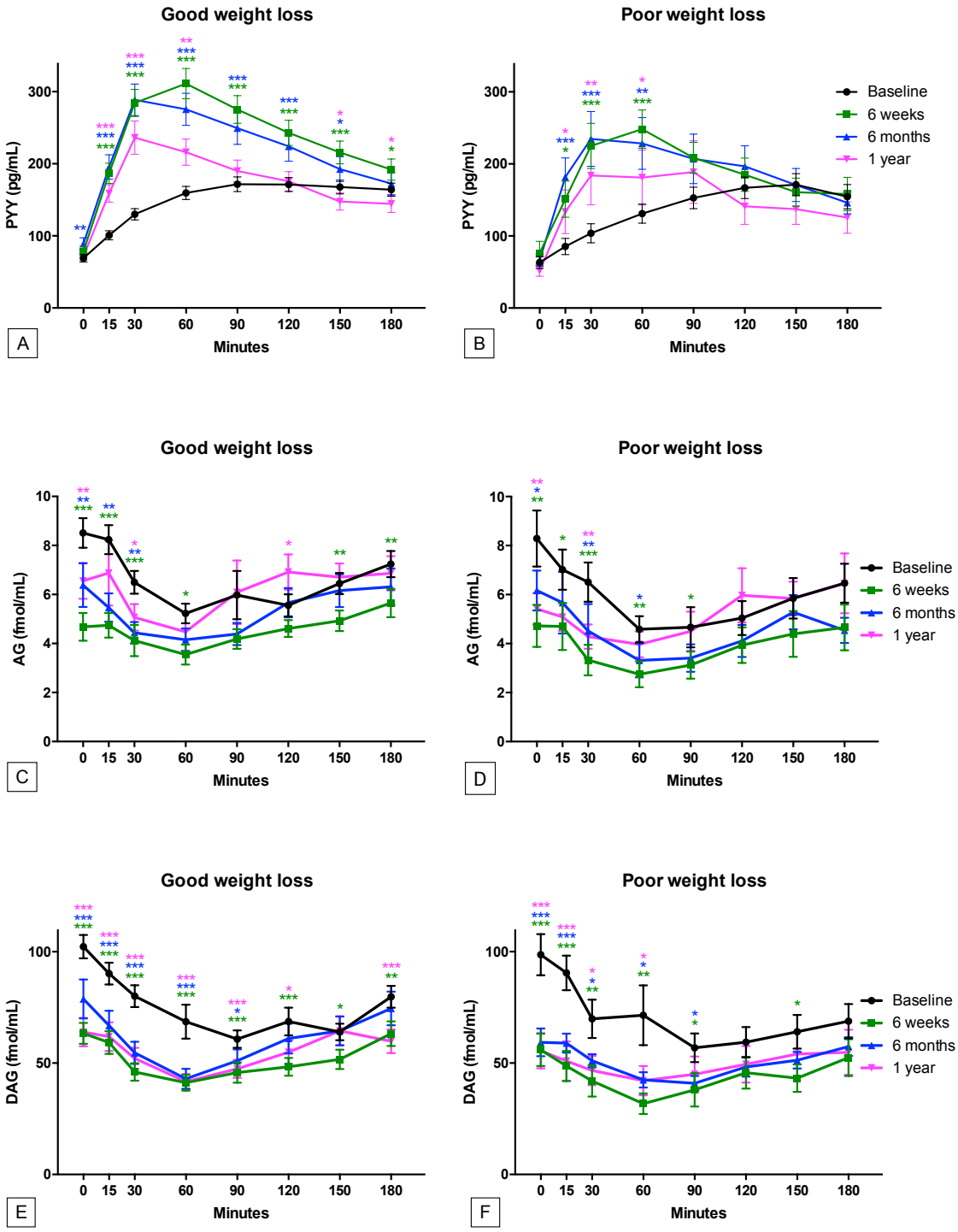


Figure 3.8 Comparison of fasting and nutrient-stimulated PYY, AG and DAG levels between subjects with good and poor WL outcomes (A) PYY in Good WL (B) PYY in Poor WL (C) AG in Good WL (D) AG in Poor WL (E) DAG in Good WL (F) DAG in Poor WL. Results were expressed as mean ±SEM. *P <0.05, **P <0.01, ***P <0.001 in green represents the comparison between baseline and 6 weeks, in blue represents the comparison between baseline and 6 months and in pink represents the comparison between baseline and 1 year

DAG: There was also a significant drop of fasting DAG and $AUC_{0\text{-}180}$ DAG after bariatric surgery in both good and poor weight loss groups in consistent with the figures seen in AG (Figure 3.7G-H, Appendix 8). The postprandial suppression of DAG, $\Delta AUC_{0\text{-}180}$, also significantly decreased after the surgery in both groups (Figure 3.7I, Appendix 8). The statistically significant difference in fasting, $AUC_{0\text{-}180}$, and $\Delta AUC_{0\text{-}180}$ between groups was not observed.

In response to the MMTT, postprandial DAG in good weight loss group reduced considerably from 0 to 180 minutes at 6 weeks, from 0 to 90 minutes at 26 weeks, and from 0 to 120 minutes and at 180 minutes for 1-year visit (Figure 3.8E). Regarding the poor weight loss subjects, the significant drop was seen from 0 to 90 minutes and at 150 minutes for 6 weeks, from 0 to 90 minutes for 26 weeks, and from 0 to 60 minutes for 1 year (Figure 3.8F).

AG:DAG ratio: There was no statistically significant alteration in fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG, and $\Delta AUC_{0\text{-}180}$ AG:DAG from pre-surgery in both groups, and nor were the significant differences between groups (Appendix 8).

Stratification of 1-year weight loss outcomes (Poor vs Good weight loss) by type of surgery

Again, as the type of bariatric surgery markedly impacts on the post-operative alteration of PYY and ghrelin levels, we compared the gut hormone profiles between good and poor weight loss groups in each type of surgery in order to eliminate the effects of the surgery.

Following LSG, the levels of post-operative $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY were greater than pre-operative; nonetheless, the changes only reached the statistical significance in good weight loss. The significant difference in these parameters between groups was not observed, only a marginally significant difference in $\Delta AUC_{0\text{-}180}$ PYY between groups (Table 3.4). After LRYGB, the value of all PYY parameters were higher than baseline with the levels in good weight loss more pronounced than poor weight loss. There was no statistically significant difference between groups; however, the number of subjects were too small to compare (Table 3.5).

Table 3.4 PYY, AG and DAG changes after laparoscopic sleeve gastrectomy (LSG) in patients with poor weight loss versus good weight loss at 1 year

	Poor weight loss (n = 12)					Good weight loss (n = 44)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	56	46	60	40	0.4	60	61	65	48	0.2	0.52
	(46, 105)	(34, 70)	(49, 78)	(34, 60)		(43, 81)	(43, 79)	(52, 84)	(35, 77)		
n	12	10	10	9		44	40	38	39		
AUC_{0-180} PYY, pg x min/mL	26,359	27,760	33,009	22,297	0.61	23,378	33,450	28,505	22,495	<0.001	0.21
	(16,531, 33,757)	(22,048, 45,066)	(16,683, 40,478)	(10,476, 44,267)		(19,172, 27,825)	(27,437, 40,489)	(20,393, 40,169)	(18,366, 29,238)		
n	12	10	10	9		43	39	38	38		
Δ AUC_{0-180} PYY, pg x min/mL	11,121	18,182	18,979	15,345	0.3	12,758	22,650	14,886	13,553	<0.001	0.06
	(12,758, 17,348)	(9,929, 25,198)	(6,139, 28,441)	(3,474, 35,888)		(8,936, 17,348)	(15,143, 30,338)	(9,152, 28,462)	(6,015, 19,132)		
n	12	10	10	9		43	39	38	38		
Fasting AG, fmol/mL	6.3 (4.3, 9.6)	3.7 (2.2, 6)	5.1 (3.4, 6.9)	4.8 (3.2, 5.6)	0.04	8 (4.8, 10.9)	2.9 (1, 4.9)	2.7 (1.7, 5.6)	3.4 (2.1, 6.2)	<0.001	0.42
n	11	10	9	10		44	40	35	39		
AUC_{0-180} AG, fmol x min/mL	975	537	503	717	0.02	945	549	622	707	<0.001	0.99
	(578, 1,515)	(362, 720)	(457, 921)	(623, 935)		(698, 1,497)	(375, 1,028)	(388, 1,038)	(547, 1,012)		
n	10	10	9	10		43	39	35	39		
Δ AUC_{0-180} AG, fmol x min/mL	-263	-228	-373	-43	0.25	-392	66	45	56	<0.001	0.44
	(-652, -147)	(-334, 78)	(-655, 0)	(-302, 136)		(-739, -87)	(-131, 244)	(-322, 287)	(-193, 230)		
n	10	10	9	10		43	39	35	39		
Fasting DAG, fmol/mL	96 ± 34	52 ± 21	62 (42, 74)	44 (37, 51)	<0.001	99 ± 40	45 ± 17	48 (32, 61)	37 (28, 48)	<0.001	0.79

n	11	10	10	10		44	40	37	39		
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	13,264 ± 5,337	6,945 (5,035, 8,993)	8,446 (7,096, 9,570)	6,769 (5,357, 7,940)	<0.001	13,242 ± 5,886	5,967 (4,180, 7,680)	7,535 (4,208, 9,903)	6,093 (4,817, 7,935)	<0.001	0.95
n	10	10	10	10		43	39	37	38		
Δ $AUC_{0\text{-}180}$ DAG, fmol x min/mL	-4,204 ± 1,741	-2,196 (-3,311, -1,444)	-2,708 (-4,156, -250)	-1,342 ± 1,108	0.02	-4,770 ± 4,760	-1,381 (-3,158, -254)	-1,687 (-2,840, -3)	-342 ± 1,505	<0.001	0.6
n	10	10	10	10		43	39	37	38		
Fasting AG:DAG	0.063 (0.046, 0.124)	0.066 (0.049, 0.177)	0.076 (0.059, 0.122)	0.111 (0.093, 0.121)	0.63	0.077 (0.058, 0.113)	0.075 (0.025, 0.127)	0.065 (0.037, 0.115)	0.088 (0.052, 0.145)	0.54	0.84
n	11	10	9	10		44	40	35	39		
$AUC_{0\text{-}180}$ AG:DAG	0.078 (0.046, 0.099)	0.069 (0.054, 0.16)	0.056 (0.049, 0.121)	0.119 (0.098, 0.13)	0.43	0.088 (0.061, 0.11)	0.092 (0.05, 0.146)	0.09 (0.057, 0.148)	0.108 (0.092, 0.161)	0.006	0.95
n	10	10	9	10		43	39	35	38		
Δ$AUC_{0\text{-}180}$ AG:DAG	0.095 (0.032, 0.195)	0.073 (-0.129, 0.13)	0.108 (-0.051, 0.158)	0.105 (-0.136, 0.321)	0.99	0.063 (-0.001, 0.14)	-0.046 (-0.224, 0.055)	-0.004 (-0.136, 0.159)	0.052 (-0.205, 0.217)	0.46	0.88
n	10	10		10		43	39		38		

Table 3.5 PYY, AG and DAG changes after laparoscopic Roux-en-Y gastric bypass (LRYGB) in patients with poor weight loss versus good weight loss at 1 year

	Poor weight loss (n = 3)					Good weight loss (n = 24)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	47 ± 16	108 ± 68	87 ± 54	71 ± 53	0.21	76 ± 51	99 ± 80	115 ± 88	92 ± 53	0.13	0.8
n	3	3	3	2		24	22	21	19		
AUC_{0-180} PYY, pg x min/mL	22,434 ± 2,205	46,764 ± 14,617	49,525 ± 22,559	29,676 ± 15,427	0.08	30,890 ± 15,294	58,516 ± 25,368	56,423 ± 31,151	46,885 ± 20,496	<0.001	0.98
n	3	3	3	2		24	22	21	19		
Δ AUC_{0-180} PYY, pg x min/mL	14,037 ± 1,717	27,285 ± 5,226	33,877 ± 18,598	16,949 ± 5,931	0.11	17,284 ± 11,781	40,700 ± 24,206	35,795 ± 27,330	30,349 ± 19,386	<0.001	0.7
n	3	3	3	2		24	22	21	19		
Fasting AG, fmol/mL	10.6 ± 2.9	5.7 ± 4.3	7.4 ± 3.1	8.3 ± 3	0.16	8.7 ± 4.5	7.3 ± 5.9	10.1 ± 8.7	11.7 ± 6.6	0.02	0.48
n	3	3	3	2		23	22	20	20		
AUC_{0-180} AG, fmol x min/mL	1,117 ± 199	852 ± 557	1,048 ± 463	1,632 ± 554	0.09	1,196 ± 584	1,062 ± 519	1,348 ± 881	1,630 ± 1,123	0.03	0.96
n	3	3	3	2		23	22	20	20		
Δ AUC_{0-180} AG, fmol x min/mL	-798 ± 465	-174 ± 220	-285 ± 153	141 ± 21	0.04	-360 ± 683	-249 ± 756	-466 ± 1,018	-474 ± 823	0.71	0.44
n	3	3	3	2		23	22	20	20		
Fasting DAG, fmol/mL	109 ± 42	68 ± 43	73 ± 13	108 ± 25	0.18	108 ± 49	97 ± 41	132 ± 87	111 ± 58	0.03	0.26

n	3	3	3	2		23	22	20	20		
AUC_{0-180} DAG, fmol x min/mL	9,959 ± 1,389	9,478 ± 6,886	10,320 ± 2,127	18,634 ± 4,781	0.1	12,619 ± 5,456	12,937 ± 6,505	15,849 ± 10,113	15,405 ± 7,978	0.03	0.23
n	3	3	3	2		23	22	20	20		
Δ AUC_{0-180} DAG, fmol x min/mL	-9,683 ± 6,362	-2,738 ± 1,253	-2,756 ± 1,374	-717 ± 416	0.09	-6,863 ± 5,969	-4,504 ± 2,994	-7,973 ± 7,694	-4,569 ± 3,587	0.02	0.16
n	3	3	3	2		23	22	20	20		
Fasting AG:DAG	0.101 ± 0.014	0.08 ± 0.009	0.1 ± 0.032	0.076 ± 0.01	0.41	0.101 ± 0.109	0.078 ± 0.054	0.087 ± 0.077	0.109 ± 0.035	0.5	0.91
n	3	3	3	2		23	22	20	20		
AUC_{0-180} AG:DAG	0.112 ± 0.009	0.094 ± 0.012	0.098 ± 0.026	0.087 ± 0.008	0.4	0.117 ± 0.099	0.095 ± 0.053	0.09 ± 0.034	0.11 ± 0.071	0.48	0.96
n	3	3	3	2		23	22	20	20		
ΔAUC_{0-180} AG:DAG	0.089 ± 0.02	0.043 ± 0.071	0.16 ± 0.178	-0.247 ± 0.173	0.03	0.056 ± 0.232	0.032 ± 0.151	0.102 ± 0.238	0.201 ± 0.404	0.18	0.15
n	3	3	3	2		23	22	20	20		

There was a significant fall in the levels of fasting AG, $AUC_{0\text{-}180}$ AG and the postprandial suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) after LSG from baseline in both groups with no significant difference between groups (Table 3.4). Post-LRYGB, the fasting AG and $AUC_{0\text{-}180}$ AG in good weight loss statistically significantly increased from baseline with no significant difference between groups (Table 3.5).

Regarding the DAG profiles, following LSG, subjects with good weight loss outcome at 1 year showed non-significantly lower fasting DAG and $AUC_{0\text{-}180}$ DAG, but non-significantly higher $\Delta AUC_{0\text{-}180}$ DAG than poor weight loss subjects (Table 3.4). In LRYGB, all DAG parameters (fasting DAG, $AUC_{0\text{-}180}$ DAG, and the suppression of postprandial DAG) in both groups slightly dropped post-operatively, and then rose to the pre-surgery levels or greater (Table 3.5). Nonetheless, the differences in all of these parameters between groups did not achieve statistical significance.

There was no statistically significant difference in fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG and $\Delta AUC_{0\text{-}180}$ AG:DAG between patients with good and poor weight-loss outcomes after LRYGB and LSG (Table 3.4, Table 3.5).

3.3.1.4.4 Highest versus lowest quartiles of weight loss

We further compared patients who achieved 1-year PWL in the highest quartile (PWL = 33.9 [31.2, 38]%) to lowest quartile (PWL = 18.7 [12.8, 20]%). Table 3.6 shows baseline characteristics between the two groups. There was no statistically significant difference in age, gender, pre-operative BW, and BMI between groups. The number of patients undergoing LSG and patients having T2D in the lowest quartile was statistically significantly greater than the highest quartile (85.7% vs 57.1%, P = 0.04 for LSG and 52.4% vs 19%, P = 0.02 for T2D, Table 3.6). No difference between groups in any gut hormone profiles was observed at the pre-surgery.

After surgery, the levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY significantly rose from pre-surgery in both groups, with the figures in the highest quartile being higher than the lowest quartile (Table 3.7). Table 3.8 specifically compared $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks and 6 months between groups. The $AUC_{0\text{-}180}$ PYY in the highest quartile was significantly greater than the lowest quartile (P = 0.03).

There was a marked fall in fasting AG, $AUC_{0\text{-}180}$ AG, and the postprandial suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) after surgery compared to pre-surgery in both groups, and no statistically significant difference between groups observed in this study. DAG followed the same pattern as AG (Table 3.7). The significant post-operative changes in fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG and $\Delta AUC_{0\text{-}180}$ AG:DAG from pre-surgery in each group and the significant differences of these parameters between groups were not observed (Table 3.7).

Table 3.6 Baseline characteristics of patients with lowest and highest quartile of weight loss

	Total (n = 85)	Percentage weight loss	
		Highest quartile (n = 21)	lowest quartile (n = 21)
Age, years	45.9 ± 11.6	41.8 ± 13.5	48.1 ± 11.7
n	85	21	21
Female, %	90.6	90.5	81
BW, kg	125.5 ± 22.5	125.1 ± 23.3	125.7 ± 20
n	84	21	21
BMI, kg/m²	44.5 (41.1, 50.5)	43.2 (39.9, 47.7)	43.1 (40.8, 50.1)
n	84	21	21
T2D, %	32.9	19	52.4
RYGB, %	31.8	42.9	14.3
SBP, mmHg	125 ± 14	123 (116, 137)	121 (113, 128)
n	85	21	21
DBP, mmHg	67 (62, 76)	69 (63, 77)	66 (60, 76)
n	85	21	21
PR, bpm	74 ± 15	76 (66, 85)	64 (60, 78)
n	84	21	21
HbA_{1c}, mmol/mol	35 (33, 38)	37.5 ± 5.9	43.2 ± 10.5
n	82	20	20
Fasting PYY, pg/mL	60 (40, 85)	57 (42, 83)	61 (38, 102)
n	83	21	21
$AUC_{0\text{-}180}$ PYY, pg x min/mL	24,218 (19,578, 30,379)	24,884 (20,905, 31,658)	23,174 (18,166, 30,564)
n	82	21	21
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	13,039 (8,768, 19,542)	16,358 (10,132, 20,296)	11,135 (7,055, 19,321)
n	82	21	21
Fasting AG, fmol/mL	7.8 (4.9, 11.5)	8.8 (5.6, 11.4)	6.5 (4.8, 11.1)
n	81	20	20
$AUC_{0\text{-}180}$ AG, fmol x min/mL	1,035 (679, 1,498)	1,127 ± 562	987 ± 393
n	79	20	19
$\Delta AUC_{0\text{-}180}$ AG, fmol x min/mL	-361 (-792, -92)	-543 ± 695	-481 ± 643
n	79	20	19
Fasting DAG, fmol/mL	102 ± 41	109 ± 54	99 ± 34
n	82	20	20
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	11,776 (8,381, 17,063)	12,162 ± 6,298	13,633 ± 5,381
n	80	20	19

$\Delta AUC_{0\text{-}180}$ DAG,	-4,925	-6,672	-4,442
fmol x min/mL	(-7,788, -2,623)	(-8,919, -3,112)	(-5,680, -1,517)
n	80	20	19
Fasting AG:DAG	0.077 (0.055, 0.115)	0.086 (0.055, 0.121)	0.064 (0.047, 0.112)
n	81	20	20
$AUC_{0\text{-}180}$ AG:DAG	0.088 (0.062, 0.112)	0.093 (0.071, 0.121)	0.071 (0.047, 0.102)
n	79	20	19
$\Delta AUC_{0\text{-}180}$ AG:DAG	0.062 (0.024, 0.14)	0.07 (0.027, 0.19)	0.04 (-0.015, 0.094)
n	79	20	19

Table 3.7 Weight loss parameters, glycaemic indices, PYY, AG, DAG and AG:DAG profiles after bariatric surgery, divided patients by lowest and highest quartile of weight loss

	Highest quartile (n = 21)					Lowest quartile (n = 21)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
BW, kg	125.1 ± 23.3	111.5 ± 20.9	88.5 (78.1, 105,2)	80.8 (69.3, 92.9)	<0.001	125.7 ± 20	115.6 ± 19	98.5 (88.2, 120.5)	106 (87.9, 121.6)	<0.001	<0.001
n	21	21	20	21		21	21	20	21		
BMI, kg/m²	43.2 (39.9, 47.7)	39.4 (34.9, 43.1)	30.7 (29.6, 35.9)	29.1 ± 3.6	<0.001	43.1 (40.8, 50.1)	39.6 (37.1, 47)	36.2 (33.5, 42.6)	37.6 ± 5.3	<0.001	<0.001
n	21	21	20	21		21	21	20	21		
PWL, %	0	9.7 (8.9, 12.3)	27.3 ± 4.8	33.9 (31.2, 38)	<0.001	0	7.1 (6.4, 9.2)	16.5 ± 4	18.7 (12.8, 20)	<0.001	<0.001
n	21	21	20	21		21	21	20	21		
WCV, kg/week	0	-2.4 (-2.98, -2.23)	-1.02 ± 0.37	-0.27 (-0.51, -0.18)	<0.001	0	-1.83 (-2.22, -1,59)	-0.51 ± 0.24	-0.03 (-0.17, 0.07)	<0.001	0.05
n	21	21	20	20		21	21	20	20		
HbA_{1c}, mmol/mol	37.5 ± 5.9	34.1 ± 4.8	33.4 ± 3.6	34 (31.5, 35)	<0.001	43.2 ± 10.5	36.8 ± 6.1	36.3 ± 5.5	38 (33, 41)	<0.001	0.44
n	20	15	19	20		20	16	19	19		
Fasting PYY level, pg/mL	57 (42, 83)	58 (48, 96)	72 (49, 104)	48 (34, 76)	0.11	61 (38, 102)	60 (36, 76)	64 (53, 77)	46 (35, 71)	0.65	0.72
n	21	19	18	17		21	19	19	17		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	24,884 (20,905, 31,658)	37,783 (31,954, 52,035)	42,190 ± 18,274	23,818 (20,585, 40,700)	<0.001	23,174 (18,166, 30,564)	30,120 (24,086, 45,353)	31,889 ± 15,387	22,069 (14,761, 37,329)	0.007	0.1
n	21	19	18	16		21	19	19	17		
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	16,358 (10,132, 20,296)	26,580 (20,018, 33,591)	28,044 ± 16,406	15,832 (12,455, 26,328)	<0.001	11,135 (7,055, 19,321)	20,842 (9,973, 28,512)	20,078 ± 13,738	12,211 (4,162, 23,112)	0.007	0.25
n	21	19	18	16		21	19	19	17		

Fasting AG,	8.8	4.6	5.4	6.5		6.5	3.3	4.6	4.7		
fmol/mL	(5.6, 11.4)	(1.8, 8)	(1.7, 10.1)	(2.2, 8)	0.049	(4.8, 11.1)	(2.4, 4.9)	(3, 8.1)	(3.4, 6)	<0.001	0.87
n	20	19	17	18		20	19	18	18		
$AUC_{0\text{-}180}$ AG,	1,127 ± 562	652	910	916		987 ± 393	549	722	717		
fmol x min/mL		(391, 1,070)	(455, 1,331)	(577, 1,388)	0.17		(371, 829)	(469, 1,061)	(619, 1,003)	0.002	0.53
n	20	19	17	18		19	19	18	18		
$\Delta AUC_{0\text{-}180}$ AG,	-543 ± 695	-113 ± 449	-151 ± 518	-17		-481 ± 643	-80 ± 291	-250 ± 562	-11		
fmol x min/mL				(-279, 199)	0.04				(-254, 134)	0.01	0.91
n	20	19	17	18		19	19	18	18		
Fasting DAG,	109 ± 54	53	55	49		99 ± 34	47	63	44		
fmol/mL		(36, 105)	(36, 124)	(28, 87)	0.009		(32, 77)	(42, 72)	(38, 59)	<0.001	0.99
n	20	19	18	18		20	19	19	18		
$AUC_{0\text{-}180}$ DAG,	12,162 ± 6,298	7,680	8,854	7,663		13,633 ± 5,381	6,740	8,163	7,456		
fmol x min/mL		(3,858, 13,874)	(4,935, 15,183)	(5,471, 13,693)	0.48		(5,257, 8,536)	(7,114, 10,075)	(5,940, 9,415)	<0.001	0.06
n	20	19	18	17		19	19	19	18		
$\Delta AUC_{0\text{-}180}$ DAG,	-6,672	-2,792 ± 4,908	-2,826	-1,118		-4,442	-2,070 ± 1,654	-1,958	-975		
fmol x min/mL	(-8,919, -3,112)		(-4,326, -2,826)	(-4,318, 106)	0.001	(-5,680, -1,517)		(-3,770, -22)	(-1,671, -152)	0.004	0.17
n	20	19	18	17		19	19	19	18		
Fasting AG:DAG	0.086	0.079	0.064	0.097		0.064	0.07	0.073	0.1		
	(0.055, 0.121)	(0.044, 0.15)	(0.036, 0.11)	(0.051, 0.149)	0.3	(0.047, 0.112)	(0.053, 0.127)	(0.057, 0.12)	(0.071, 0.116)	0.82	0.31
n	20	19	17	18		20	19	18	18		
$AUC_{0\text{-}180}$ AG:DAG	0.093	0.07	0.096	0.11		0.071	0.088	0.072	0.1		
	(0.071, 0.121)	(0.05, 0.13)	(0.074, 0.143)	(0.083, 0.17)	0.08	(0.047, 0.102)	(0.058, 0.108)	(0.051, 0.128)	(0.09, 0.121)	0.2	0.25
n	20	19	17	17		19	19	18	18		
$\Delta AUC_{0\text{-}180}$ AG:DAG	0.07	0.005	0.011	0.123		0.04	-0.005	0.111	-0.023		
	(0.027, 0.19)	(-0.104, 0.096)	(-0.094, 0.105)	(-0.173, 0.215)	0.22	(-0.015, 0.094)	(-0.099, 0.11)	(-0.006, 0.316)	(-0.225, 0.128)	0.23	0.2
n	20	19	17	17		19	19	18	18		

Table 3.8 Comparison of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks and 6 months between patients with highest and lowest quartile of PWL

	Highest quartile	**Lowest quartile**	**P-value**
At 6 weeks			
$AUC_{0\text{-}180}$ PYY, pg x min/mL **n**	37,783 (31,954, 52,035) 19	30,120 (24,086, 45,353) 19	0.03
Δ $AUC_{0\text{-}180}$ PYY, pg x min/mL **n**	26,580 (20,018, 33,591) 19	20,842 (9,973, 28,512) 19	0.06
At 6 months			
$AUC_{0\text{-}180}$ PYY, pg x min/mL **n**	42,190 ± 18,274 18	31,889 ± 15,387 19	0.07
Δ $AUC_{0\text{-}180}$ PYY, pg x min/mL **n**	28,044 ± 16,406 18	20,078 ± 13,738 19	0.12

3.3.2 Subgroup analysis in patients with T2D

3.3.2.1 Baseline characteristics of patients with T2D

Twenty-eight patients had been diagnosed with T2D prior to the surgery. The majority of patients were female (82.1%) and underwent LSG (64.3%). The median of age, diabetes duration and HbA_{1c} levels were 51.9 (41.3, 58.1) years old, 3 (1, 6) years and 49.2 (43.2, 54.1) mmol/mol. The mean BW and BMI were 124.1 ± 23.9 kg and 45.7 ± 6.6 kg/m^2, respectively. The average SBP, DBP and PR were 126 ± 12 mmHg, 69 ± 9 mmHg and 74 ± 16 bpm (Table 3.9).

BW, BMI and HbA_{1C} in LSG group were greater than LRYGB group (128.5 ± 24.9 vs 116.2 ± 20.8 kg, 46.3 ± 7.4 vs 44.7 ± 5.2 kg/m^2 and 51.4 [44.8, 60.7] vs 45.9 [41, 53] mmol/mol); however, the difference did not achieve statistical significance (Table 3.9). Subjects in LRYGB had marginally significantly higher duration of diabetes than LSG (5.5 (2.8, 7) vs 2 (1, 5.6), P = 0.06, Table 3.9). In terms of gut hormones parameters, there was no significant difference between groups.

Patients who met the criteria of diabetes remission at 1 year were older, pre-surgery had BW and BMI slightly higher than patients with no diabetes remission (51.9 [42.2, 58.2] vs 46.6 [31.7, 60.5] years old, 126 ± 23.8 vs 115.2 ± 24.6 kg, and 45.9 ± 5.7 vs 44.9 ± 10.8 kg/m^2, respectively). Nevertheless, the differences between groups were not statistically significant (Table 3.9). A hundred percent of non-remitters were female, whilst around 80% were female in remitters. The percentage of remitters undergoing LRYGB was twice as many as non-remitters. The difference in duration of diabetes between groups was marginally significant (6 [5.3, 8] years in non-remitters vs 2 [1, 6] years in remitters, P = 0.06, Table 3.9). The significant difference in all gut hormones profiles between groups was not observed.

Table 3.9 Pre-surgery characteristics of patients with T2D

	Total (n = 28)	**Type of surgery**		**Diabetes remission at 1 year**	
		LRYGB (n = 10)	**LSG (n = 18)**	**Non-remitters (n = 5)**	**Remitters (n = 23)**
Age, years	51.9 (41.3, 58.1)	51 (39.3, 61.7)	53.4 (43.6, 57.7)	46.6 (31.7, 60.5)	51.9 (42.2, 58.2)
n	28	10	18	5	23
Female, %	82.1	80	83.3	100	78.3
BW, kg	124.1 ± 23.9	116.2 ± 20.8	128.5 ± 24.9	115.2 ± 24.6	126 ± 23.8
n	28	10	18	5	23
BMI, kg/m^2	45.7 ± 6.6	44.7 ± 5.2	46.3 ± 7.4	44.9 ± 10.8	45.9 ± 5.7
n	28	10	18	5	23
RYGB, %	35.7	100	0	20	39.1
SBP, mmHg	126 ± 12	130 ± 10	123 ± 13	125 ± 13.7	126 ± 12
n	28	10	18	5	23
DBP, mmHg	69 ± 9	69 ± 9	69 ± 10	62 (60, 78)	69 (63, 77)
n	28	10	18	5	23
Pulse rate, bpm	74 ± 16	81 (71, 93)	65 (61, 79)	86 ± 24	72 ± 13
n	28	10	18	5	23
Duration of diabetes, years	3 (1, 6)	5.5 (2.8, 7)	2 (1, 5.6)	6 (5.3, 8)	2 (1, 6)
HbA_{1c},	49.2	45.9	51.4	53	47.5
mmol/mol	(43.2, 54.1)	(41, 53)	(44.8, 60.7)	(48.1, 67.8)	(41, 54.1)
n	28	10	18	5	23
Fasting PYY level, pg/mL	84 (61, 110)	100 ± 66	83 ± 27	104 (71, 140)	84 (51, 106)
n	28	10	18	5	23
$AUC_{0\text{-}180}$ PYY, pg x min/mL	30,046 ± 12,136	34,280 ± 16,776	27,556 ± 7,953	31,541 ± 16,337	29,707 ± 11,440

n	27	10	17	5	22
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	13,723 ± 9,700	16,278 ± 12,236	12,221 ± 7,886	12,657 ± 11,857	13,966 ± 9,454
n	27	10	17	5	22
Fasting AG, fmol/mL	7.6 (5.2, 2.2)	9.1 (5.3, 12.6)	6.4 (5.2, 11.6)	5.3 (5.2, 8.8)	7.6 (5.1 12.2)
n	27	10	17	4	23
$AUC_{0\text{-}180}$ AG, fmol x min/mL	1,107 ± 490	1,246 (790, 1,555)	965 (581, 1,565)	1,057 (583, 1,549)	1,022 (683, 1,569)
n	26	10	16	4	22
$\Delta AUC_{0\text{-}180}$ AG, fmol x min/mL	-469 ± 649	-328 (-856, -11)	-354 (-674, -144)	-319 (-358, 398)	-384 (-790, -134)
n	26	10	16	4	22
Fasting DAG, fmol/mL	106 (75, 118)	118 ± 53	99 ± 36	110 (69, 154)	106 (75, 116)
n	27	10	17	4	23
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	12,812 (8,235, 15,645)	13,115 ± 5,133	13,301 ± 6,720	14,082 ± 8,385	13,074 ± 5,774
n	26	10	16	4	22
$\Delta AUC_{0\text{-}180}$ DAG, fmol x min/mL	-5,325 (-8,787, -3,625)	-8,113 ± 8,428	-4,927 ± 3,548	-5,359 (-8,191, -4,044)	-5,325 (-8,787, -2,676)
n	26	10	16	4	22
Fasting AG:DAG	0.076 (0.05, 0.102)	0.074 (0.051, 0.105)	0.076 (0.046, 0.141)	0.067 (0.035, 0.097)	0.076 (0.051, 0.12)
n	27	10	17	4	23
$AUC_{0\text{-}180}$ AG:DAG	0.078 (0.062, 0.111)	0.093 (0.064, 0.125)	0.07 (0.06, 0.106)	0.074 (0.046, 0.143)	0.078 (0.063, 0.111)
n	26	10	16	4	22
$\Delta AUC_{0\text{-}180}$ AG:DAG	0.054 (0.026, 0.13)	0.05 (0.028, 0.13)	0.059 (0.018, 0.166)	0.044 (-0.098, 0.083)	0.057 (0.026, 0.137)
n	26	10	16	4	22

3.3.2.2 Weight loss outcome in people with T2D

The difference in PWL over time between LRYGB and LSG was marginally statistically significant with the PWL after LRYGB slightly greater than the LSG over time; at 6 weeks 8.7 (7, 9.9)% in LRYGB vs 9.3 (7.1, 11.7)% in LSG; at 6 months 20 ± 6.2% in LRYGB vs 19.4 ± 4.3% in LSG and at 1 year 25.6 ± 7.1% in LRYGB vs 21 ± 5.9 in LSG, P = 0.06, Figure 3.9A, **Error! Reference source not found.**. There was a statistically significant reduction in BW a nd BMI at 1 year compared to pre-surgery in both groups (P <0.001 for both BW and BMI, Appendix 9). The difference in BW and BMI between groups did not reach statistical significance (P = 0.25, Figure 3.9C, Appendix 9).

In terms of WCV, at 6 weeks LSG produced a greater weight loss rate than LRYGB (-2.54 ± 0.87 vs -1.9 ± 0.43 kg/week); however, the rate was then comparable at 6 months (-0.6 ± 0.3 kg/week in LSG vs -0.59 ± 0.38 kg/week in LRYGB), and then the weight loss rate in LRYGB was greater than LSG up to 1 year (-0.25 ± 0.15 vs -0.11 ± 0.18 kg/week). Hence, the rate of weight loss in LRYGB was overall statistically significantly greater than in LSG over time (P <0.01, Figure 3.9E, Appendix 9).

Interestingly, the weight loss parameters, PWL and WCV, were comparable between remitters and non-remitter (Figure 3.9B, F, Appendix 10). There was a significant decrease in BW and BMI at 1 year compared to pre-surgery in both remitters and non-remitters groups (P <0.001 for BW and BMI in each group, Appendix 10) with no statistically significant difference between groups (Figure 3.9D, Appendix 10).

3.3.2.3 HbA_{1c} changes after bariatric surgery in patients with T2D

The levels of HbA_{1c} significantly fell from baseline in both LRYGB and LSG groups (P <0.001 for both, Appendix 9). However, the statistically significant difference between groups was not seen.

In non-remitters, there was no statistically significant reduction in HbA_{1c} levels after the surgery from baseline. In contrast, the HbA_{1c} in remitters group dramatically dropped over 1-year time (P <0.001, Appendix 10).

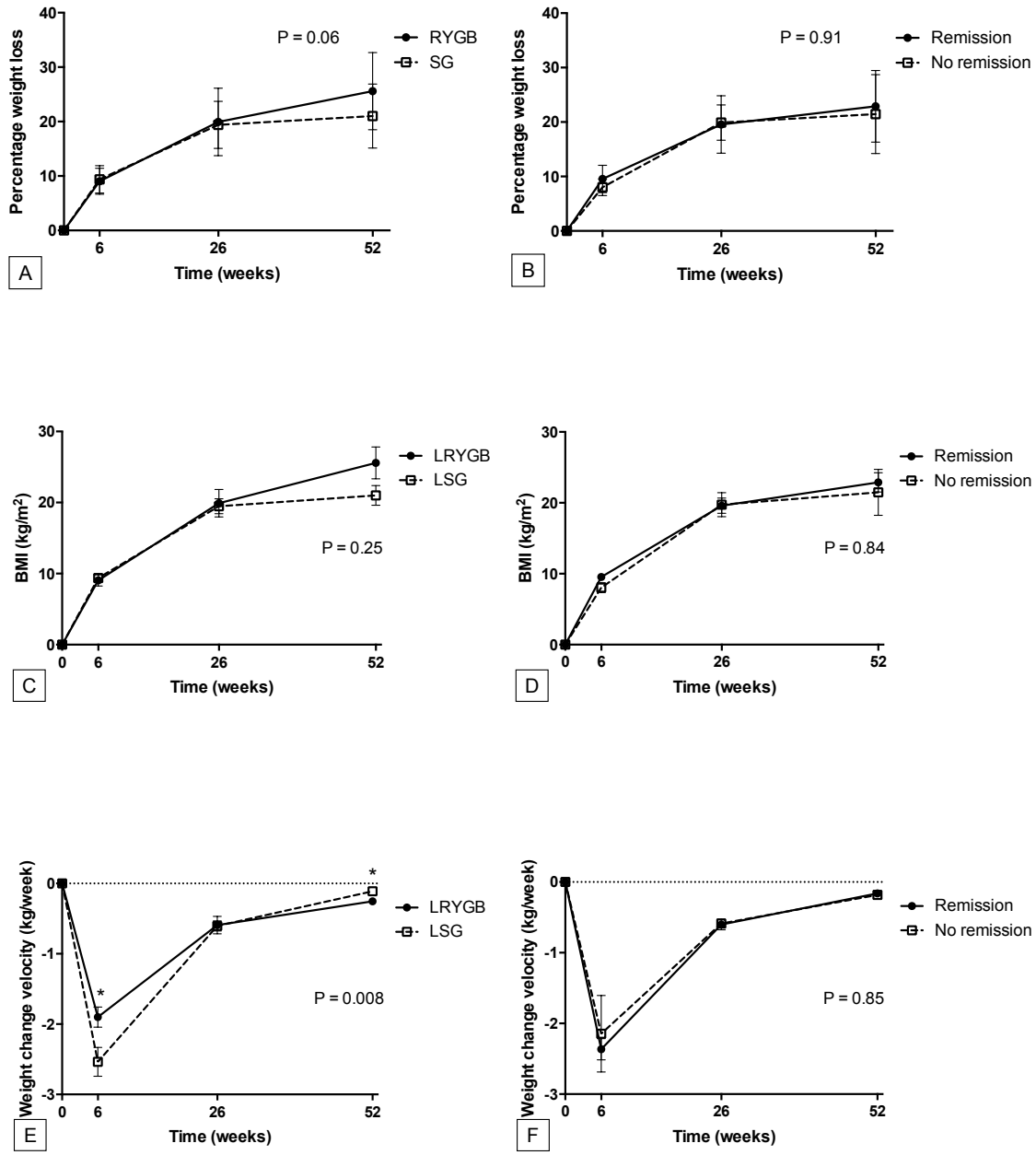


Figure 3.9 Percentage weight loss in subjects with T2D at each visit (A) T2D subjects divided by type of surgery, (B) T2D subjects divided by diabetes remission at 1 year, BMI in subjects with T2D at each visit (C) T2D subjects divided by type of surgery, (D) T2D subjects divided by diabetes remission at 1 year, weight change velocity in subjects with T2D at each visit (E) T2D subjects divided by type of surgery, (F) T2D subjects divided by diabetes remission at 1 year; * P<0.05, ** P<0.01, *** P<0.001 of the comparison between groups at each visit; P-value from mixed model analysis

3.3.2.4 PYY and ghrelin changes after bariatric surgery in patients with T2D

3.3.2.4.1 LRYGB versus LSG in patients with T2D

PYY: Following both types of surgery, there was neither significant changes in fasting PYY from pre-surgery in each group nor differences between groups (Appendix 9, Figure 3.10A). In contrast, the $AUC_{0\text{-}180}$ PYY considerably increased from the pre-surgery in both groups (P <0.001 for LRYGB, P <0.01 for LSG) with the level in LRYGB significantly higher than LSG at all visits (P <0.01, Figure 3.10B, Appendix 9). The $\Delta AUC_{0\text{-}180}$ PYY also marked increased from baseline in both groups (P <0.01 for both); however, the difference between groups was only marginally significant over time (P = 0.05, Figure 3.10C, Appendix 9).

A significant augmentation was observed during the MMTT. In LRYGB, the PYY levels rose substantially from 15 to 180 minutes at 6 weeks and 26 weeks, and from 15 to 90 minutes at 52 weeks (Figure 3.11A). In LSG, the augmentation was significantly seen from 30 to 120 minutes at 6 weeks, from 30 to 90 minutes at 26 and 52 weeks (Figure 3.11B). The augmentation in LRYGB was apparently more pronounced than LSG.

AG: Following LSG, the circulating levels of AG substantially dropped from baseline, making the levels of fasting AG and $AUC_{0\text{-}180}$ AG were statistically significantly lower than after the LRYGB at all visits (Figure 3.10D-E, Appendix 9). The $\Delta AUC_{0\text{-}180}$ AG in LSG was statistically significantly higher than baseline, meaning less postprandial suppression (P = 0.02, Appendix 9), but there was no a significant change in LRYGB. The statistically significant difference of $\Delta AUC_{0\text{-}180}$ AG between groups was not observed (Figure 3.10F, Appendix 9).

Figure 3.11C-D show the postprandial AG levels during the MMTT. AG levels in LSG group were not as fluctuated as in LRYGB. The AG levels were statistically significantly less than baseline from 0 to 180 minutes at 6 and 26 weeks, and from 0 to 60 minutes, at 120 and 180 minutes for 1-year visit (Figure 3.11FD). On the other hand, in LRYGB, the AG levels considerably rose at 150 minutes at 26 weeks and from 120 to 180 minutes at 1 year (Figure 3.11C).

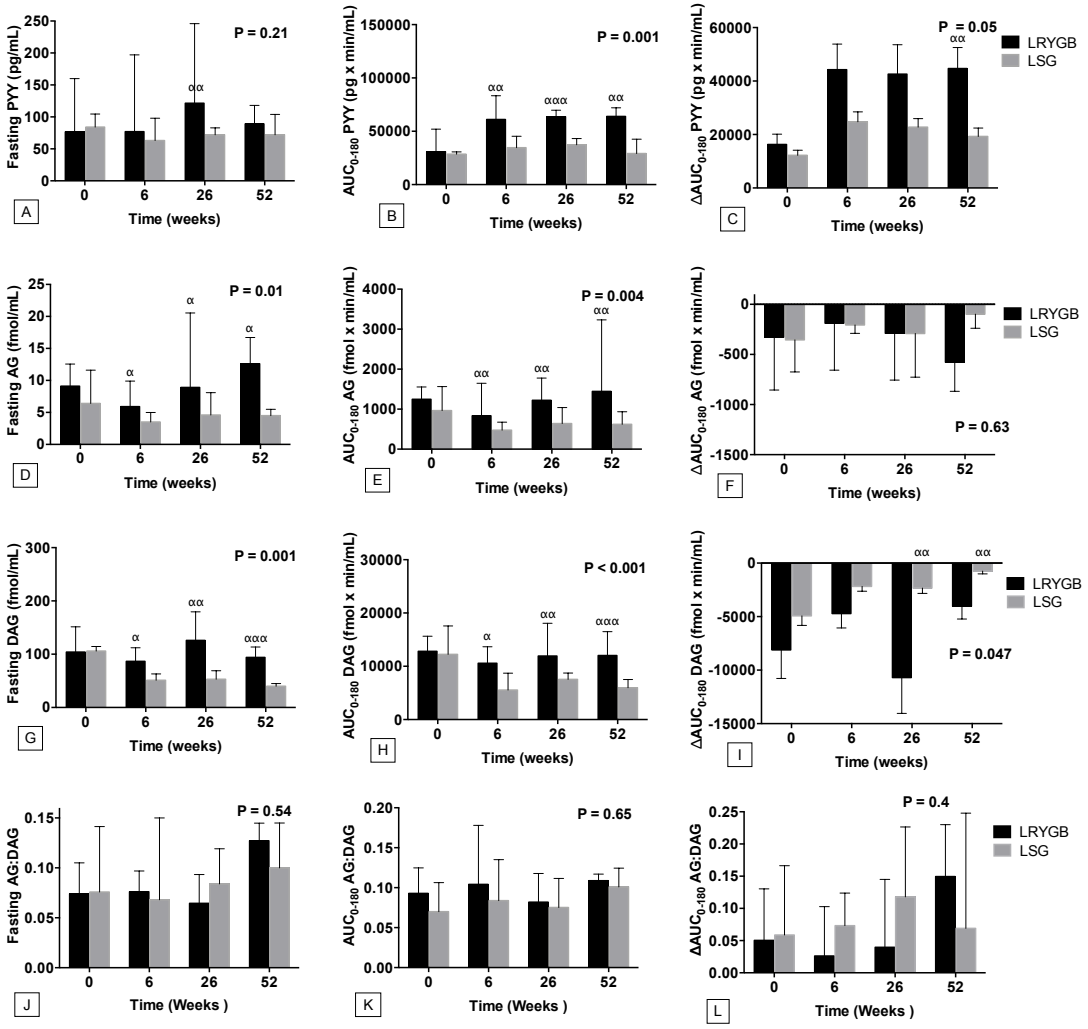


Figure 3.10 Comparison of PYY, AG and DAG parameters at each visit in patients with T2D divided by type of bariatric surgery (A) fasting PYY (B) $AUC_{0\text{-}180}$ PYY (C) $\Delta AUC_{0\text{-}180}$ PYY (D) fasting AG (E) $AUC_{0\text{-}180}$ AG (F) $\Delta AUC_{0\text{-}180}$ AG (G) fasting DAG (H) $AUC_{0\text{-}180}$ DAG (I) $\Delta AUC_{0\text{-}180}$ DAG (J) fasting AG:DAG (K) $AUC_{0\text{-}180}$ AG:DAG (L) $\Delta AUC_{0\text{-}180}$ AG:DAG. Results were expressed as mean ±SEM or median (interquartile range), according to their distribution. $^{\alpha}P < 0.05$, $^{\alpha\alpha}P < 0.01$, $^{\alpha\alpha\alpha}P < 0.001$ of the comparison between groups at each visit, P from mixed model analysis of the comparison between groups over 1 year.

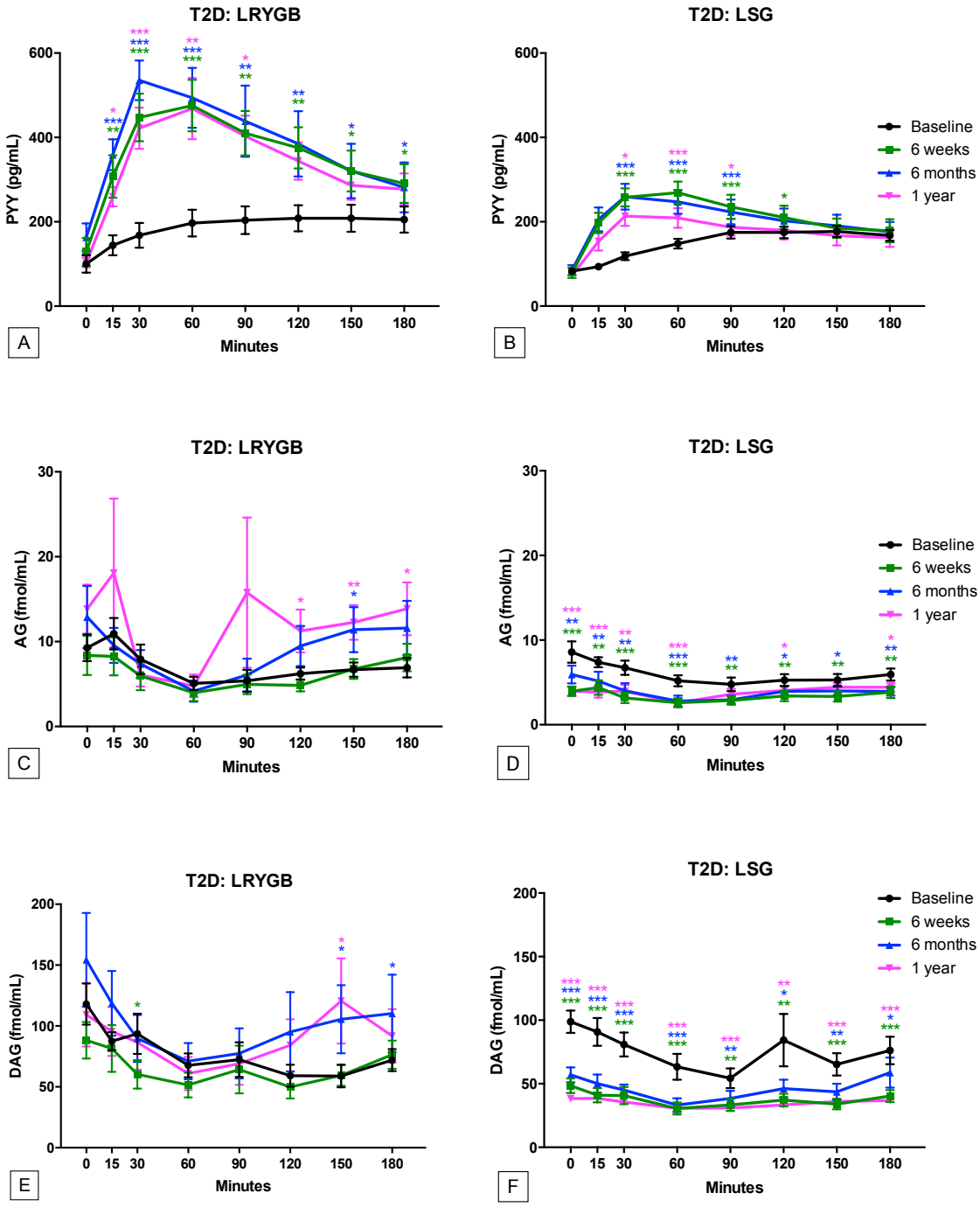


Figure 3.11 Comparison of fasting and nutrient-stimulated gut hormones levels in T2D subjects, divided by type of bariatric surgery (A) PYY in LRYGB (B) PYY in LSG (C) AG in LRYGB (D) AG in LSG (E) DAG in LRYGB (F) DAG in LSG. Results were expressed as mean ±SEM. *P <0.05, **P <0.01, ***P <0.001 in green represents the comparison between baseline and 6 weeks, in blue represents the comparison between baseline and 6 months and in pink represents the comparison between baseline and 1 year

DAG: The temporal alteration of DAG levels showed a similar pattern to the AG levels. The fasting DAG levels and $AUC_{0\text{-}180}$ DAG in LSG were statistically significantly lower than LRYGB at all visits after surgery (P = 0.001 for fasting DAG, P <0.001 for $AUC_{0\text{-}180}$ DAG Figure 3.10G-H, Appendix 9). In contrast, the postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) in LSG were statistically significantly less than LRYGB postoperatively (Figure 3.10I, Appendix 9).

In patients after LSG, the meal led to a marked suppression of DAG. There was a marked drop from 0 to 180 minutes at all visits (Figure 3.11F). In LRYGB, the significant fall was seen at 30 minutes at 6 weeks. Then, there was a substantial rise from 150 to 180 minutes at 26 weeks, and at 150 minutes at 1 year (Figure 3.11E). Overall, LRYGB showed a greater fluctuation of postprandial DAG levels than LSG.

AG:DAG ratio: There was no statistically significant difference in post-operative fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG and $\Delta AUC_{0\text{-}180}$ AG:DAG from pre-surgery in both groups, and the differences of these parameters between groups also were not seen (Figure 3.10J-L, Appendix 9).

3.3.2.4.2 Remitters versus non-remitters

PYY: There was no significant change in fasting PYY levels from pre-surgery in both groups and the levels in non-remitters were non-significantly greater than remitters (Figure 3.12A, Appendix 10). Following the surgery, patients with diabetes remission had a statistically significantly greater $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY than pre-surgery, whereas these changes were not seen in non-remitters (Appendix 10, Figure 3.12B-C). The post-operative levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in remitters were non-significantly greater than non-remitters (Appendix 10, Figure 3.12B-C). Nonetheless, there were only 5 subjects in the non-remitters group.

A substantial augmentation of post-operative meal-stimulated plasma PYY concentrations in remitters was evident (Figure 3.13A-B). A marked rise was seen from 15 to 180 minutes at 6 weeks, from 15 to 150 minutes at 26 weeks and from 15 to 120 minutes at 52 weeks (Figure 3.13A). In no remission group, a statistically significant increase was observed only at 15 minutes for 6 weeks (Figure 3.13B).

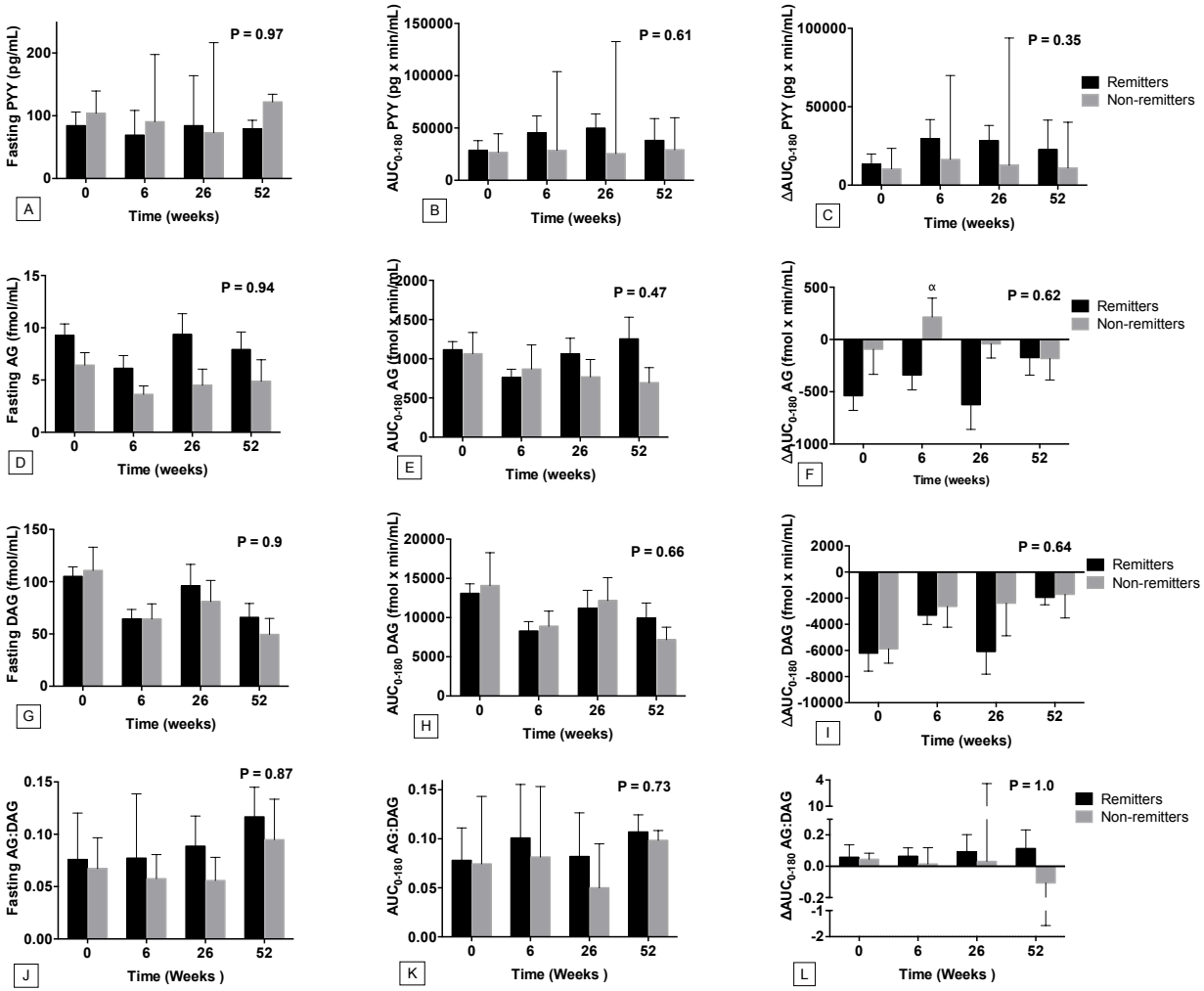


Figure 3.12 Comparison of PYY, AG and DAG parameters at each visit between remitters vs non-remitters (A) fasting PYY (B) $AUC_{0\text{-}180}$ PYY (C) $\Delta AUC_{0\text{-}180}$ PYY (D) fasting AG (E) $AUC_{0\text{-}180}$ AG (F) $\Delta AUC_{0\text{-}180}$ AG (G) fasting DAG (H) $AUC_{0\text{-}180}$ DAG (I) $\Delta AUC_{0\text{-}180}$ DAG (J) fasting AG:DAG (K) $AUC_{0\text{-}180}$ AG:DAG (L) $\Delta AUC_{0\text{-}180}$ AG:DAG. Results were expressed as mean ±SEM or median (interquartile range), according to their distribution. $^{\alpha}P < 0.05$, $^{\alpha\alpha}P < 0.01$, $^{\alpha\alpha\alpha}P < 0.001$ of the comparison between groups at each visit, P from mixed model analysis of the comparison between groups over 1 year.

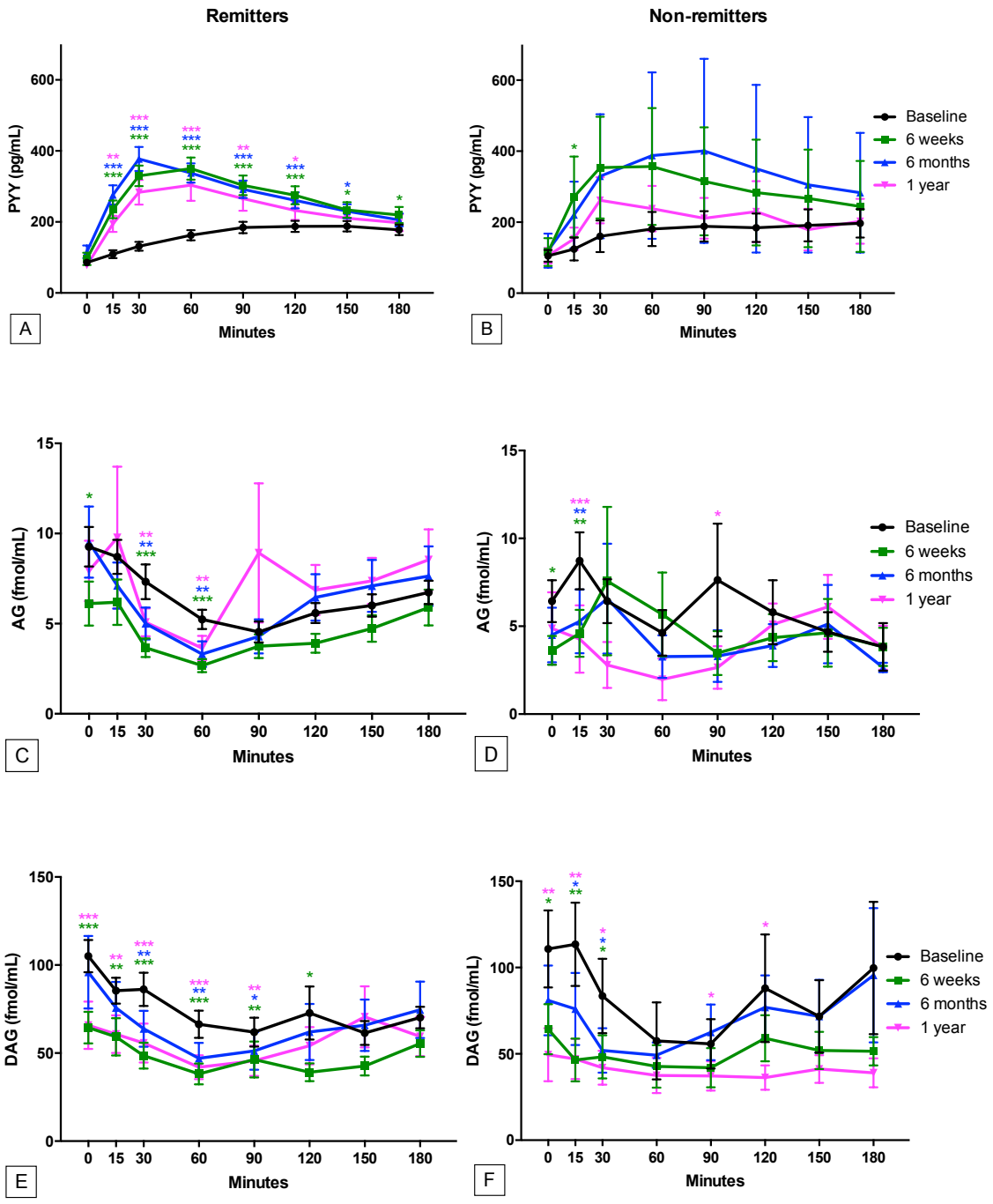


Figure 3.13 Comparison of fasting and nutrient-stimulated gut hormones levels in T2D subjects, divided by diabetes remission at 1 year (A) PYY in remission (B) PYY in no remission (C) AG in remission (D) AG in no remission (E) DAG in remission (F) DAG in no remission. Results were expressed as mean ±SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ in green represents the comparison between baseline and 6 weeks, in blue represents the comparison between baseline and 6 months and in pink represents the comparison between baseline and 1 year

AG: The levels of fasting AG dropped from pre-surgery in both groups, even though they were not statistically significant (Figure 3.12D, Appendix 10). There was a statistically significant drop of $AUC_{0\text{-}180}$ AG from pre-surgery in non-remitters (P = 0.02) and a marginally significant fall from pre-surgery in remitters (P = 0.08). In remitters, the fasting AG and postprandial suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) were non-significantly greater than non-remitters (Appendix 10, Figure 3.12F).

During the MMTT, a marked suppression was observed at 0 minute and from 30 to 60 minutes for 6 weeks, and from 30 to 60 minutes for 26 and 52 weeks in patients with T2D remission (Figure 3.13C). In non-remitters, the suppression was seen from 0 to 15 minutes at 6 weeks, at 15 minutes for 26 weeks, and at 15 and 90 minutes for 52 weeks (Figure 3.13D).

DAG: A statistically significant decrease of fasting DAG, $AUC_{0\text{-}180}$ DAG and postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) from pre-surgery was observed in both groups with no statistical difference between groups over time (Appendix 10, Figure 3.12G-H). Of note, the postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) in remitters was non-significantly greater that non-remitters over time (Appendix 10, Figure 3.12I).

The levels of postprandial DAG in remitters was flatter than in non-remitters. A marked suppression was seen from 0 to 120 minutes at 6 weeks, from 30 to 90 minutes at 26 weeks, and from 0 to 90 minutes at 52 weeks in the remission group (Figure 3.13E). In terms of the non-remitters group, there was a considerable drop from 0 to 30 minutes at 6 weeks, from 15 to 30 minutes at 26 weeks, and from 0 to 30, 90 – 120 minutes at 52 weeks (Figure 3.13F).

AG:DAG ratio: The levels of fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG and postprandial suppression of AG:DAG ($\Delta AUC_{0\text{-}180}$ AG:DAG) in remitters were non-significantly greater than non-remitters at pre-surgery and at all post-operative visits (Figure 3.12J-L, Appendix 10).

Stratification of diabetes remission (Non-remitters vs Remitters) by type of surgery

There were 4 non-remitters and 14 remitters undergoing LSG, whereas 1 non-remitter and 9 remitters had LRYGB.

Following LSG, the levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in remitters were statistically significantly greater than pre-surgery. When compared with non-remitters, $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY were higher. However, the differences did not reach statistical significance (Table 3.10). In LRYGB, the post-operative $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in remission group were statistically significantly more than pre-surgery. There was only one patient with no diabetes remission, making the analysis non-applicable (Table 3.11).

After LSG, the levels of fasting AG and $AUC_{0\text{-}180}$ AG in both groups were lower than pre-surgery as well as the postprandial suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) (Table 3.10). Nevertheless, the changes from pre-surgery only achieved statistical significance in remitters, and the differences of these parameters between groups were not observed (Table 3.10). The levels of fasting AG in remitters were non-significantly greater than non-remitters. Table 3.11 showed AG parameters after LRYGB. The changes in fasting AG, $AUC_{0\text{-}180}$ AG and the postprandial suppression of AG from pre-surgery were not statistically significant in remission group over 1 year. There was only 1 subject in the no diabetes remission group; thus, the comparisons within the group and between groups were not applicable.

In terms of DAG profiles, there was a statistically significant decrease in post-LSG fasting DAG, $AUC_{0\text{-}180}$ DAG and postprandial suppression of DAG ($\Delta AUC_{0\text{-}180}$ DAG) in both groups from pre-surgery with no statistically significant difference between groups (Table 3.10). Following the LRYGB, these changes were not statistically significant and the comparison between groups was not performed (Table 3.11).

Overall, the ratios of post-operative fasting AG:DAG, $AUC_{0\text{-}180}$ AG:DAG and $\Delta AUC_{0\text{-}180}$ AG:DAG in remitters were non-significantly greater than non-remitters following LSG. Nevertheless, following the LRYGB, the comparison between groups was not performed (Table 3.10, Table 3.11).

Table 3.10 PYY and ghrelin changes after laparoscopic sleeve gastrectomy (LSG) in remitters versus non-remitters

	Non-remitters (n = 4)					Remitters (n = 14)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	92 ± 26	78 ± 25	72 ± 2	101 ± 53	0.65	80 ± 28	62 (44, 97)	73 (57, 94)	66 ± 27	0.39	0.38
n	4	3	3	3		14	12	12	12		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	24,432 ± 4,350	28,103 ± 1,288	23,508 ± 6,930	27,152 ± 3,982	0.57	28,517 ± 8,681	41,729 ± 17,023	39,491 (29,423, 39,491)	33,666 ± 15,033	<0.01	0.35
n	4	3	3	3		13	12	12	12		
Δ $AUC_{0\text{-}180}$ PYY, pg x min/mL	7,936 ± 6,238	14,116 ± 4,174	10,546 ± 7,242	8,867 ± 5,850	0.58	13,539 ± 8,074	27,439 ± 14,881	24,327 (15,713, 30,856)	21,893 ± 11,901	<0.01	0.51
n	4	3	3	3		13	12	12	12		
Fasting AG, fmol/mL	5.2 ± 0.1	2.8 ± 0.5	3 ± 1.2	3.5 ± 3.7	0.35	7.3 (5.1, 12.6)	4.2 ± 2.3	6.6 ± 4.2	4.1 ± 2	<0.001	0.48
n	3	3	3	3		14	12	11	12		
$AUC_{0\text{-}180}$ AG, fmol x min/mL	916 ± 562	620 ± 451	617 ± 400	576 ± 368	0.01	1,065 ± 497	584 ± 366	708 ± 349	711 ± 328	<0.001	0.75
n	3	3	3	3		13	12	11	12		
Δ $AUC_{0\text{-}180}$ AG, fmol x min/mL	-27 ± 567	110 ± 361	71 ± 189	-45 ± 378	0.95	-415 (-785, -151)	-217 (-310, 21)	-471 ± 583	-24 ± 241	0.01	0.44
n	3	3	3	3		13	12	11	12		
Fasting DAG, fmol/mL	114 ± 54	55 ± 28	71 ± 43	35 ± 14	<0.01	96 ± 33	47 ± 22	54 ± 17	39 ± 8	<0.001	0.55
n	3	3	3	3		14	12	12	12		
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	15,677 ± 9,497	8,830 ± 4,712	12,474 ± 7,165	6,415 ± 3,449	0.04	12,753 ± 6,299	5,980 ± 2,705	6,763 ± 2,440	6,085 ± 1,268	<0.001	0.27

n	3	3	3	3		13	12	12	12		
Δ $AUC_{0\text{-}180}$ DAG, fmol x min/mL	-4,876 ± 1,240	-1,049 ± 294	-200 ± 3,015	68 ± 958	0.02	-4,939 ± 3,934	-2,449 ± 1,894	-2,860 ± 1,226	-954 ± 954	<0.01	0.63
n	3	3	3	3		13	12	12	12		
Fasting AG:DAG	0.055 ± 0.031	0.059 ± 0.024	0.05 ± 0.021	0.08 ± 0.071	0.82	0.107 ± 0.07	0.126 ± 0.111	0.13 ± 0.107 P = 0.04	0.103 ± 0.038	0.73	0.82
n	3	3	3	3		14	12	11	12		
$AUC_{0\text{-}180}$ AG:DAG	0.063 ± 0.024	0.069 ± 0.021	0.048 ± 0.004	0.088 ± 0.018	0.02	0.096 ± 0.055	0.114 ± 0.075	0.108 ± 0.066 P = 0.01	0.116 ± 0.043	0.53	0.69
n	3	3	3	3		13	12	11	12		
Δ$AUC_{0\text{-}180}$ AG:DAG	0.002 ± 0.126	-0.052 ± 0.285	5.98 ± 10.4	-0.683 ± 1.17	0.41	0.065 ± 0.183	0.054 ± 0.161	7.66 ± 25.1	0.047 ± 0.952	0.35	1.0
n	3	3	3	3		13	12	11	12		

Table 3.11 PYY and ghrelin changes after laparoscopic Roux-en-Y gastric bypass (LRYGB) in remitters versus non-remitters

	No remission (n = 1)					Remission (n = 9)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
Fasting PYY level, pg/mL	158	230	264	118	NA	94 ± 66	74 (55, 153)	95 (85, 215)	101 ± 33	0.44	NA
n	1	1	1	1		9	9	9	6		
AUC_{0-180} PYY, pg x min/mL	59,978	128,715	166,343	69,536	NA	31,425 ± 14,996	60,603 ± 16,616	61,511 ± 9,487	62,345 ± 20,018	<0.001	NA
n	1	1	1	1		9	9	9	6		
Δ AUC_{0-180} PYY, pg x min/mL	31,538	87,315	118,823	48,338	NA	14,582 ± 11,666	39,438 ± 27,842	34,087 ± 23,716	44,095 ± 22,643	<0.01	NA
n	1	1	1	1		9	9	9	6		
Fasting AG, fmol/mL	10	6	8.9	9.1	NA	9.2 ± 5.3	5.8 (3.4, 11.2)	7.7 (4.9, 24.3)	14.5 ± 8.6	0.34	NA
n	1	1	1	1		9	9	8	7		
AUC_{0-180} AG, fmol x min/mL	1,505	1,612	1,222	1,054	NA	1,186 ± 507	829 (613, 1,566)	1,178 (809, 1,814)	2,181 ± 1,615	0.07	NA
n	1	1	1	1		9	9	8	7		
Δ AUC_{0-180} AG, fmol x min/mL	-291	530	-378	-590	NA	-467 ± 653	-203 (-680, -9)	-284 (-817, -130)	-426 ± 1,181	0.84	NA
n	1	1	1	1		9	9	8	7		
Fasting DAG, fmol/mL	101	92	112	92	NA	120 ± 56	88 ± 50	134 (70, 197)	96 (55, 115)	0.07	NA
n	1	1	1	1		9	9	8	7		
AUC_{0-180} DAG, fmol x min/mL	9,296	9,131	11,195	9,475	NA	13,539 ± 5,255	11,355 ± 6,961	13,626 (9,902, 18,948)	12,782 (10,909, 17,383)	0.11	NA

n	1	1	1	1		9	9	8	7		
Δ $AUC_{0\text{-}180}$ DAG, fmol x min/mL	-8,830	-7,375	-8,912	-7,008	NA	-8,033 ± 8,935	-4,420 ± 4,407	-6,776 (-22,910, -3,530)	-3,595 ± 3,441	0.07	NA
n	1	1	1	1		9	9	8	7		
Fasting AG:DAG	0.099	0.065	0.079	0.099	NA	0.075 ± 0.035	0.077 (0.069, 0.106)	0.064 (0.044, 0.094)	0.136 ± 0.025	0.33	NA
n	1	1	1	1		9	9	8	7		
$AUC_{0\text{-}180}$ AG:DAG	0.162	0.177	0.109	0.111	NA	0.091 ± 0.035	0.105 ± 0.053	0.092 ± 0.035	0.106 (0.089, 0.117)	0.25	NA
n	1	1	1	1		9	9	8	7		
Δ$AUC_{0\text{-}180}$ AG:DAG	0.033	-0.072	0.042	0.084	NA	0.052 (0.028, 0.132)	0.046 ± 0.171	0.036 (0.012, 0.184)	0.216 ± 0.609	0.62	NA
n	1	1	1	1		9	9	8	7		

3.3.3 Association of pre-operative factors with 1-year PWL, weight-loss outcomes and diabetes remission after LRYGB and LSG

With regard to the above longitudinal data, the significant postprandial alterations of PYY and ghrelin levels occurred in the first hour following a test meal. We thus created additional parameters representing the first-hour changes during a MMTT ($AUC_{0\text{-}60}$ and Δ $AUC_{0\text{-}60}$) and examined their associations with 1-year PWL, weight-loss outcomes and diabetes remission after primary LRYGB and LSG.

3.3.3.1 Association of pre-operative parameters with 1-year PWL

In order to examine whether clinical factors, PYY and ghrelin parameters at pre-surgery would be able to predict weight loss response to bariatric surgery at 1 year, multiple linear regression analysis was performed using 1-year PWL as a dependent variable. The analysis was adjusted with age at surgery, gender, type of surgery and T2D status, and it revealed that none of pre-operative parameters were associated with 1-year weight loss (Table 3.12).

3.3.3.2 Association of pre-operative parameters with 1-year weight-loss outcomes (good and poor weight loss)

Logistic regression analysis was performed in order to examine whether or not any clinical factors, PYY and ghrelin parameters would be able to predict 1-year weight-loss outcomes defined by PWL at 20% (good weight loss; $\geq$20%, Poor weight loss; <20%). None of pre-operative parameters showed statistically significant association with 1-year weight-loss outcomes (Table 3.13).

Table 3.12 Association study of 1-year PWL with pre-operative parameters adjusted for age at surgery, type of bariatric surgery, gender and presence of T2D

Pre-operative parameters	β	95% CI	R^2	P-value
BW	0.01	-0.07, 0.09	0.123	0.79
BMI	0.05	-0.21, 0.32	0.352	0.68
Fasting PYY level, pg/mL	0.03	-0.01, 0.07	0.148	0.13
AUC_{0-60} PYY, pg x min/mL	4.3×10^{-4}	-6.9×10^{-5}, 1×10^{-3}	0.153	0.09
AUC_{0-180} PYY, pg x min/mL	1×10^{-4}	-4.8×10^{-5}, 2.5×10^{-4}	0.141	0.18
Δ AUC_{0-60} PYY, pg x min/mL	3.6×10^{-4}	-4×10^{-4}, 1×10^{-3}	0.13	0.35
Δ AUC_{0-180} PYY, pg x min/mL	5×10^{-5}	-1.4×10^{-4}, 2.4×10^{-4}	0.124	0.61
Fasting AG, fmol/mL	4×10^{-3}	-0.32, 0.33	0.082	0.98
AUC_{0-60} AG, fmol x min/mL	-1×10^{-3}	-8×10^{-3}, 7×10^{-3}	0.08	0.89
AUC_{0-180} AG, fmol x min/mL	1.1×10^{-4}	-3×10^{-3}, 3×10^{-3}	0.095	0.94
Δ AUC_{0-60} AG, fmol x min/mL	-1×10^{-3}	-0.01, 9×10^{-3}	0.08	0.82
Δ AUC_{0-180} AG, fmol x min/mL	4×10^{-4}	-2×10^{-3}, 3×10^{-3}	0.096	0.74
Fasting DAG, fmol/mL	0.02	-0.02, 0.06	0.091	0.38
AUC_{0-60} DAG, fmol x min/mL	3.1×10^{-4}	-4.3×10^{-4}, 1×10^{-3}	0.088	0.41
AUC_{0-180} DAG, fmol x min/mL	6.3×10^{-5}	-2.2×10^{-4}, 3.5×10^{-4}	0.097	0.66
Δ AUC_{0-60} DAG, fmol x min/mL	-1.4×10^{-4}	-1×10^{-3}, 1×10^{-3}	0.08	0.8
Δ AUC_{0-180} DAG, fmol x min/mL	-1.4×10^{-4}	-4.5×10^{-4}, 1.8×10^{-4}	0.104	0.4
Fasting AG:DAG	-2.4	-21.9, 17	0.083	0.81
AUC_{0-60} AG:DAG	-6	-26.5, 14.5	0.084	0.56
AUC_{0-180} AG:DAG	-3.4	-26.4, 19.6	0.096	0.77
Δ AUC_{0-60} AG:DAG	0.3	-1.4, 2	0.081	0.74
Δ AUC_{0-180} AG:DAG	-0.5	-5.2, 4.3	0.095	0.84

Table 3.13 Association study of 1-year weight-loss outcomes (good VS poor) with pre-operative parameters adjusted for age at surgery, gender, type of surgery and presence of T2D

Pre-operative parameters	OR	95% CI	P-value
BW	0.99	0.96, 1.02	0.56
BMI	0.98	0.89, 1.08	0.98
Fasting PYY level, pg/mL	1	1, 1	0.57
AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.14
AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.53
ΔAUC_{0-60} PYY, pg x min/mL	1	1, 1	0.37
ΔAUC_{0-180} PYY, pg x min/mL	1	1, 1	0.77
Fasting AG, fmol/mL	1	0.9, 1.1	0.86
AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.67
AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.62
Δ AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.78
Δ AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.56
Fasting DAG, fmol/mL	1	1, 1	0.86
AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.76
AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.74
Δ AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.98
Δ AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.96
Fasting AG:DAG	3	4×10^{-4}, 22,026	0.81
AUC_{0-60} AG:DAG	2.2	2.2×10^{-4}, 2.2×10^{4}	0.87
AUC_{0-180} AG:DAG	5.3	5.5×10^{-5}, 514,593	0.78
Δ AUC_{0-60} AG:DAG	0.9	0.5, 1.5	0.66
Δ AUC_{0-180} AG:DAG	0.6	0.1, 3.4	0.59

3.3.3.3 Association of pre-operative parameters with 1-year PWL in patients with T2D

In this cohort, the statistically significant association of clinical factors, PYY and ghrelin parameters with 1-year PWL in patients with T2D was not observed (Table 3.14).

3.3.3.4 Association of pre-operative parameters with diabetes remission at 1 year

None of clinical factors, PYY and ghrelin parameters were associated with diabetes remission at 1 year (Table 3.15).

Table 3.14 Association study of 1-year PWL in patients with T2D adjusted with age at surgery, type of bariatric surgery and gender

Pre-operative parameters	β	95% CI	R^2	P-value
BW	-0.01	-0.14, 0.13	0.234	0.89
BMI	-0.12	-0.55, 0.32	0.243	0.59
Fasting PYY level, pg/mL	-1×10^{-3}	-0.07, 0.07	0.233	0.98
AUC_{0-60} PYY, pg x min/mL	2.6×10^{-4}	-6.6×10^{-4}, 1.2×10^{-3}	0.245	0.56
AUC_{0-180} PYY, pg x min/mL	2.5×10^{-5}	-2.5×10^{-4}, 3×10^{-4}	0.234	0.85
Δ AUC_{0-60} PYY, pg x min/mL	4.2×10^{-4}	-7.2×10^{-4}, 1.6×10^{-3}	0.253	0.45
Δ AUC_{0-180} PYY, pg x min/mL	2.9×10^{-5}	-2.5×10^{-4}, 3.1×10^{-4}	0.234	0.83
Fasting AG, fmol/mL	0.04	-0.45, 0.52	0.259	0.88
AUC_{0-60} AG, fmol x min/mL	-1.6×10^{-3}	-0.01, 0.01	0.261	0.78
AUC_{0-180} AG, fmol x min/mL	2×10^{-3}	-4×10^{-3}, 7×10^{-3}	0.272	0.54
Δ AUC_{0-60} AG, fmol x min/mL	-4.1×10^{-3}	-0.02, 0.01	0.271	0.55
Δ AUC_{0-180} AG, fmol x min/mL	4.9×10^{-4}	-3.5×10^{-3}, 4.5×10^{-3}	0.261	0.8
Fasting DAG, fmol/mL	0.02	-0.04, 0.07	0.27	0.57
AUC_{0-60} DAG, fmol x min/mL	2.8×10^{-4}	-8.7×10^{-4}, 1.4×10^{-3}	0.267	0.62
AUC_{0-180} DAG, fmol x min/mL	8.3×10^{-5}	-3.4×10^{-4}, 5×10^{-4}	0.264	0.69
Δ AUC_{0-60} DAG, fmol x min/mL	-2.5×10^{-4}	-2.2×10^{-3}, 1.7×10^{-3}	0.261	0.8
Δ AUC_{0-180} DAG, fmol x min/mL	-7×10^{-5}	-5.1×10^{-4}, 3.7×10^{-4}	0.262	0.75
Fasting AG:DAG	-7.2	-49.2, 34.8	0.263	0.73
AUC_{0-60} AG:DAG	-12.5	-61.6, 36.7	0.268	0.6
AUC_{0-180} AG:DAG	13.5	-40.2, 67.1	0.268	0.61
Δ AUC_{0-60} AG:DAG	-0.1	-0.4, 0.2	0.281	0.51
Δ AUC_{0-180} AG:DAG	-3.7	-18.5, 11.1	0.268	0.61

Table 3.15 Association study of diabetes remission adjusted for age at surgery, gender and type of surgery

Pre-operative parameters	OR	95% CI	P-value
BW	1	1, 1.1	0.41
BMI	1	0.9, 1.2	0.77
Fasting PYY level, pg/mL	1	1, 1	0.21
AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.15
AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.43
Δ AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.47
Δ AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.98
Fasting AG, fmol/mL	1.2	0.8, 1.7	0.35
AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.72
AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.96
Δ AUC_{0-60} AG, fmol x min/mL	1	0.99, 1	0.27
Δ AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.21
Fasting DAG, fmol/mL	1	1, 1	0.74
AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.89
AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.86
Δ AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.84
Δ AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.9
Fasting AG:DAG	4.2×10^{6}	5.1×10^{-8}, 3.5×10^{20}	0.35
AUC_{0-60} AG:DAG	77.7	2.4×10^{-11}, 2.5×10^{14}	0.77
AUC_{0-180} AG:DAG	0.5	7.9×10^{-13}, 2.6×10^{11}	0.95
Δ AUC_{0-60} AG:DAG	15.4	5×10^{-3}, 4.8×10^{4}	0.5
Δ AUC_{0-180} AG:DAG	16.6	6×10^{-3}, 4.4×10^{4}	0.49

3.3.4 Association of early post-operative (6 weeks) factors with 1-year PWL, weight-loss outcomes and diabetes remission after primary LRYGB and LSG

3.3.4.1 Association of 6-week parameters with 1-year PWL

In order to examine whether 6-weeks parameters including clinical factors and the gut hormones (PYY and ghrelin) would be able to predict weight loss response to bariatric surgery at 1 year, multiple linear regression analysis was performed using 1-year PWL as a dependent variable. The analysis was adjusted with age at surgery, gender, type of surgery and the presence of T2D. Six-week PWL and 6-week WCV were significantly associated with 1-year PWL (β = 1.21, P <0.001 for 6-week PWL and β = -3.84, P <0.001 for 6-week WCV, Table 3.16). In addition, postprandial PYY parameters at 6 weeks demonstrated a significant association with 1-year weight loss ($\beta = 3.2 \times 10^{-4}$, P = 0.02 for 6-week $AUC_{0\text{-}60}$ PYY, $\beta = 9.4 \times 10^{-5}$, P = 0.04 for 6-week $AUC_{0\text{-}180}$ PYY and $\beta = 8.5 \times 10^{-5}$, P = 0.04 for 6-week Δ $AUC_{0\text{-}180}$ PYY, Table 3.16).

3.3.4.2 Association of 6-week parameters with 1-year weight-loss outcomes (good and poor weight loss)

Logistic regression analysis was performed in order to examine whether or not any clinical factors, PYY and ghrelin parameters would be able to predict 1-year weight-loss outcomes defined by PWL at 20% (good weight loss; ≥20%, poor weight loss; <20%). The analysis was adjusted with age at surgery, gender, type of surgery and the presence of T2D. Only 6-week PWL was statistically significantly associated with good weight loss at 1 year (adjusted OR = 1.52, P = 0.01, Table 3.17)

Table 3.16 Association study of 1-year PWL adjusted for age at surgery, type of bariatric surgery, gender and the presence of T2D

6-week parameters	β	95% CI	R^2	P-value
BW	-0.02	-0.11, 0.06	0.358	0.58
BMI	-0.07	-0.34, 0.2	0.357	0.61
PWL	1.21	0.61, 1.63	0.303	<0.001
WCV	-3.84	-5.88, -1.8	0.261	<0.001
Fasting PYY level, pg/mL	0.02	-0.02, 0.05	0.072	0.35
AUC_{0-60} PYY, pg x min/mL	3.2×10^{-4}	4.4×10^{-5}, 1×10^{-3}	0.128	0.02
AUC_{0-180} PYY, pg x min/mL	9.39×10^{-5}	3×10^{-6}, 1.85×10^{-4}	0.115	0.04
Δ AUC_{0-60} PYY, pg x min/mL	3.4×10^{-4}	1.5×10^{-5}, 1×10^{-3}	0.116	0.04
Δ AUC_{0-180} PYY, pg x min/mL	8.45×10^{-5}	-1.5×10^{-5}, 1.84×10^{-4}	0.099	0.09
Fasting AG, fmol/mL	0.03	-0.4, 0.46	0.061	0.89
AUC_{0-60} AG, fmol x min/mL	-1×10^{-3}	-0.01, 9×10^{-3}	0.061	0.89
AUC_{0-180} AG, fmol x min/mL	1.1×10^{-4}	-4×10^{-3}, 4×10^{-3}	0.06	0.96
Δ AUC_{0-60} AG, fmol x min/mL	-1×10^{-3}	-0.01, 8×10^{-3}	0.062	0.76
Δ AUC_{0-180} AG, fmol x min/mL	-2.5×10^{-4}	-4×10^{-3}, 3×10^{-3}	0.061	0.88
Fasting DAG, fmol/mL	0.02	-0.02, 0.06	0.091	0.38
AUC_{0-60} DAG, fmol x min/mL	7.6×10^{-4}	-3.3×10^{-4}, 1.9×10^{-3}	0.086	0.17
AUC_{0-180} DAG, fmol x min/mL	6.3×10^{-5}	-2.2×10^{-4}, 3.5×10^{-4}	0.097	0.66
Δ AUC_{0-60} DAG, fmol x min/mL	3.9×10^{-4}	-1.5×10^{-3}, 2.3×10^{-3}	0.063	0.69
Δ AUC_{0-180} DAG, fmol x min/mL	-1.4×10^{-4}	-4.5×10^{-4}, 7.3×10^{-4}	0.063	0.65
Fasting AG:DAG	-2.5	-27.8, 22.8	0.061	0.84
AUC_{0-60} AG:DAG	-7.5	-26.6, 11.6	0.069	0.44
AUC_{0-180} AG:DAG	-9.7	-33.3, 13.9	0.07	0.41
Δ AUC_{0-60} AG:DAG	0.1	-0.2, 0.4	0.067	0.48
Δ AUC_{0-180} AG:DAG	0.7	-4.8, 6.2	0.061	0.8

Table 3.17 Association study of 1-year weight-loss outcomes (good VS poor) with 6-week parameters adjusted for age at surgery, gender, the presence of T2D and type of surgery

6-week parameters	OR	95% CI	P-value
BW	0.98	0.95, 1.01	0.27
BMI	0.95	0.87, 1.05	0.34
PWL	1.52	1.1, 2.1	0.01
WCV	0.46	0.18, 1.18	0.11
Fasting PYY level, pg/mL	1.01	0.99, 1.02	0.49
AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.1
AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.11
ΔAUC_{0-60} PYY, pg x min/mL	1	1, 1	0.15
ΔAUC_{0-180} PYY, pg x min/mL	1	1, 1	0.2
Fasting AG, fmol/mL	0.98	0.83, 1.14	0.77
AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.7
AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.63
Δ AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.47
Δ AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.47
Fasting DAG, fmol/mL	1	0.98, 1.02	0.94
AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.81
AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.85
Δ AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.59
Δ AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.69
Fasting AG:DAG	3.24	5.2×10^{-4}, 2×10^{4}	0.79
AUC_{0-60} AG:DAG	5.8	1.7×10^{-3}, 2×10^{4}	0.67
AUC_{0-180} AG:DAG	5.09	5.4×10^{-4}, 4.8×10^{4}	0.73
Δ AUC_{0-60} AG:DAG	1	0.9, 1.1	0.71
Δ AUC_{0-180} AG:DAG	0.32	0.03, 3.32	0.34

We subsequently examined the association of the changes in 6-week PYY and ghrelin parameters from pre-surgery with the weight-loss outcomes. Table 3.18 shows odds ratios of the association of PYY and ghrelin's changes at 6 weeks from pre-surgery (diff $AUC_{0\text{-}180}$ PYY/AG/DAG and diff $\Delta AUC_{0\text{-}180}$ PYY/AG/DAG) with weight-loss outcomes (good or poor weight loss) at 1 year. Only the increase in $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks from pre-surgery (diff $\Delta AUC_{0\text{-}180}$ PYY) was marginally significantly associated with good weight loss at 1 year (crude odds ratio [95% confidence interval, CI] = 1.05 [1.00, 1.10], P = 0.06, adjusted odds ratio [95% CI] = 1.05 [0.99, 1.10], P = 0.09, Table 3.18).

Next, the cut-point of the change in $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks from pre-surgery (diff $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks – pre-surgery) indicating poor weight-loss outcome at 1 year was examined using the ROC curve and we found that the increase in 6-week $\Delta AUC_{0\text{-}180}$ PYY from pre-surgery less than 16,000 pg x min/mL significantly correlated with 6.13 times higher chance of being poor weight loss at 1 year, with a sensitivity of 87%, a specificity of 49%, a positive predictive value (PPV) of 27%, and a negative predictive value (NPV) of 94% (Table 3.19). Furthermore, the chance rose to 7.65 times after adjustment with age at surgery, gender, the presence of T2D and type of surgery (Table 3.20). Table 3.20 furthermore demonstrated odds ratios of factors associated with the poor weight-loss outcomes at 1-year.

In conclusion, the presence of T2D and the rise in 6-week $\Delta AUC_{0\text{-}180}$ PYY from pre-surgery less than 16,000 pg x min/mL are associated with a higher chance of being poor weight loss at 1 year (adjusted odds ratio [95% CI] = 4.31 [1.09, 17.04], P = 0.04 for T2D, and adjusted odds ratio [95% CI] = 7.65 [1.41, 41.56], P = 0.02 for the diff $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks – pre-surgery, Table 3.20).

Table 3.18 Odds ratios of the association of the changes in 6-week $AUC_{0\text{-}180}$ PYY/AG/DAG and 6-week $\Delta AUC_{0\text{-}180}$ PYY/AG/DAG from pre-surgery with weight-loss outcomes (good or poor weight loss) at 1 year

Hormones	Total (n = 83)	Poor weight loss (n = 15)	Good weight loss (n = 68)	P-value*	Crude odds ratio (95% CI) (1,000 units)	P-value	Adjusted odds ratio (95% CI) (1,000 units)	P-value
diff $AUC_{0\text{-}60}$ PYY, pg x min/mL at 6 weeks – pre-surgery	5,723 (3,038, 8,773)	5,333 (1,689, 7,743)	5,787 (3,454, 8,773)	0.39	1.06 (0.94, 1.2)	0.36	1.09 (0.94, 1.27)	0.25
diff $AUC_{0\text{-}180}$ PYY, pg x min/mL at 6 weeks – pre-surgery	12,503 (2,066, 22,962)	6,158 (279, 16,845)	14,039 (3,719, 23,408)	0.13	1.04 (0.99, 1.09)	0.11	1.05 (0.99, 1.12)	0.1
diff $\Delta AUC_{0\text{-}60}$ PYY, pg x min/mL at 6 weeks – pre-surgery	5,871 (2,340, 8,770)	5,348 (1,049, 7,530)	5,916 (2,874, 9,330)	0.26	1.00 (1.00, 1.00)	0.18	1.00 (1.00, 1.00)	0.16
diff $\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL at 6 weeks – pre-surgery	12,439 (1,605, 21,498)	6,678 (666, 13,178)	12,990 (2,811, 27,520)	0.046	1.05 (1.00, 1.10)	0.06	1.05 (0.99, 1.10)	0.09
diff $AUC_{0\text{-}60}$ AG, fmol x min/mL at 6 weeks – pre-surgery	-175 (-275, -65)	-175 (-250, -70)	-165 (-296, -65)	0.88	1.17 (0.05, 27.7)	0.92	1.02 (0.04, 27.6)	0.99
diff $AUC_{0\text{-}180}$ AG, fmol x min/mL at 6 weeks – pre-surgery	-270 (-589, -66)	-459 (-524, -38)	-246 (-609, -66)	0.83	1.23 (0.29, 5.25)	0.78	0.97 (0.18, 5.17)	0.98
diff $\Delta AUC_{0\text{-}60}$ AG, fmol x min/mL at 6 weeks – pre-surgery	44 (-56, 175)	44 (-89, 184)	49 (-50, 177)	0.68	1.00 (1.00, 1.00)	0.92	1.00 (1.00, 1.00)	0.83

diff $\Delta AUC_{0\text{-}180}$ AG, fmol x min/mL at 6 weeks – pre-surgery	288 (-33,786)	168 (-93, 701)	338 (-33, 786)	0.66	1.00 (0.43, 2.32)	0.99	0.93 (0.38, 2.26)	0.87
diff $AUC_{0\text{-}60}$ DAG, fmol x min/mL at 6 weeks – pre-surgery	-2,045 ± 2,008	-2,344 ± 1,784	-1,983 ± 2,059	0.43	1.09 (0.82, 1.46)	0.55	1.02 (0.71, 1.45)	0.92
diff $AUC_{0\text{-}180}$ DAG, fmol x min/mL at 6 weeks – pre-surgery	-3,186 (-6,733, -893)	-5,363 (-9,584, -2,055)	-3,161 (-6,516, -625)	0.28	1.04 (0.93, 1.16)	0.54	1.00 (0.87, 1.16)	0.97
diff $\Delta AUC_{0\text{-}60}$ DAG, fmol x min/mL at 6 weeks – pre-surgery	455 ± 1,301	499 ± 1,224	446 ± 1,326	0.89	1.00 (1.00, 1.00)	0.89	1.00 (1.00, 1.00)	0.91
diff $\Delta AUC_{0\text{-}180}$ DAG, fmol x min/mL at 6 weeks – pre-surgery	2,752 (-61, 5,526)	2,477 (648, 4,593)	2,759 (-61, 5,526)	0.95	1.00 (0.87, 1.13)	0.94	1.00 (0.87, 1.15)	0.97
diff $AUC_{0\text{-}60}$ AG:DAG at 6 weeks – pre-surgery	0.005 ± 0.107	0.011 ± 0.044	0.003 ± 0.116	0.51	1.96 (0.01, 504)	0.81	0.45 (0.00, 397)	0.82
diff $AUC_{0\text{-}180}$ AG:DAG at 6 weeks – pre-surgery	0.008 (-0.022, 0.034)	0.01 (-0.024, 0.03)	0.006 (-0.022, 0.036)	0.85	4.96 (0.00, 26,603)	0.72	1.08 (0.00, 24,759)	0.99
diff $\Delta AUC_{0\text{-}60}$ AG:DAG at 6 weeks – pre-surgery	-0.981 ± 6.017	-1.174 ± 4.193	-0.94 ± 6.36	0.98	0.99 (0.91, 1.09)	0.9	1.03 (0.93, 1.13)	0.62
diff $\Delta AUC_{0\text{-}180}$ AG:DAG at 6 weeks – pre-surgery	-0.064 (-0.243, 0.036)	-0.043 (-0.12, 0.041)	-0.08 (-0.252, 0.035)	0.41	1.54 (0.37, 6.4)	0.55	1.24 (0.3, 5.23)	0.77

* P-value of the comparison between groups. Adjusted odds ratio represents odds ratio of each parameter adjusted with age at surgery, gender, the presence of T2D and type of surgery

Table 3.19 The cut-point of diff $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks – pre-surgery predicting poor weight-loss outcome at 1 year

	AuROC	Sensitivity	Specificity	PPV	NPV	Odds Ratio
diff $\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL at 6 weeks – baseline	0.68 (0.51, 0.85)					
≤ 15,000	0.63 (0.50, 0.76)	73 (45, 92)	53 (40, 65)	26 (14, 41)	90 (76, 97)	3.09 (0.94, 10.11)
≤ 16,000	0.68 (0.77, 0.78)	87 (60, 98)	49 (36, 61)	27 (15, 42)	94 (81, 99)	6.13 (1.42, NA)
≤ 17,000	0.66 (0.55, 0.77)	87 (60, 98)	46 (34, 58)	26 (15, 40)	94 (80, 99)	5.45 (1.26, NA)

Table 3.20 Odds ratios of factors associated with poor weight-loss outcome at 1 year

	Total (n = 83) n (%)	**Poor weight loss (n = 15) n (%)**	**Good weight loss (n = 68) n (%)**	**P-value**	**Crude odds ratio (95% CI) (1,000 units)**	**P-value**	**Adjusted odds ratio (95% CI) (1,000 units)**	**P-value**
diff $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks – baseline ≤ 16,000 pg x min/mL	48 (58)	13 (87)	35 (51.4)	0.01	6.13 (1.28, 29.25)	0.02	7.65 (1.41, 41.56)	0.02
Age at surgery, years	46 ± 12	46 ± 12	46 ± 12	0.97	0.99 (0.95, 1.05)	0.97	0.99 (0.93, 1.05)	0.7
Males	8 (10)	3 (20)	5 (7.35)	0.15	3.15 (0.66, 14.97)	0.15	1.68 (0.29, 9.87)	0.56
Type 2 diabetes	28 (34)	8 (53)	20 (29)	0.08	2.74 (0.88, 8.58)	0.08	4.31 (1.09, 17.04)	0.04
LSG	56 (67)	12 (80)	44 (65)	0.37	2.18 (0.56, 8.50)	0.26	1.66 (0.36, 7.63)	0.52

Adjusted odds ratio represents odds ratio of each parameter adjusted with other four parameters

3.3.4.3 Association of 6-week parameters with 1-year PWL in patients with T2D

There was no a statistically significant association of 6-week BW, BMI, weight-loss parameters (6-week PWL and WCV) and PYY and ghrelin profiles with 1-year PWL observed in patients with T2D (Table 3.21).

3.3.4.4 Association of 6-week parameters with diabetes remission

Logistic regression analysis was performed in order to examine whether or not any clinical factors, PYY and ghrelin parameters would be able to predict diabetes remission at 1 year. The analysis was adjusted with age at surgery, gender and type of surgery, and revealed that the greater postprandial suppression of AG, meaning more negative value of $\Delta AUC_{0\text{-}180}$ AG, was statistically significantly associated with the higher chance of having diabetes remission. (adjusted OR = 0.99 [0.99, 1], P = 0.04, Table 3.22).

Table 3.21 Association study of 1-year PWL in patients with T2D adjusted with age at surgery, type of bariatric surgery and gender

6-weeks parameters	β	95% CI	R^2	P-value
BW	-0.04	-0.17, 0.1	0.294	0.57
BMI	-0.18	-0.6, 0.25	0.308	0.4
PWL	0.52	-0.49, 1.53	0.32	0.3
WCV	-0.57	-3.98, 2.84	0.288	0.73
Fasting PYY level, pg/mL	0.02	-0.02, 0.06	0.291	0.41
AUC_{0-60} PYY, pg x min/mL	1.2×10^{-4}	-2.9×10^{-4}, 5.3×10^{-4}	0.279	0.55
AUC_{0-180} PYY, pg x min/mL	2.1×10^{-5}	-1.1×10^{-4}, 1.5×10^{-4}	0.269	0.75
Δ AUC_{0-60} PYY, pg x min/mL	2.4×10^{-5}	-4.2×10^{-4}, 4.7×10^{-4}	0.266	0.91
Δ AUC_{0-180} PYY, pg x min/mL	-9.6×10^{-6}	-1.5×10^{-4}, 1.3×10^{-4}	0.266	0.88
Fasting AG, fmol/mL	0.02	-0.53, 0.57	0.266	0.93
AUC_{0-60} AG, fmol x min/mL	3.3×10^{-4}	-0.01, 0.02	0.265	0.96
AUC_{0-180} AG, fmol x min/mL	2×10^{-3}	-4×10^{-3}, 8×10^{-3}	0.281	0.52
Δ AUC_{0-60} AG, fmol x min/mL	-4.8×10^{-4}	-0.01, 0.01	0.266	0.94
Δ AUC_{0-180} AG, fmol x min/mL	6.5×10^{-4}	-3.5×10^{-3}, 4.8×10^{-3}	0.269	0.75
Fasting DAG, fmol/mL	0.05	-0.03, 0.14	0.329	0.18
AUC_{0-60} DAG, fmol x min/mL	6.2×10^{-3}	-9.3×10^{-4}, 2.2×10^{-3}	0.29	0.42
AUC_{0-180} DAG, fmol x min/mL	3×10^{-4}	-2.8×10^{-4}, 8.8×10^{-4}	0.306	0.29
Δ AUC_{0-60} DAG, fmol x min/mL	-2.4×10^{-3}	-5.7×10^{-3}, 9.4×10^{-4}	0.339	0.15
Δ AUC_{0-180} DAG, fmol x min/mL	-4.1×10^{-4}	-1.3×10^{-3}, 4.9×10^{-4}	0.297	0.35
Fasting AG:DAG	5.6	-25.8, 37.1	0.27	0.71
AUC_{0-60} AG:DAG	-2.3	-37.9, 33.2	0.266	0.89
AUC_{0-180} AG:DAG	-8.8	-49.9, 32.3	0.272	0.66
Δ AUC_{0-60} AG:DAG	-0.1	-0.4, 0.2	0.281	0.51
Δ AUC_{0-180} AG:DAG	-0.01	-14.9, 14.8	0.265	1.0

Table 3.22 Association study of diabetes remission with 6-week parameters adjusted for age at surgery, gender and type of surgery

6-week parameters	OR	95% CI	P-value
BW	1	1, 1.1	0.57
BMI	1	0.8, 1.2	0.99
PWL	1.5	0.9, 2.6	0.16
WCV	0.6	0.1, 3.1	0.52
Fasting PYY level, pg/mL	0.96	0.92, 1	0.053
AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.31
AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.44
Δ AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.84
Δ AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.96
Fasting AG, fmol/mL	1.61	0.7, 3.72	0.27
AUC_{0-60} AG, fmol x min/mL	0.99	0.98, 1	0.14
AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.35
Δ AUC_{0-60} AG, fmol x min/mL	0.97	0.94, 1	0.11
Δ AUC_{0-180} AG, fmol x min/mL	0.99	0.99, 0.99	0.04
Fasting DAG, fmol/mL	0.96	0.96, 1.04	0.86
AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.97
AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.62
Δ AUC_{0-60} DAG, fmol x min/mL	1	1, 1	0.75
Δ AUC_{0-180} DAG, fmol x min/mL	1	1, 1	0.64
Fasting AG:DAG	2.1×10^{13}	4.2×10^{-8}, 1×10^{34}	0.21
AUC_{0-60} AG:DAG	7×10^{-6}	7×10^{-15}, 7.3×10^{3}	0.26
AUC_{0-180} AG:DAG	37.6	4.6×10^{-9}, 3.1×10^{11}	0.76
Δ AUC_{0-60} AG:DAG	28.9	0.3, 2.6×10^{3}	0.14
Δ AUC_{0-180} AG:DAG	32	0.04, 2.4×10^{4}	0.3

3.4 Discussion

3.4.1 Longitudinal pattern of weight loss and circulating PYY and ghrelin changes in patients undergoing primary LRYGB and LSG

Our study has demonstrated that the range of 6-month and 1-year PWL is wide and follows a normal distribution, in agreement with the previous study by Manning et al (Manning et al., 2015b). This indicates that biology has a major role in underlying the weight reduction after bariatric surgery rather than the malabsorption and restriction from the surgery itself.

Weight loss

The superiority of weight loss after LRYGB over LSG at 1 year seen in our cohort was consistent with many previous studies (Schauer et al., 2017, Li et al., 2016, Arterburn et al., 2018, Shoar and Saber, 2017). Furthermore, the STAMPEDE trial has revealed that the superiority remained up to 5 years after surgery (Schauer et al., 2017). Nonetheless early and late post-operative complications; for example, wound infection, leakages, bleeding, nutrients deficiency, etc. found in LRYGB are greater than LSG (Zhang et al., 2015, Li et al., 2014). Thus, carefully weighing risk-benefit ratio is crucial to select a particular type of bariatric surgery for each individual. Personalised bariatric surgery hence would be an ideal.

We also found that 1-year PWL in patients with T2D was significantly lower than patients without the diabetes, and the percentage of patients with T2D at baseline experiencing poor weight loss at 1 year was almost 2-fold as much as good weight loss, suggesting that T2D might diminish the weight-loss outcomes following bariatric surgery. Previous studies by Luo et al. has shown that individuals without T2D achieved 1.6 times greater weight loss at $\geq$50% excess body weight loss (Luo, 2020), and Arterburn et al. has also demonstrated less PWL in individuals with diabetes (Arterburn et al., 2018). In addition, T2D also attenuated BW reduction following lifestyle intervention and pharmacotherapy (Pi-Sunyer, 2005, Wing et al., 1987). The reasons underlying this are still not yet well elucidated.

PYY and ghrelin parameters at pre-surgery

At baseline, patients with T2D exhibited a statistically significantly greater fasting PYY levels and $AUC_{0\text{-}180}$ PYY than subjects without T2D, in spite of the comparable BW and BMI. In contrast, the post-prandial rise of PYY concentrations ($\Delta AUC_{0\text{-}180}$ PYY) was similar in both groups. The higher level of serum fasting PYY in T2D subjects than non-T2D subjects with matched BMI was in line with findings from previous studies by English et al. (English et al., 2006), Ukkola et al. (Ukkola et al., 2011) and Olivan et al. (Olivan et al., 2009), indicating that the high levels of serum fasting PYY could be independently associated with T2D, not the adiposity.

The real mechanisms explaining this finding is not well understood. Hyper-secretion and diminished clearance of PYY in T2D were attributed to be the cause of elevated fasting PYY levels (English et al., 2006). In addition, acute and chronic administration of metformin can raise fasting PYY levels in women with polycystic ovarian syndrome (PCOS) (Tsilchorozidou et al., 2008), and an in vitro study has shown that metformin could directly activate PYY secretion from human intestinal epithelium (Sun et al., 2019). In this study, 61% of patients with T2D had metformin pre-operatively, thus this might be the reason explaining the greater levels of pre-operative fasting PYY and $AUC_{0\text{-}180}$ PYY in individuals with T2D than those without T2D.

However, at 6-week post-surgery when only 29% of patients with T2D continued metformin, the greater levels of $AUC_{0\text{-}180}$ PYY in T2D than non-T2D were more striking than at pre-surgery ($P = 0.04$). Of note, the postprandial augmentation of PYY levels ($\Delta AUC_{0\text{-}180}$ PYY) in T2D were also higher than non-T2D, which was not seen at the pre-surgery. Furthermore, these occurred with a slightly drop of the fasting PYY levels in patients with diabetes at 6 weeks. Hence, we hypothesise that PYY resistance in patients with T2D could be another potential mechanism, and that bariatric surgery perhaps can solve this condition. The improvement in PYY sensitivity could lead to the reduction in fasting PYY levels and the enhancement in meal stimulated PYY secretion observed in the present study particularly at 6 weeks may well resulted from the improvement in PYY secretion after bariatric surgery.

Previously, several studies have shown that PYY resistance in obesity was unlikely (Batterham and Bloom, 2003); however, the study of PYY resistance and function of its receptors in T2D patients is lacking. A study in rodents revealed that insulin-resistant rats had higher ghrelin, PYY and insulin levels than insulin-sensitive littermates and this was found independent of BW (Antunes Lda et al., 2014). In contrast, a study in first-degree relatives of subjects with T2D compared with matched-subjects without such family history showed that low circulating levels of PYY correlated with hyperinsulinemia and insulin resistance (Boey et al., 2006); however, subjects with established T2D were not tested in this trial. Hence, more studies elucidating this issue are now warranted.

PYY and ghrelin parameters at post-surgery

LRYGB vs. LSG

In the gut, PYY is expressed predominantly within L-cells of the ileum and colon, and to a lesser extent, in the stomach, duodenum and jejunum. Our study showed that the meal-stimulated PYY parameters represented by $AUC_{0\text{-}180}$ and $\Delta AUC_{0\text{-}180}$ in both LRYGB and LSG groups were significantly higher than pre-surgery at every post-operative visit. These can be explained by the increase in nutrients exposure to the hindgut L-cells, leading to enhanced circulating PYY and GLP-1 levels (Karra et al., 2010).

However, the $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in LRYGB were significantly greater than LSG over time. These findings of a great PYY response following LRYGB are in keeping with the differences in anatomy of LRYGB (accelerating gastric emptying, excluding nutrients from proximal small intestine and transferring nutrients directly from stomach to jejunum) and LSG (accelerating gastric emptying and entry of nutrients to duodenum and proximal part of small intestine, thus no alteration in the food passage). The more rapid delivery of nutrients to the hindgut L-cells after LRYGB is potentially a reason of the greater levels of PYY (Morinigo et al., 2006).

In our cohort, fasting PYY levels following LRYGB were greater than LSG in contrast to findings from previous studies by Yousseif et al. (Yousseif et al., 2014) and Alamuddin et al. (Alamuddin et al., 2017) where they found no significant difference in fasting PYY levels between LRYGB and LSG. Nevertheless, if we look at the figures of PYY in Alamuddin's

study, we can see that the rise in fasting PYY levels from pre-surgery after LRYGB were higher than LSG (13.5 $\pm$ 6.4 pg/mL after LRYGB vs 0.98 $\pm$ 7.3 pg/mL after LSG at 6 months and 12 $\pm$ 7.6 pg/mL after LRYGB vs 10.4 $\pm$ 8.5 pg/mL after LSG at 18 months). The small sample size could be a reason why they did not get the statistical significance. Our findings probably indicate that the surgery affects both basal circulating PYY levels and post-prandial PYY.

Owing to the significant difference in anatomy of LRYGB and LSG, there are discrepancies in the changes of post-operative AG and DAG between the two procedures. In LSG, the majority of ghrelin producing cells (*X/A*-like cells) located in gastric fundus are removed, whereas these cells are preserved in LRYGB but are bypassed from the nutrient passage. A number of previous studies showed that ghrelin considerably reduced post-SG, whilst conflicting results were observed following RYGB. In our study, there was a significant decrease in fasting AG and DAG and $AUC_{0\text{-}180}$ AG and DAG after LSG since 6-week post-operatively and persisted until 1 year, consistent with previous studies (Alamuddin et al., 2017, Chandarana et al., 2011, Peterli et al., 2009, Bohdjalian et al., 2010).

In contrast, the levels of fasting AG and DAG and $AUC_{0\text{-}180}$ AG and DAG after LRYGB reduced at 6 weeks and then rose to more than baseline at 1 year in agreement with studies by (Chandarana et al., 2011, Alamuddin et al., 2017, Falken et al., 2011, Stoeckli et al., 2004). Santiago-Fernández et al. concluded that the alteration in post-operative circulating ghrelin levels depended on type of surgery; the ghrelin levels declined after SG but increased after RYGB (Santiago-Fernandez et al., 2017).

In terms of postprandial suppression of AG and DAG, the suppression in LSG was significantly smaller and the graph was flatter than LRYGB. This could be because of the lower levels of fasting AG and DAG in LSG. The differences in the ghrelin profiles between LSG and LRYGB potentially come from: first, the removal of gastric fundus leads to a greater decrease in ghrelin levels after LSG; second, although in LRYGB the ghrelin producing cells are excluded from the nutrient contact, these cells still actively produce the hormone probably via the vagus nerve signalling (the secretion of ghrelin is independent from *X/A*-like cell nutrient-sensing) (Chronaiou et al., 2012, Sundbom et al., 2007). Given that the 1-year PWL following LRYGB was greater than LSG in our cohort, it

suggests that the suppression of orexigenic hormone, ghrelin, may not play a major role in weight-loss success after bariatric surgery, but mainly results from the anatomical alteration after surgery.

Interestingly, the levels of fasting AG:DAG and the postprandial suppression of AG:DAG ($\Delta AUC_{0\text{-}180}$ AG:DAG) were statistically comparable between LRYGB and LSG. This could be due to equality of magnitude changes in AG and DAG between groups. Only the levels of $AUC_{0\text{-}180}$ AG:DAG that was statistically significantly different between groups (the levels after LSG was greater than LRYGB). This tells us that following LSG the reduction in $AUC_{0\text{-}180}$ DAG was markedly higher than $AUC_{0\text{-}180}$ AG, compared to LRYGB.

T2D vs. non-T2D

Studies comparing the differences in fasting and nutrient-stimulated gut hormones after bariatric surgery, particularly PYY and ghrelin, between T2D and non-T2D subjects are lacking. We found that subjects with T2D had greater levels of fasting and postprandial PYY representing by $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY than non-T2D subjects. Since the type of surgery has a marked impact on post-operative PYY and ghrelin, we examined patients with and without T2D stratified by the type of surgery. The fasting and postprandial PYY in T2D were still higher than non-T2D with the figures more-pronounced after LRYGB up to 1 year, even though the difference did not reach statistical significance in both categories of surgery. the small sample size could be the explanation.

It is unknown whether abnormalities in PYY physiology, for example, PYY resistance or dysfunction of its receptors or impaired secretion, results in the pathogenesis of T2D and/or whether the plasma PYY levels are regulated by glucose homeostasis. Nevertheless, at pre-surgery, patients with T2D had significantly greater fasting levels of PYY than patients without diabetes; whereas, at post-surgery, the levels of meal-stimulated PYY ($AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY) in T2D were significantly higher than non T2D. The higher pre-operative fasting PYY levels in patients with T2D together with a slightly drop of the fasting PYY after surgery could indicate PYY resistance which improved post-operatively, and this pattern was not observed in patients without diabetes. The higher levels of postprandial PYY in patients with T2D after bariatric surgery could indicate

the improvement in PYY secretion response to a meal. These findings hence suggest that the bariatric surgery may well improve PYY secretion and sensitivity physiologically, and that abnormal PYY physiology could be one of the mechanisms underlying T2D. Further studies scrutinising the physiopathology of PYY in patients with T2D are now required.

The evidence of beneficial effects of ghrelin on glucose homeostasis is heterogeneous. Previous studies showed that ghrelin can increase secretion of insulin counter-regulatory hormones, inhibit adiponectin (an insulin-enhancing hormone), suppress phosphatidylinositol-3-kinase (a step in hepatic insulin signalling cascade) and diminish insulin secretion (Thaler and Cummings, 2009), whereas some evidence demonstrated that the rise in plasma AG and DAG was associated with diabetes remission after RYGB, and that ghrelin gene products maintained intracellular calcium homeostasis, leading to β-cells protection from apoptosis (Yang et al., 2014).

Furthermore, in vivo and in vitro evidence have demonstrated that DAG had beneficial effects on insulin sensitivity, glucose metabolism and inhibition of lipolysis, whereas AG inhibited insulin secretion (Benso et al., 2012, Gasco et al., 2010) via; first, an association with a rise in circulating FFA levels (Huda et al., 2009) which diminished insulin sensitivity; second, intracellular lipid retention leading to enlarged fat laden adipocytes and indirect immuno-modulatory response which ultimately led to insulin resistance (Churm et al., 2017). Castorina et al discovered that there was an overproduction of ghrelin by the stomach in patients with obesity and T2D (Castorina et al., 2021).

In our cohort, fasting AG and DAG, $AUC_{0\text{-}180}$ AG and DAG and the postprandial suppression of AG and DAG ($\Delta AUC_{0\text{-}180}$ AG and DAG) considerably declined in both T2D and non-T2D groups after the surgery. When compared these parameters between groups, the levels of fasting AG and the postprandial suppression of AG and DAG in T2D seemed to be greater than non-T2D. However, the differences were not statistically significant. The greater post-meal suppression of AG and DAG potentially resulted from the higher levels of fasting AG and DAG in patients with T2D. We then stratified T2D and non-T2D subjects by type of surgery and found that the pattern of AG and DAG evolution remained in the same direction as prior to the stratification. Given that the pre-operative AG parameters were comparable between T2D and non-T2D, and 82% (23/28) of patients with T2D

experienced T2D remission after the surgery, the rising levels of post-operative AG could be associated with the diabetes remission.

In terms of AG:DAG ratio, patients with T2D had non-statistically significantly greater levels of fasting AG:DAG and postprandial suppression of AG:DAG ($\Delta AUC_{0\text{-}180}$ AG:DAG) than patients without diabetes. The greater magnitude of the decrease in fasting DAG and $\Delta AUC_{0\text{-}180}$ DAG than fasting AG and $\Delta AUC_{0\text{-}180}$ AG in T2D group, compared to non-T2D group might be the explanation. However, after stratifying patients by the type of surgery, the pattern was not seen. This could be due to the small sample size after the stratification.

Good weight loss vs. Poor weight loss

We also identified patients with good and poor weight loss at 1 year after bariatric surgery, since this is the time when maximal PWL is usually achieved (Sjostrom et al., 2007). Both groups experienced significantly increases in nutrient-stimulated PYY post-operatively. There was no significant difference in PYY parameters between groups. However, if we take a closer look at the details, the rise in $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks in good weight loss was significantly higher than poor weight loss. After stratification by the type of surgery, the 6-week $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in good weight loss were higher than poor weight loss following LSG, whilst the $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in good weight loss were greater than poor weight loss at every time-point following LRYGB

Regarding ghrelin profiles, the levels of post-operative fasting AG and DAG and $AUC_{0\text{-}180}$ AG and DAG were significantly lower than baseline in both groups. In poor weight loss subjects, the plasma levels of fasting AG and DAG and the post-meal suppression of AG ($\Delta AUC_{0\text{-}180}$ AG) seemed to be higher than subjects with good weight loss. After stratifying by type of surgery, the pattern of ghrelin evolution remained the same as the whole population in LSG group. On the other hand, following the LRYGB, there was an opposite pattern to LSG. This may well support that the post-operative ghrelin concentrations are primarily influenced by the type of surgery, rather than relating to weight reduction.

Our findings suggest there is a trend of higher levels of PYY in good-weight-loss than poor-weight-loss subjects. The significant difference in AG and DAG parameters between the two groups was not observed. As mentioned earlier, previous studies by le Roux et al., demonstrated that the nutrient-stimulated PYY and GLP-1 levels were attenuated in poor weight loss compared to good weight loss (le Roux et al., 2007) and Dirksen et al. showed a larger release of GLP-1 and a greater suppression of ghrelin in good weight loss than poor weight loss people whereas PYY did not differ between groups (Dirksen et al., 2013). The inconsistent results are possibly due to the varied definitions of good weight loss, time from surgery, definition of a suboptimal response to surgery and the type of bariatric procedure (the two previous studies had LRYGB whereas our study had both LSG and LRYGB). Also, the relatively small sample size could be another reason.

Highest quartile of PWL vs. Lowest quartile of PWL

We then compared PYY and ghrelin profiles between subjects in the highest quartile and lowest quartile of weight loss at 1 year. At baseline, the BW and BMI were comparable between groups, suggesting that these two parameters did not contribute to weight-loss outcomes in our cohort. The higher incidence of T2D and LSG in the lowest quartile group indicates that they potentially have a negative impact on the weight loss. Post-surgery, the levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in the highest quartile groups were non-significantly greater than the lowest quartile group at every visit. Of note, the postprandial PYY concentrations at 6 weeks in the highest quartile group were significantly higher than the lowest quartile group, suggesting that the early rise in postprandial PYY levels could be associated with the weight-loss success after bariatric surgery.

Subgroup analysis in patients with T2D

In terms of the subgroup analysis in patients with T2D, we found that T2D subjects undergoing LSG had greater BMI than LRYGB. This could be due to the fact that LSG is a preferable option for subjects with extreme obesity and multiple co-morbidities, since it is a simpler operation and has a lower risk than LRYGB (Li et al., 2014, Zhang et al., 2015). Furthermore, we found that the percentage of diabetes remitters undergoing LRYGB was higher than LSG in line with previous studies revealing that the rate of diabetes remission

following LRYGY was greater than LSG (Aminian, 2020, Madadi et al., 2019, Aminian et al., 2017).

Remitters vs. non-remitters

Several scores were previously reported to be able to predict diabetes remission after bariatric surgery; for example, DiaRem score (Still et al., 2014a), DiaBetter score (Pucci et al., 2018), ABCD score (Lee et al., 2013) and Individualized Metabolic Surgery (IMS) score (Aminian et al., 2017). Basically, the predicting pre-operative parameters for the diabetes remission include younger age, higher BMI, lower number of anti-diabetes medication, shorter duration of diabetes, lower HbA_{1c} and higher C-peptide levels. In agreement with these trials, we found that remitters possessed slightly greater BMI and shorter duration of diabetes.

Even in the T2D subgroup analysis, the PWL following LRYGB was greater than LSG over 1 year in agreement with the whole study population, supporting that LRYGB leads to better weight-loss outcome at 1 year. Weight loss has been attributed to be a key role in improvement of T2D and diabetes remission following dieting and exercise (Lean et al., 2018, Gregg et al., 2012). However, in bariatric surgery, it is yet still conflicting since the procedure produces a substantial glycaemic improvement beginning within a few weeks post-operatively prior to the significant weight loss (Pories et al., 1995). Our finding of a comparable PWL over 1 year between remitters and non-remitters indicates that other factors; for example, gut hormones, microbiome, neural signalling, etc. potentially are more important mediators in determining the diabetes remission (Batterham and Cummings, 2016).

In terms of the improvement of glycaemia after bariatric surgery, the Swedish Obesity Subjects study revealed that bariatric surgery was associated with 72% remission of T2D at 2 years and 36% at 10 years (Sjostrom, 2013). Owing to the different anatomy between LRYGB and LSG, it differentially influences meal-stimulated circulating levels of some gut hormones known to impact upon glycaemia and insulin secretion (Yousseif et al., 2014). Chandarana and colleagues have thrown light on how PYY benefits on glycaemia (Chandarana et al., 2013). They have found that PYY3-36 enhanced nutrient-stimulated

insulin secretion by promoting local effects of GLP-1. In addition, a study in an animal model by Ramracheya et al. also showed that the normalisation of glycaemia in diabetes mice undergone RYGB was resulted from the restoration of insulin and glucagon secretion in diabetic islets by PYY (Ramracheya et al., 2016).

We observed that patients with T2D after both LSG and LRYGB had a significantly greater nutrient-stimulated PYY post-operatively compared to pre-surgery. Patients with T2D following LRYGB exhibited higher PYY levels namely fasting PYY, $AUC_{0\text{-}180}$ and $\Delta AUC_{0\text{-}180}$ than LSG, although the statistically significant difference between groups was observed only in $AUC_{0\text{-}180}$ PYY. In addition, the percentage of T2D patients achieving remission in LRYGB was more pronounced than LSG, even though the number did not reach statistical significance. In the light of advantages of PYY on glycaemia mentioned earlier, our findings again support that LRYGB is potentially a preferable surgical option for patients with diabetes.

The pattern of AG and DAG changes following LRYGB and LSG in T2D subgroup population was in line with the results form the whole population analysis, supporting that the differences in anatomy between LRYGB and LSG primarily influence the post-operative levels.

We investigated the difference in PYY and ghrelin parameters between T2D remitters and non-remitters. Interestingly, it is noticeable that the levels of post-operative fasting PYY in non-remitters were slightly greater than remitters. On the contrary, there was an attenuated response of nutrient-stimulated PYY after the surgery in non-remitters, compared to remitters. This supports our hypotheses that the high fasting levels of PYY could relate to PYY resistance and thus disadvantage glucose homeostasis, whereas the high levels of postprandial PYY may well indicate great PYY secretion and thus improve glycaemia. A larger trial investigating the differential levels of PYY between diabetes remitters and non-remitters, and the mechanism underlying it are now warranted.

In terms of ghrelin, the levels of fasting AG and DAG, $AUC_{0\text{-}180}$ AG and DAG were lower than pre-surgery at every follow-up visit following LSG; whilst the levels dropped at 6 weeks and then gradually rose to pre-operative levels or more at 1 year after LRYGB. This

again indicates that the type of surgery has a huge impact on the circulating levels of AG and DAG. The postprandial suppression of AG was similar between groups; whereas the greater DAG postprandial suppression in LRYGB than LSG could be due to the higher levels of fasting DAG in LRYGB.

Furthermore, our findings revealed that the levels of fasting AG in remitters were higher than non-remitters at pre-surgery and all post-operative visits, even though the difference did not achieve statistical significance. The greater fasting AG:DAG in remitters than non-remitters suggests that the proportion of fasting AG levels were higher than fasting DAG levels in remitters, compared to non-remitters. These findings support Yang and colleagues' study that the rise in plasma AG and DAG was associated with diabetes remission (Yang et al., 2014).

We also observed that the postprandial AG and DAG suppression was more pronounce in remitters than non-remitters, in accordance with a study by Samat and colleagues suggesting that enhanced suppression of nutrient-stimulated plasma ghrelin perhaps engenders weight reduction and favourable glycaemia (Samat et al., 2013). Alternatively, the higher postprandial AG suppression in remitters may well just resulted from the higher levels of its fasting values. The higher postprandial suppression of $AUC_{0\text{-}180}$ AG:DAG in remitters than non-remitters indicates that the proportion of postprandial suppression in AG was greater than DAG in remitters, compared to non-remitters.

With regard to ghrelin, further studies are now warranted to examine its genuine pathophysiology on glycaemic homeostasis in patients with obesity and diabetes. The levels of fasting AG in diabetes remitters greater than non-remitter indicates the advantages of AG on the remission of diabetes. The over-secretion of ghrelin in patients with obesity and T2D found in a previous study (Castorina et al., 2021) is perhaps a compensated mechanism of hyperinsulinemia, and the genuine function of ghrelin is potentially to maintain the normoglycaemic state.

3.4.2 Factors associated with 1-year PWL, weight-loss outcomes and diabetes remission after primary LRYGB and LSG

Several pre-operative clinical predictors of weight loss response to bariatric surgery were previously identified such as baseline BMI, gender, age and early post-operative weight loss velocity (Still et al., 2014b, Contreras et al., 2013, Ma et al., 2006, Ochner et al., 2013, Ortega et al., 2012, Scozzari et al., 2012, Manning et al., 2015b). Nevertheless, the evidence showing that gut hormones are able to predict weight loss after bariatric surgery is scarce and limited to only RYGB. We thus investigated the association between pre-operative and early post-operative (6 weeks) clinical parameters, PYY, AG and DAG profiles and 1-year PWL after LRYGB and LSG by a multiple linear regression using 1-year PWL as a dependent variable with the adjustment for age, gender, surgical type and the presence of T2D.

We found that T2D status and type of bariatric surgery were significantly associated with 1-year PWL after adjusted with age at surgery, gender and another one of them. None of the rest of baseline clinical parameters, particularly BW, BMI, age and gender showed the association. In addition, the correlation between preoperative PYY, AG and DAG parameters and 1-year PWL was not observed in agreement with previous studies by Werling et al. and Morinigo et al. (Morinigo et al., 2008, Werling et al., 2014) showing that the pre-surgery assessment of PYY and ghrelin do not correlate with weight loss following RYGB. In T2D subgroup analysis, the statistically significant association of clinical factors, PYY and ghrelin parameters with 1-year PWL in patients with T2D was also not observed.

Logistic regression analysis was performed in order to examine whether or not any clinical factors, PYY and ghrelin parameters at pre-surgery adjusted with age at surgery, gender and type of surgery would be able to predict 1-year good weight-loss outcome defined by PWL at $\geq$20%. None of pre-operative parameters showed statistically significant association with 1-year good weight-loss outcome. The logistic regression analysis was furthered conducted for identifying pre-operative factors associated with 1-year diabetes remission adjusted with age at surgery, gender and type of surgery, and any associations were not seen.

Next, we further examined whether or not body-weight changes and gut hormones profiles in the early phase after surgery (6 weeks) would be able to predict the PWL at 1 year. We found that the PWL, WCV and PYY profiles at 6 weeks were significantly associated with the 1-year PWL, suggesting that the early changes are the key of predicting weight loss success in the longer term in addition to some baseline parameters such as diabetes, older age, male gender and higher BMI reported from previous studies. Furthermore, WCV had the highest magnitude of association with the 1-year weight loss in agreement with a previous study by Manning et al. (Manning et al., 2015b).

The 6-week PWL also correlated with 1-year good weight-loss outcome by a logistic regression analysis, adjusted with age at surgery, gender, type of surgery and the presence of T2D. These findings highlight that pre-operative factors are not a good predictor for 1-year weight loss, whilst the early post-operative (6 weeks) weight loss parameters had a better association, aligning with a number of previous studies (Silveira et al., 2020, Mor et al., 2012, Hindle et al., 2017, Manning et al., 2015b). Belligoli et al. proposed that post-surgical behavioural factors more influenced on BW after bariatric surgery than pre-surgical factors (Belligoli et al., 2020).

Of note, our finding of the association between the increase in postprandial PYY at 6 weeks and 1-year weight-loss outcome is consistent with a study by Morinigo et al, which they found that the 6-week PYY response to a MMTT significantly correlated with the percent excess weight loss at 3 years in 35 patients with severe obesity following LRYGB (Morinigo et al., 2008). These findings support the idea of PYY underpinning weight-loss success after bariatric surgery. In animal model, Chandarana and colleagues compared PYY levels in wild-type and PYY-knockout (*PYYKO)* mice undergoing either gastrointestinal bypass (GIBP) or sham operation. T wild-type mice with GIBP had significantly less BW than their littermates with sham operation, whereas there was no significant difference in BW between *PYYKO*-mice with GIBP and *PYYKO*-mice with sham operation. Moreover, the wild-type mice with GIBP had significantly greater fasting and postprandial PYY than wild-type mice with sham operation (Chandarana et al., 2011).

We then investigated if the changes in PYY, AG and DAG at each visit from pre-surgery related with weight-loss outcome, and we found that the change in $\Delta AUC_{0\text{-}180}$ PYY (diff

$\Delta AUC_{0\text{-}180}$ PYY) at 6 weeks from pre-surgery was significantly associated with 1-year PWL. The rise in $\Delta AUC_{0\text{-}180}$ PYY at 6 weeks less than 16,000 pg x min/mL from pre-surgery was connected with 8 times greater chance of being poor weight loss defined by PWL <20% at 1 year. To the best of our knowledge, this is the first trial determining the relation between the extent of the change in postprandial PYY response to a MMTT after bariatric surgery (LYGB and LSG) and the chance of developing poor weight loss at 1 year.

PYY (3-36) was proposed to be a key mechanism of weight loss by reducing food intake, modulating energy expenditure and enhancing eating habit (Teubner and Bartness, 2013, Ballantyne, 2006, Batterham et al., 2002). It modulates neuronal activity of arcuate neurons in hypothalamus via NPY2R. The reasons why there was a variability observed in the change in post-operative $\Delta AUC_{0\text{-}180}$ PYY has not been elucidated. Genetics, a subtle discrepancy in surgical technique leading to the difference in nutrient delivery to hindgut L-cells, life-style, exercise and dietary pattern could be the explanation.

The reasons why the post-op increase in $AUC_{0\text{-}180}$ PYY related with the 1-year weight loss was only observed at the 6-week values could be explained by the more influence on weight loss by biology during the first 6 months, whereas lifestyle, eating habit and environmental factors might play a more important role in the longer-term weight reduction.

Using the early post-operative parameters to identify patients who are highly likely to have poor weight-loss outcome allows us to provide them early additional support; for example, eating behaviours, psychological interventions, diet, exercise, and pharmacotherapy (Silveira et al., 2020). Wharton and colleagues have reported that 3.0 mg of liraglutide could assist people with poor weight loss or weight regain to lose a decent weight (Wharton et al., 2019), so does topiramate plus phentermine.(Istfan et al., 2020).

Regarding the T2D subgroup analysis, the weight-loss parameters were not associated with diabetes remission in our cohort, highlighting that other factors e.g. body composition, the alteration of gut hormones, microbiome, neural signalling, etc. have more impact on determining the diabetes remission (Batterham and Cummings, 2016).

There was a statistically significant association between $\Delta AUC_{0\text{-}180}$ AG and diabetes remission at 1 year. This is in line with our results in the longitudinal study mentioned earlier in this chapter that the postprandial AG suppression in remitters was greater than non-remitters. Thus, emphasising that the suppression of postprandial AG significantly benefits glucose homeostasis.

Several limitations of the present study are worth noting. First, sample size calculation was not performed. However, this study could be reported as a preliminary article and could be used for sample size calculation for further relevant studies. Second, MMTTs were conducted using a fixed calorie meal not an adjusted energy content for each individual. This might be not an ideal; nonetheless, it is more practical, generally used and widely accepted (Shankar et al., 2016). Third, some patients with T2D were on antidiabetic agents before and after surgery which might have had an impact on gut hormone levels. However, after surgery the antidiabetic agents were ceased in most of the patients, only 2 (7%) continued the medication.

3.5 Conclusion

In our cohort, T2D and LSG were associated with less weight loss at 1 year, and shorter duration of diabetes and LRYGB were associated with better chance in achieving diabetes remission. The early post-operative changes in BW (PWL and WCV) and PYY at 6 weeks showed an association with 1-year weight loss. Our findings suggest that PYY benefits weight-loss outcome and diabetes remission following LRYGB and LSG. It hence is a hope for being further developed as an anti-obesity and anti-diabetes medication. In addition, PYY resistance and dysfunction of its receptors in T2D individuals is currently an interesting concept and thus further pathophysiological study of the PYY in T2D should be meticulously established. The changes in AG and DAG primarily related to the type of bariatric surgery. Nevertheless, the significant association between AG and glucose homeostasis was observed in this study, particularly with the diabetes remission. Future research should focus on the role of ghrelin on glycaemia.

Chapter 4

Factors associated with type 2 diabetes (T2D) remission after LRYGB and LSG

Chapter 4 Factors associated with type 2 diabetes (T2D) remission after LRYGB and LSG

The magnetic resonance imaging (MRI) study in this chapter was performed by Dr. Naomi Sakai, a research associate in the Centre for Medical Imaging at the Division of Medicine, University College London. The metabolomics study was also conducted by Dr. Marco G. Alves, a research associate in the Unit for Multidisciplinary Research in Biomedicine (UMIB), Institution of Biomedical Science Abel Salazar (ICBAS), University of Porto. Kusuma Chaiyasoot was responsible for study design, patient recruitment, conducting MMTTs, laboratory work, data collection, data analysis and critical discussion.

4.1 Introduction

Type 2 diabetes (T2D) is a chronic progressive impairment in glucose homeostasis and is characterised by high levels of blood glucose. It leads to a range of health problems such as T2D-related macro- and micro-vascular complications, mental issues, nerve damage, etc. The condition thus causes a significant global financial burden. It affected more than 422 million adults worldwide in 2014, with a projection of 650 million people by 2040 (WHO, 2016, Cummings and Rubino, 2018). Nevertheless, many studies have shown that this condition can be reversed through sustained weight loss, achieved by profound energy restriction (Lean et al., 2018) or bariatric surgery (Ramos-Levi and Rubio, 2017). Bariatric surgery brings about 70 – 89% of diabetes remission at 1 year (Dixon et al., 2013, Chikunguwo et al., 2010), although the rate is less durable in the long-term. The remission rate decreases to 45% at 5 years (Salminen et al., 2018) and 36% at 10 years (Sjostrom, 2013).

A number of clinical factors such as age, duration of diabetes, pre-operative use of anti-diabetic medication, a pre-operative HbA_{1c}, and type of bariatric surgery have been reported to be related to diabetes remission after bariatric surgery (Hopkins et al., 2020, Aminian, 2020). Several scores were also described as predictors for T2D remission after bariatric surgery such as DiaRem score (Still et al., 2014a), DiaBetter score (Pucci et al., 2018) and ABCD score (Lee et al., 2013). In addition, a range of biological mechanisms

were reported to be associated with diabetes remission following bariatric surgery, including the alteration of gut anatomy and physiology such as altered bile acid metabolism, microbiome, gut hormones, neural signalling and gastrointestinal (GI) nutrient-sensing, and the reprogramming of GI glucose control as well as the weight loss that leads to reduced hepatic and pancreatic fat content (Batterham and Cummings, 2016, Lim et al., 2011). Nonetheless, the mechanisms of diabetes remission are incompletely understood, and the studies examining this are limited.

Nannipieri et al. have shown that in patients with T2D remission after RYGB and SG β-cell function, represented by β-cell glucose sensitivity, improved at 15 days and persisted up to 1 year post-surgery, whereas insulin sensitivity improved at 1 year. Furthermore, they found that a restored GLP-1 response was the key determinant of diabetes remission. PYY, glucagon, ghrelin, amylin and pancreatic polypeptide were not different between groups (Nannipieri et al., 2013). In contrast, some evidence postulate that PYY is a key mediator of diabetes remission after the surgery (Guida et al., 2019). Recently, Taylor and colleagues have demonstrated that the diabetes remission needs reduction in hepatic and pancreatic triglycerides, but it essentially depends on the capacity of the β-cells to recover (Taylor et al., 2018). In addition, the association between visceral adiposity and T2D remission is debatable. A study in Chinese patients with a BMI <35 kg/m^2 revealed that high visceral fat area was associated with T2D remission at 1 year after RYGB, whereas a negative correlation between pre-operative ratio of visceral adipose tissue (VAT) to subcutaneous adipose tissue (SAT) and the remission was reported in a study in Korean patients with severe obesity (Kim et al., 2011, Yu et al., 2015a).

Metabolomics provides a tool to study dynamic changes in metabolomes, and to investigate mechanisms contributing to certain medical conditions as well as identifying biomarkers for prognostic evaluations (Luo et al., 2016). Previous studies have demonstrated that the reduction in circulating levels of branched-chain amino acids (BCAAs) correlated with improvement in glucose homeostasis after bariatric surgery, which were independent of weight reduction (Lips et al., 2014, Laferrere et al., 2011). Interestingly, studies report a clear link between BCAAs and VAT, where the catabolism of BCAAs was downregulated (Pakiet et al., 2020, Boulet et al., 2015, Su et al., 2015).

Metabolites related to tricarboxylic acid (TCA) cycle and fatty-acid metabolism have also been reported to be associated with the remission of T2D (Samczuk et al., 2018, Zhao et al., 2017).

Hence, this study was conducted to test the hypothesis that the differences in factors previously described as mechanisms of T2D improvement could be found between people who did and did not achieve non-diabetic glucose homeostasis post bariatric surgery. Insulin sensitivity, β-cell function, gut hormones, hepatic and pancreatic fat, visceral adipose tissue (VAT), body composition, and systemic metabolomics were compared between 2 groups namely:

1. Remitters
2. Their age, gender, type of surgery, duration of diabetes, baseline BW, BMI and PWL matched non-remitters

4.2 Material and Methods

4.2.1 Subjects

The electronic clinical database of bariatric patients at the UCLH Bariatric Centre for Weight Management and Metabolic Surgery was retrospectively reviewed. Patients who had had T2D at surgery and were at more than 1 year following a primary bariatric procedure at the time of the study were identified. The inclusion criteria include adults (age ≥18 years old) who at the time of surgery had T2D for <10 years. Next, subjects who met the criteria of diabetes remission (Buse et al., 2009) namely non-diagnostic levels of HbA_{1C} (<48 mmol/mol) and fasting plasma glucose (5.6 – 6.9 mmol/l) were allocated to the 'remitters' group. A matched group of patients without T2D remission for age, gender, type of bariatric surgery, BW, BMI, PWL and duration of T2D was created and called the 'non-remitters' group. The exclusion criteria were:

1. Current use of GLP-1 agonists
2. Types of diabetes other than T2D
3. Active cardiovascular diseases, cancers, renal or hepatic impairment
4. Pacemaker and metallic implants (in view of contraindications to MRI scanning)
5. Conversion or reversion of bariatric surgery

6. Active hypoglycaemia

Patients were approached by a research investigator by phone, asking about T2D history; for example, if they still had T2D, what medications they were taking and how the diabetes was controlled. An MRI safety check was also performed verbally. The detail of the study was explained and for those who agreed to participate in the study were given an appointment.

An appointment letter, a participant information sheet for the mixed-meal tolerance test (MMTT) (appendix 3), a patient information sheet for the MRI scan (appendix 4) and an MRI safety check list (appendix 5) were sent to the patients by post. Patients were very welcome to contact the research investigator by email and phone, if they required more information or had any questions.

Prior to the test day, patients were asked to refrain from alcohol for 24 hours and to fast overnight for a minimum of 12 hours prior to the test, usually from 08:00pm to 08:00am. They were allowed to drink water during the fasting period. On arrival, the second informed consent was signed by patients (appendix 2). The MMTT was performed at 08:00am on the study day as outlines in the 'Mixed-meal tolerance test (MMTT)' section, chapter 2.

4.2.2 Anthropometric measurement

BW was measured while patients wore indoor clothing without shoes and heavy accessories using a calibrated weighing sale (Seca 877, Seca, UK). Height was determined by a wall-mounted stadiometer (242 Measuring Rod, Seca, UK). BMI was calculated by dividing BW (kg) by the square of height (in metres) and PWL was calculated by the following formula: PWL = ([baseline BW – BW at each study visit]/ baseline BW) x 100. BIA (Tanita DC-430 MA S, Manchester, UK) was performed after a 12-hour fast with patients wearing light indoor clothing. They were then asked to step on the 4 electrodes with bare feet and arms straight down during measurement.

4.2.3 Magnetic resonance imaging (MRI)

After the MMTT, patients underwent a quantitative MRI scan of the abdomen and pelvis at the UCLH Macmillan Cancer Centre. Where possible, the MRI scan was on the same day as the MMTT, otherwise, it was scheduled within 1 week. Individual organ fat was quantified (liver, pancreas) and body composition measurements were assessed using the quantitative MR images.

For the organ fat quantification and measurements of body composition, the MRI scans were anonymised and analysed independently by two readers (both radiologists). The readers were blinded to the identity of the patients and the T2D remission status.

Liver, pancreatic, and skeletal muscle fat were measured as percentages (proton density fat fraction); body composition parameters included the ratio of visceral adipose tissue area to subcutaneous adipose tissue area (VAT:SAT ratio), and indices of total body fat mass, total body fat free mass and skeletal muscle (adjusted for patient height) were produced.

4.2.4 Gut hormone study

Details of samples collection and processing were described in 'Sample collection and processing' section in Chapter 2. In brief, the levels of insulin, PYY, active GLP-1, AG, DAG and FGF-19 were compared between diabetes remitters and non-remitters. Plasma samples from EDTA blood treated with 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF) (Fluka, UK), aprotinin (Trasylol, Bayer, UK) and HCl were used for AG assays as well as plasma samples from EDTA blood treated with aprotinin (Trasylol, Bayer, UK) and HCl were for DAG assays, plasma samples from EDTA blood treated with aprotinin were for insulin and FGF-19 assays, and plasma samples from EDTA blood treated with DPP4- inhibitor and aprotinin were for PYY and active GLP-1 assays. Details of each hormone assays was narrated in 'Gut hormone assays' section, Chapter 2.

4.2.5 Definition of gut hormone parameters

8. Fasting levels: gut hormone levels measured after a 12-hour fast

9. $AUC_{0\text{-}180}$: an area under the curve (AUC) of a gut hormone measured during a MMTT from time 0 – 180 minutes, calculated by the trapezoid rule
10. $AUC_{0\text{-}60}$: an AUC of a gut hormone measured during a MMTT from time 0 – 60 minutes
11. $\Delta AUC_{0\text{-}180}$: an AUC calculated by subtracting the fasting (0-minute) hormone level from every time-point level during the MMTT. In other words, it is an AUC calculated from t0 – t 0, t15 – t0, t30 – t0, t60 – t0, t90 – t0, t120 – t0, t150 – t0, and t180 – t0 levels, using the trapezoid rule
12. $\Delta AUC_{0\text{-}60}$: a ΔAUC from time 0 – 60 minutes

4.2.6 Insulin sensitivity and β-cell function

QUICKI score, calculated as 1/ (log [fasting plasma insulin] + log [fasting plasma glucose]), and 1/ fasting insulin were selected to indicate insulin sensitivity, whilst fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR), computed from (fasting plasma glucose x fasting plasma insulin)/ 22.5 in molar units, represents insulin resistance. In addition, HOMA-β (= [20 x fasting plasma insulin] / [fasting plasma glucose – 3.5] in molar units) was utilised as an indicator of β-cell function. Area under the curve (AUC) of insulin was also produced using the trapezoid rule. Owing to the absence of postprandial plasma glucose levels measured in this study, we applied to use the $AUC_{0\text{-}30}$ insulin to also reflect the ability of β-cell function since the first-phase insulin secretion occurring at 30 minutes post-meal (Bacha et al., 2008).

4.2.7 Metabolomics study

Details of samples collection and processing were described in 'Sample collection and processing' section in Chapter 2. Basically, a 5-mL blood in a 10-mL plain syringe (BD, Oxford, UK) was centrifuged at 1800 rpm for 10 minutes at 4℃. 350 µL of plasma samples were transferred into a 1-mL plastic tube and were kept at -80℃. All samples for metabolomics study were shipped with dry ice to be analysed at the Nightingale Health Ltd., Helsinki, Finland, using a liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) technique. Area under the curves (AUC) of serum metabolites during meal stress assays were produced using the trapezoid rule.

4.2.8 Statistical analysis

Results from the MMTT are described as fasting levels, area under the curve (AUC) and ΔAUC. The Shapiro-Wilk test was used to determine the normality of all variables. Continuous data with normal distribution was expressed as mean ± SD whilst the non-normally distributed data was presented as median (25^{th}, 75^{th} percentiles). Categorical variables were reported as percentages and χ^2 tests were used to compare between groups. The unpaired t-tests were used for the comparison of variables with normal distribution between groups. Mann-Whitney tests were used for the comparison of non-normally distributed data. Logistic regression was performed to identify factors associating with the remission of T2D.

In terms of metabolomics study, multivariate analysis was conducted to identify the most predictive metabolites for diabetes remission, measured in serum of patients during a mixed-meal tolerance test. A Discriminant Analysis based on Project of Latent Structures (PLS-DA) was performed using the AUC of the several metabolites over the duration of the MMTT as predictors of diabetes remission. To avoid multicollinearity issues, only independent variables were used (*e.g.*, the AUC of valine was included, but not the AUC of total branched-chain amino acids). Two diabetes categorisations were considered: Remission vs. No remission; and Total remission vs. Partial remission vs. No remission). The first two Latent Structures (LS) were obtained, and the corresponding component loadings for each variable. To clarify the discriminant power of the variables in the multivariate model, a Lasso penalty was applied to the variables with component loading ≤ |0.1|, using the sparse PLS-DA (sPLS-DA) method (Lê Cao et al., 2008). Subjects were then projected in the space spanned by the two first LSs according to the variable component loadings obtained by sPLS-DA. PERMANOVA was used to numerically compare sample distribution in the space spanned by the two first LSs according to the diabetes remission status (Anderson, 2017). For this method, a matrix of Euclidean distances between samples was calculated and 999 permutations were performed. P-values were corrected for multiple hypothesis testing by controlling the False Discovery Rate (FDR) with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Significance was considered whenever the adjusted-p < 0.05. These methods were

performed with R 4.0.3 (R Core Team, 2021), with the R packages mixOmics 6.14.0 (Rohart et al., 2017) and vegan 2.5-7 (Oksanen et al., 2020).

Variables with component loading ≥ |0.1| in any of the first two LSs obtained from sPLS-DA were selected for enrichment analysis using MetaboAnalyst 5.0 (Chong et al., 2019). The list of selected metabolites was used as input for comparison against The Small Molecule Pathway Database (SMPDB) of human metabolites. The proportion of hits to total number of metabolites linked to a metabolic pathway was used to calculate the pathway impact and the p-value. In this test, significance was considered when $p < 0.05$.

Univariate analysis was also performed to confirm relevant metabolites and to test variables excluded from multivariate analysis. Variables were tested for normality and homoskedasticity using Shapiro-Wilk Test and Levene's Test respectively. Parametric tests were applied if one of the assumptions was met. When grouping subjects in two groups (Remission vs. No Remission), metabolite AUCs were tested using T-test, considering equal or unequal variances according to the result of the Levene's Test. P-values were then adjusted using the Benjamini-Hochberg test. ROC curves were then obtained for variables with significant differences, using Logistic Regression. When grouping subjects in three groups (Total, Partial or No remission), One-way ANOVA was used. Overall ANOVA p-values were adjusted for the FDR using the Benjamini-Hochberg method. The p-values obtained for the family-wise comparisons were adjusted using the Tukey's Honestly Significant Difference (HSD) method. Regardless the statistical method applied, significance was considered when $p < 0.05$. These methods were performed using IBM SPSS Statistics v27.0.1.

4.3 Results

4.3.1 Patient flow

Figure 4.1 describes patient enrolment. Ninety-nine patients (62 with diabetes remission and 37 without the remission) were approached by a research investigator on a telephone call. Seventy-three patients were excluded. Of these, 42 patients declined to participate in the study, 10 did not answer the telephone call, 4 had conversion of bariatric surgery, 4 were on Liraglutide, 2 never had T2D, 2 had active hypoglycaemia, 1 had multiple cardiac stents, 1 had a mobility problem, 1 had an intestinal stoma, 1 had an active lung cancer, 1 had died since their previous follow-up, 1 had end-stage renal disease, 1 had latent autoimmune diabetes in adults (LADA), 1 had a stroke and 1 due to absence on the study day.

4.3.2 Patient characteristics at the time of surgery

The two patient cohorts, remitters and non-remitters, were comparable in terms of their demographics and BW at the time of surgery (Table 4.1). Patients in both groups had comparable age (52.5 [50.9, 55] years in non-remitters vs. 53.5 [48.6, 59.1] years in remitters, P = 0.32), BW (117.8 ± 19.4 kg in non-remitters vs. 116.1 ± 26.9 kg in remitters, P = 0.85) and BMI (42.6 ± 7.4 kg/m^2 in non-remitters vs. 41.9 ± 6.6 kg/m^2 in remitters, P = 79). 46% of non-remitters and 62% of remitters were female (P = 0.43). Approximately 2/3 of subjects underwent LRYGB and one-third had LSG in both groups (P = 0.59). The average time of having T2D in non-remitters (8.1 ± 3.7 years) was non-significantly greater than remitters (6.5 ± 4.3 years) (P = 0.34), and so does HbA_{1c} levels (62.4 ± 11.3 mmol/mol in non-remitters vs 54.3 ± 9.8 mmol/mol in remitters, P = 0.06).

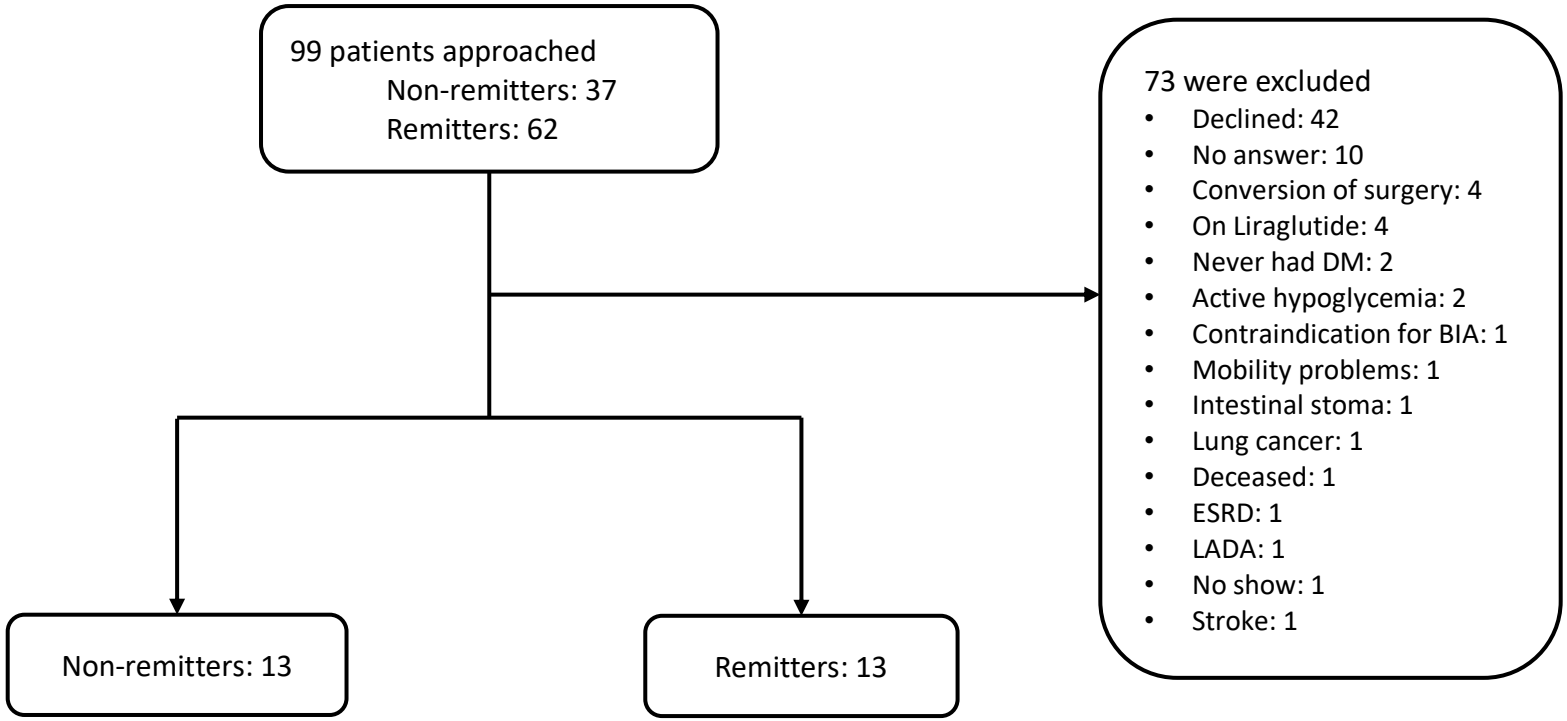


Figure 4.1 Subject enrolment

Table 4.1 Anthropometric, clinical, and metabolic features at the time of surgery

	Non-remitters (n = 13)	**Remitters (n = 13)**	**P-value**
Age, years	52.5 (50.9, 55)	53.5 (48.6, 59.1)	0.32
Female, %	46.2	61.5	0.43
Body weight, kg	117.8 ± 19.4	116.1 ± 26.9	0.85
BMI, kg/m^2	42.6 ± 7.4	41.9 ± 6.6	0.79
Type of surgery - **RYGB, %** - **SG, %** - **MGB, %**	 69.2 30.8 0	 61.5 30.8 7.7	0.59
Diabetes duration, years	8.1 ± 3.7	6.5 ± 4.3	0.34
HbA_{1c}, mmol/mol	62.4 ± 11.3	54.3 ± 9.8	0.06

Data were expressed as mean ±SD and median (P25, P75)

4.3.3 Anthropometric, clinical, and metabolic features at study

4.3.3.1 Anthropometric and clinical features

Patients in both groups were matched in age (60.2 [55.7, 61.9] years old in non-remitters vs. 58.1 [55.7, 63.2] years old in remitters, P = 0.79), BW (94.2 ± 15.7 kg in non-remitters vs. 92.6 ± 22.5 kg in remitters, P = 0.84), BMI (34.2 ± 4.8 kg/m^2 in non-remitters vs. 34 ± 5.4 kg/m^2 in remitters, P = 0.93) and PWL (20.6 ± 8.8% in non-remitters vs. 20.3 ± 5.7% in remitters, P = 0.93) (Table 4.2). Interestingly, patients with diabetes remission had a statistically significantly lower SBP than patients without the remission (123.2 ± 12.5 mmHg vs 135.7 ± 4.6 mmHg, P <0.01). There was no statistically significant difference between groups in DBP and PR (Table 4.2).

4.3.3.2 Glycaemic and lipid indices

As expected, the glucose and HbA_{1c} levels in remission group were lower than no remission (5 [4.4, 5.5] mmol/L vs. 7.4 (6.6, 9) mmol/L, P <0.001 for glucose and 40 [32, 43.5] mmol/mol vs. 52 [47.8, 62.3] mmol/mol, P <0.001 for HbA_{1c}). In addition, triglyceride (TG) level in remission patients was significantly less than no remission patients (0.9 [0.8, 1.2] mmol/L vs 1.5 [1.33, 2.18] mmol/L, P <0.01). There was no statistically significant difference between groups in total cholesterol, HDL-c, non-HDL-c, LDL-c and cholesterol-HDL ratio (Table 4.2).

4.3.3.3 Biochemical parameters

Parameters representing liver function including total bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT) and albumin were not statistically significantly different between groups (Table 4.2). There was no statistically significant difference in renal function parameters including the levels of urea and creatinine between groups (Table 4.2). Furthermore, the levels of thyroid-stimulating hormone (TSH) and free thyroxine (T_4) were comparable in both groups (Table 4.2).

Table 4.2 Anthropometric, clinical, and metabolic features at study

	Non-remitters (n = 13)	**Remitters (n = 13)**	**P-value**
Age, years	60.2 (55.7, 61.9)	58.1 (55.7, 63.2)	0.79
Body weight, kg	94.2 ± 15.7	92.6 ± 22.5	0.84
BMI, kg/m^2	34.2 ± 4.8	34 ± 5.4	0.93
Weight loss, %	20.6 ± 8.8	20.3 ± 5.7	0.93
Duration from surgery, years	6.9 ± 1.9	4.9 ± 2.1	0.02
Systolic blood pressure, mmHg	136 ± 5	123 ± 13	0.004
Diastolic blood pressure, mmHg	77 ± 8	72 ± 3	0.31
Pulse rate, bpm	77 ± 16	71 ± 12	0.3
Glycaemic and lipid indices			
Glucose, mmol/L	7.4 (6.6, 9)	5 (4.4, 5.5)	<0.001
HbA_{1c}, mmol/mol	52 (47.8, 62.3)	40 (32, 43.5)	<0.001
Cholesterol, mmol/L	4.49 ± 0.71	4.46 ± 0.85	0.93
Triglyceride, mmol/L	1.5 (1.33, 2.18)	0.9 (0.8, 1.2)	0.004
HDL-c, mmol/L	1.34 ± 0.33	1.65 ± 0.53	0.17
Non-HDL-c, mmol/L	3.15 (2.25, 4.53)	2.7 (2.3, 3.3)	0.55
LDL-c, mmol/L	2.13 ± 1.04	2.34 ± 0.78	0.63
Cholesterol-HDL ratio	3.69 ± 1.6	2.91 ± 0.86	0.19
Biochemistry			
Total bilirubin, umol/L	8.7 ± 3.6	7.6 ± 2.8	0.46
Alkaline phosphatase, IU/L	90.6 ± 20.4	81.7 ± 28.1	0.47
Alanine transaminase, IU/L	18 (15, 26)	21 (17, 31.5)	0.64
Albumin, g/L	44 (43, 45)	44 (44, 45)	0.7
Urea, mmol/L	5.6 ± 1.8	4.8 ± 1	0.22
Creatinine, umol/L	73.4 ± 21.3	74.7 ± 14.1	0.88
TSH, mIU/L	2.4 ± 1.9	2.2 ± 0.9	0.74
Free T_4, pmol/L	14.8 ± 1.4	15.3 ± 2.5	0.65

Data were expressed as mean ±SD, median (P25, P75)

Table 4.3 Number of patients with antidiabetic agents at pre-surgery and at study

Antidiabetic agents, n (%)	Non-remitters (n = 13)		Remitters (n = 13)	
	Pre-surgery	At study	Pre-surgery	At study
Metformin	12 (92%)	9 (69%)	8 (62%)	0
Sulfonylurea	6 (46%)	1 (8%)	1 (8%)	0
Pioglitazone	4 (31%)	1 (8%)	0	0
DPP-4 inhibitors	0	0	1 (8%)	0
SGLT-2 inhibitors	0	1 (8%)	0	0
GLP-1 receptor agonists	1 (8%)	0	0	0
Insulin glagine	0	1 (8%)	0	0

DPP-4, Dipeptidyl peptidase 4; SGLT-2, sodium glucose co-transporter type 2; GLP-1, glucagon-like peptide 1

4.3.3.4 Insulin sensitivity and β-cell function indices at study

With regards to meal-stimulated plasma concentrations of insulin, the level rapidly increased from 0 to 30 minutes, with a further gradual increase to reach its peak at 60 minutes, followed by a marked drop to 90 minutes. After this point, the insulin level slightly decreased up to 180 minutes (Figure 4.2). In remitters, the insulin level was statistically significantly higher than the non-remitters at 15 and 30 minutes. However, the level in remitters was statistically significantly lower than non-remitters at 90 and 120 minutes (Figure 4.2).

The levels of fasting insulin, AUC_{0-30} insulin and AUC_{0-180} insulin in remitters were greater than non-remitters (fasting insulin: 19.4 [9.2, 47] pM in remitters vs. 17.1 [7.7, 47.1] pM in non-remitters, P = 0.9; AUC_{0-30} insulin: 12,414 ± 5,550 pM x min in remitters vs. 7,071 ± 4,590 pM x min in non-remitters, P = 0.02; AUC_{0-180} insulin: 55,477 ± 27,509 pM x min in remitters vs. 44,309 ± 24,813 pM x min in non-remitters, P = 0.3) (Table 4.4, Figure 4.3A, C-D). However, the difference only achieved statistical significance for the AUC_{0-30} insulin.

In terms of insulin sensitivity/resistance and β-cell function indices, there was no significant difference in fasting insulin levels and 1/fasting insulin between groups (fasting insulin: 19.4 [9.2, 47] pM in remitters vs. 17.1 [7.7, 47.1] pM in non-remitters, P = 0.9, Table 4.4, Figure 4.3A; 1/fasting insulin: 0.07 [0.02, 0.13] pM^{-1} in non-remitters vs. 0.05 [0.02, 0.11] pM^{-1} in remitters, P = 0.76, Table 4.4, Figure 4.3B). The HOMA-IR in non-remitters was non-significant higher than remitters (1.72 [0.35, 2.86] vs. 0.54 [0.33, 1.55], P = 0.17, Table 4.4, Figure 4.3E), whilst the HOMA-β in remitters was statistically significantly greater than in non-remitters (50.5 ± 39.3% vs. 20.7 ± 16.5%, P = 0.03, Table 4.4, Figure 4.3F). The QUICKI index in remitters was slightly higher than non-remitters (0.43 [0.36, 0.48] vs. 0.35 [0.33, 0.47], P = 0.23, Table 4.4, Figure 4.3G); however, this did not reach statistical significance.

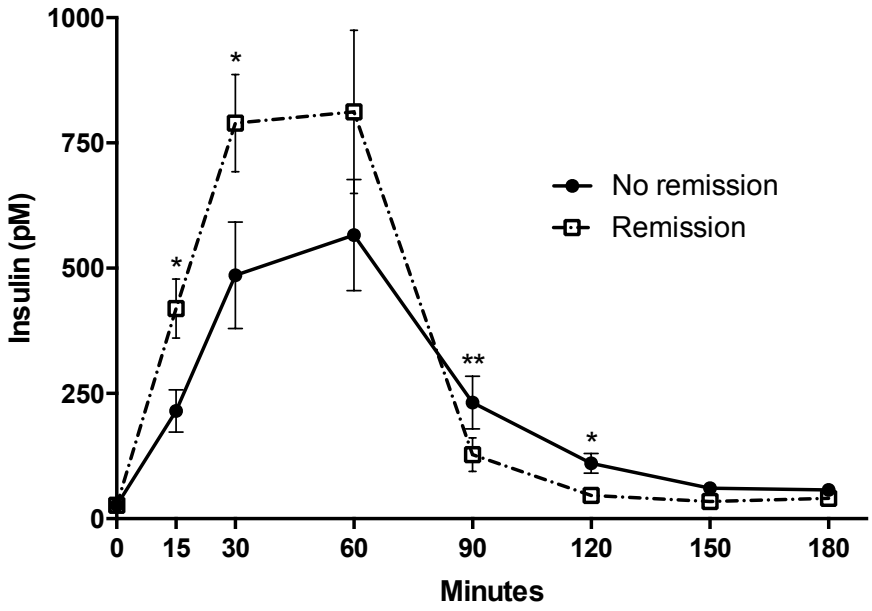


Figure 4.2 Nutrient-stimulated insulin levels. Results were expressed as mean ±SEM. *P <0.05, **P <0.01 of the comparisons between groups

Table 4.4 Insulin sensitivity and β-cell function indices at study

	No remission (n = 13)	**Remission (n = 13)**	**P-value**
Fasting insulin, pM	17.1 (7.7, 47.1)	19.4 (9.2, 47)	0.9
AUC_{0-30} insulin, pM x min	7,071 ± 4,590	12,414 ± 5,550	0.02
AUC_{0-180} insulin, pM x min	44,309 ± 24,813	55,477 ± 27,509	0.3
1/ fasting insulin, pM^{-1}	0.07 (0.02, 0.13)	0.05 (0.02, 0.11)	0.76
HOMA-IR	1.72 (0.35, 2.86)	0.54 (0.33, 1.55)	0.17
HOMA-β	20.7 ± 16.5	50.5 ± 39.3	0.03
QUICKI	0.35 (0.33, 0.47)	0.43 (0.36, 0.48)	0.23

Data were expressed as mean ±SD, median (P25, P75)

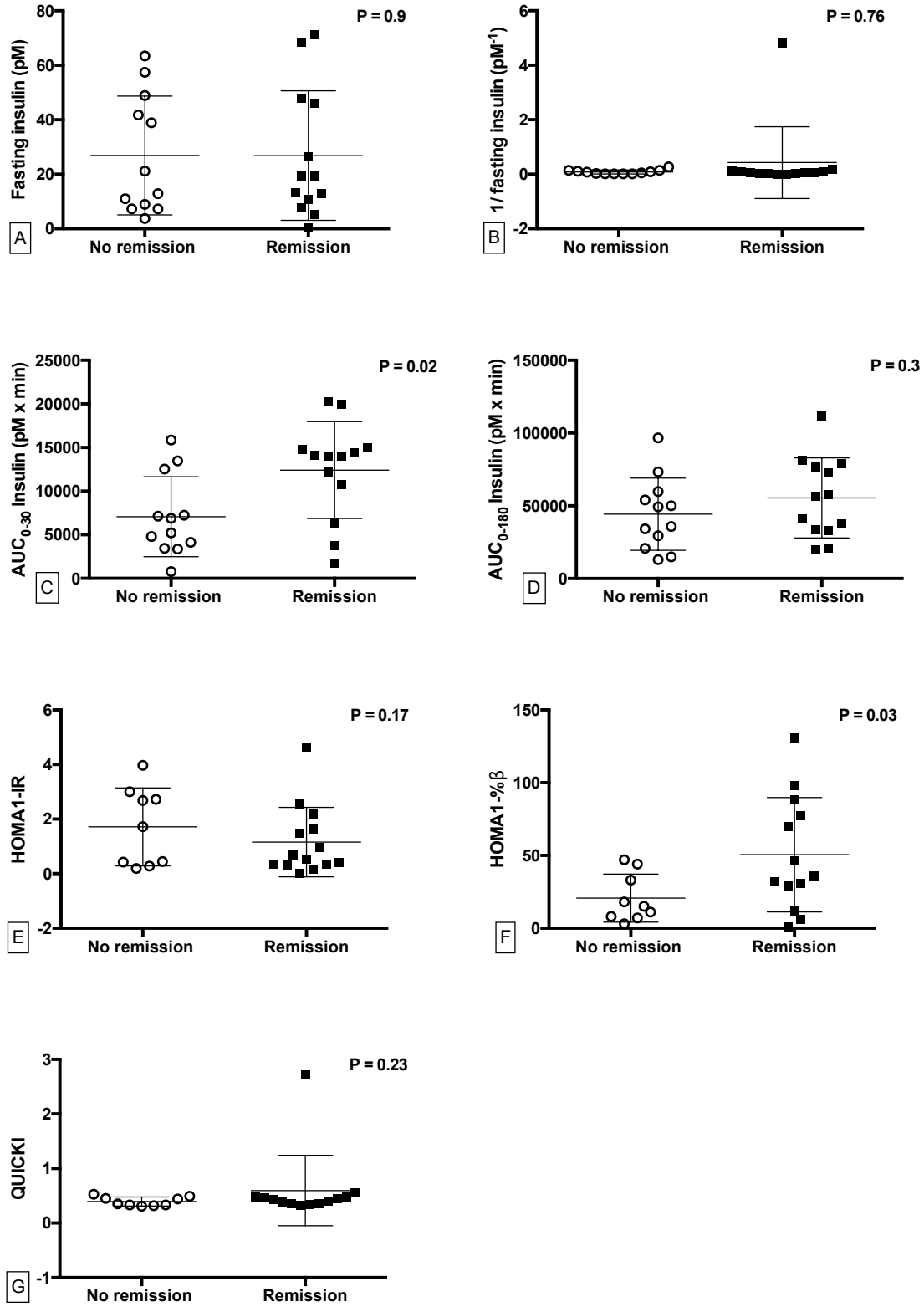


Figure 4.3 Comparisons of insulin sensitivity indices between non-remitters and remitters: (A) fasting insulin, (B) 1 /fasting insulin, (C) $AUC_{0\text{-}30}$ insulin, (D) $AUC_{0\text{-}180}$ insulin, (E) HOMA-IR, (F) HOMA-β and (G) QUICKI

4.3.3.5 Gut hormone profiles at study

PYY

During the MMTT, the PYY level markedly rose to reach its highest at 60 minutes and then gradually dropped until 180 minutes. There was no significant difference in PYY levels at any time-points during the 180-minute MMTT between groups (Figure 4.4A). The fasting PYY and $AUC_{0\text{-}180}$ PYY in non-remitters seemed to be higher than remitters (fasting PYY: 139 $\pm$ 78 pg/mL in non-remitters vs. 99 $\pm$ 36 pg/mL in remitters, P = 0.11; $AUC_{0\text{-}180}$ PYY: 52,099 [41,150, 77,329] pg x min/mL in non-remitters vs. 42,864 [35,972, 65,183] pg x min/mL in remitters, P = 0.43, Table 4.5, Figure 4.5A-B). After subtracting fasting PYY, the $\Delta AUC_{0\text{-}180}$ PYY was not significantly different between groups ($\Delta AUC_{0\text{-}180}$ PYY: 28,578 [23,235, 44,232] pg x min/mL in non-remitters vs. 29,170 [19,003, 38,671] pg x min/mL in remitters, P = 0.8, Table 4.5, Figure 4.5C).

Active GLP-1

The nutrient-stimulated active GLP-1 levels during the MMTT followed the similar pattern as PYY, which the hormone significantly increased from time 0 minute to its highest at 30 minutes. The levels then plummeted up to 90 minutes and the reduction of active GLP-1 levels gradually continued until 180 minutes. The levels of active GLP-1 were statistically comparable in both remitters and non-remitters groups at every time-points during the MMTT (Figure 4.4B). The fasting levels of active GLP-1 in non-remitters was non-significantly higher than remitters (4.8 [1.6, 8.1] pM vs. 2.9 [1, 9.1] pM, P = 0.46, Table 4.5, Figure 4.5D). Nevertheless, the levels of $AUC_{0\text{-}180}$ active GLP-1 and $\Delta AUC_{0\text{-}180}$ active GLP-1 were not different between groups ($AUC_{0\text{-}180}$ active GLP-1: 4,782 [2,110, 11,639] pM x min in non-remitters vs. 4,887 [2,854, 15,109] pM x min in remitters, P = 0.69, $\Delta AUC_{0\text{-}180}$ active GLP-1: 4,808 [2,293, 11,004] pM x min vs. 3,326 [1,708, 10,342] pM x min, P = 0.61, Table 4.5, Figure 4.5E,F).

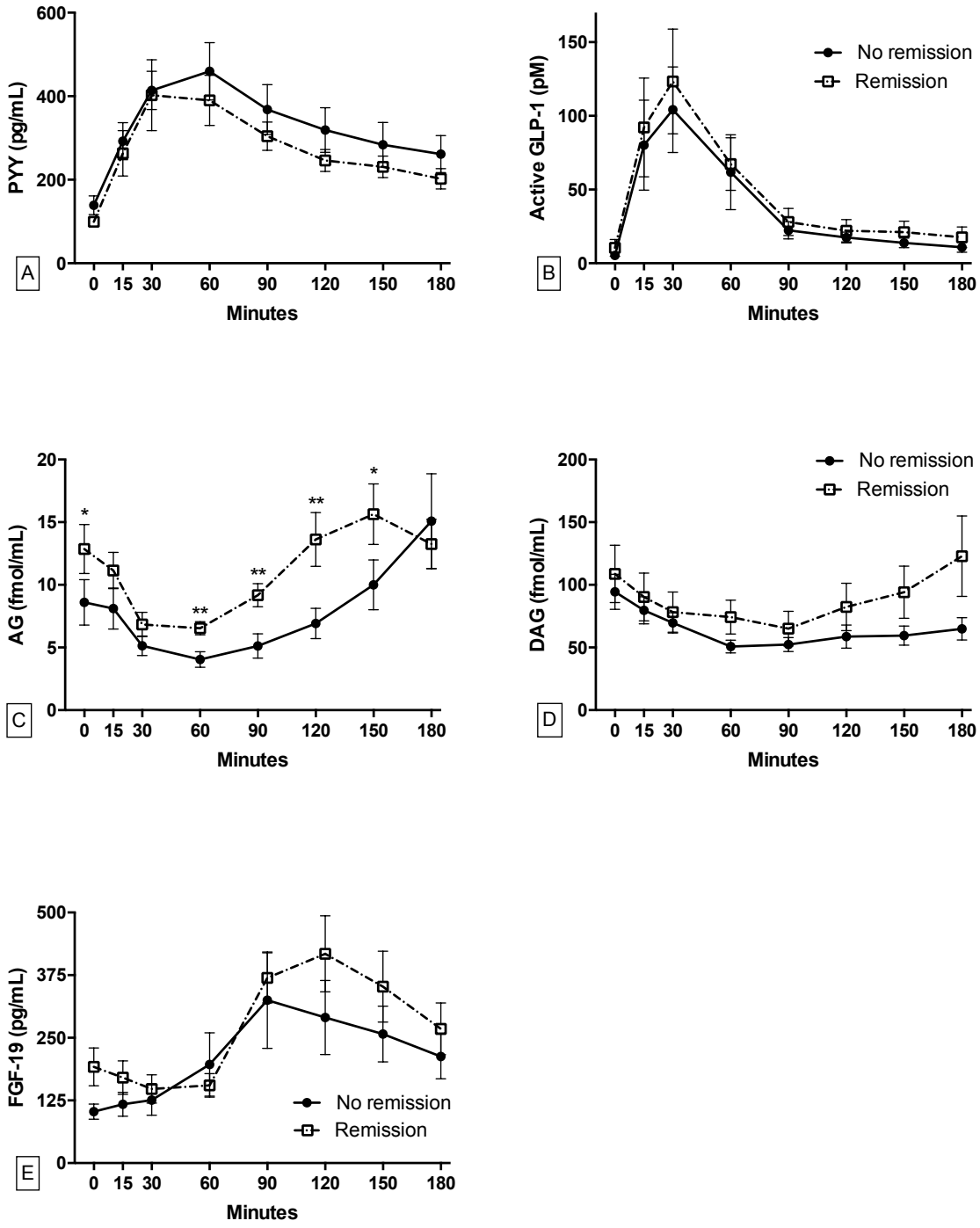


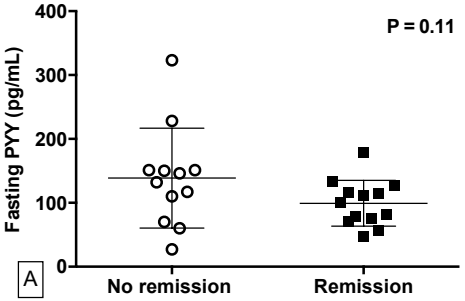
Figure 4.4 Nutrient-stimulated gut hormone levels: (A) PYY, (B) active GLP-1, (C) AG, (D) DAG and (E) FGF-19. Results were expressed as mean ±SEM. *P <0.05, **P <0.01 of the comparisons between groups

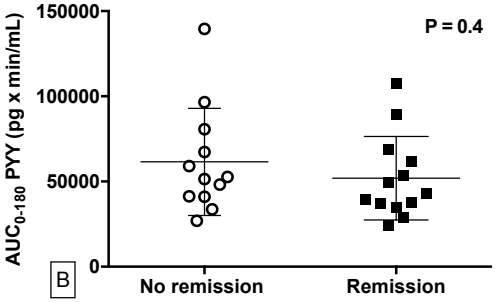
Table 4.5 Gut hormone profiles at study

	No remission (n = 13)	**Remission (n = 13)**	**P-value**
Fasting PYY, pg/mL	139 ± 78	99 ± 36	0.11
AUC_{0-60} PYY, pg x min/mL	19,750 (14,256, 29,814)	15,246 (11,614, 25,511)	0.32
AUC_{0-180} PYY, pg x min/mL	52,099 (41,150, 77,329)	42,864 (35,972, 65,183)	0.43
ΔAUC_{0-60} PYY, pg x min/mL	11,356 (9,702, 19,290)	10,783 (6,028, 16,125)	0.61
ΔAUC_{0-180} PYY, pg x min/mL	28,578 (23,235, 44,232)	29,170 (19,003, 38,671)	0.8
Fasting active GLP-1, pM	4.8 (1.6, 8.1)	2.9 (1, 9.1)	0.46
AUC_{0-60} active GLP-1, pM x min	3,022 (1,499, 6,837)	3,228 (1,566, 9,206)	0.89
AUC_{0-180} active GLP-1, pM x min	4,782 (2,110, 11,639)	4,887 (2,854, 15,109)	0.69
ΔAUC_{0-60} active GLP-1, pM x min	2,887 (1,333, 6,383)	3,202 (1,447, 6,685)	0.85
ΔAUC_{0-180} active GLP-1, pM x min	3,326 (1,708, 10,342)	4,808 (2,293, 11,004)	0.61
Fasting FGF-19, pg/mL	99.1 (62.7, 134.2)	145.2 (110, 247.2)	0.06
AUC_{0-60} FGF-19, pg x min/mL	5,869 (4,241, 8,476)	7,730 (5,967, 11,732)	0.27
AUC_{0-180} FGF-19, pg x min/mL	27,282 (18,971, 74,979)	44,141 (23,429, 77,434)	0.32
ΔAUC_{0-60} FGF-19, pg x min/mL	112 (-571, 3,322)	-954 (-4,190, -263)	0.01
ΔAUC_{0-180} FGF-19, pg x min/mL	10,600 (2,618, 44,035)	9,672 (6,071, 23,539)	0.96
Fasting AG, fmol/mL	5.3 (3.6, 15.1)	11 (6.5, 19.9)	0.04
AUC_{0-60} AG, fmol x min/mL	362 ± 195	516 ± 204	0.07
AUC_{0-180} AG, fmol x min/mL	1,311 ± 660	1,966 ± 743	0.03
ΔAUC_{0-60} AG, fmol x min/mL	-47 (-241, 4)	-128 (-546, -61)	0.14

$\Delta AUC_{0\text{-}180}$ AG, fmol x min/mL	-238 ± 668	-348 ± 737	0.7
Fasting DAG, fmol/mL	80 (49, 135)	82 (39, 176)	0.84
$AUC_{0\text{-}60}$ DAG, fmol x min/mL	3,784 (2,897, 5,946)	3,564 (2,139, 7,408)	0.85
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	10,313 (7,658, 14,629)	12,010 (6,656, 19,910)	0.56
$\Delta AUC_{0\text{-}60}$ DAG, fmol x min/mL	-1,431 ± 1,491	-1,480 ± 1,716	0.34
$\Delta AUC_{0\text{-}180}$ DAG, fmol x min/mL	-5,906 ± 5,013	-4,321 ± 5,040	0.44
Fasting AG:DAG	0.09 ± 0.04	0.14 ± 0.05	0.007
$AUC_{0\text{-}60}$ AG:DAG	0.07 (0.06, 0.12)	0.13 (0.08, .16)	0.04
$AUC_{0\text{-}180}$ AG:DAG	0.12 (0.08, 0.15)	0.16 (0.11, 0.23)	0.14
$\Delta AUC_{0\text{-}60}$ AG:DAG	0.06 ± 0.23	0.1 ± 0.16	0.65
$\Delta AUC_{0\text{-}180}$ AG:DAG	0.02 (-0.08, 0.17)	0.04 (-0.06, 0.12)	0.94

Data were expressed as mean ±SD, median (P25, P75)


Fasting PYY (pg/mL)
400
300
200
100
0
P = 0.11
A
No remission
Remission


AUC0-180 PYY (pg x min/mL)
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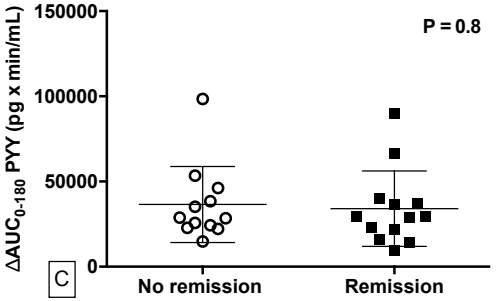
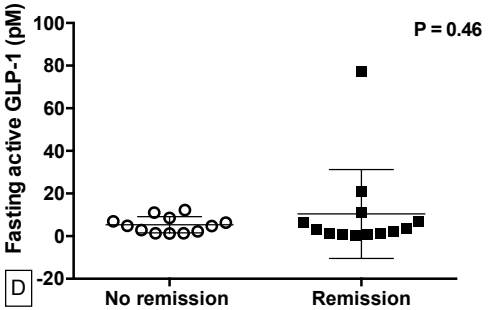
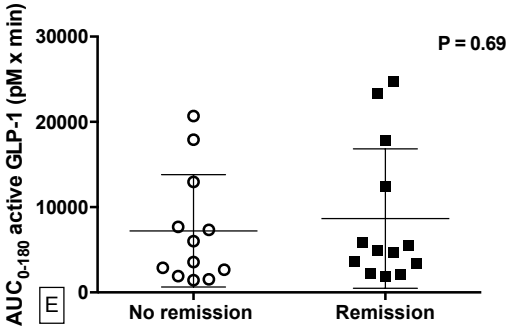
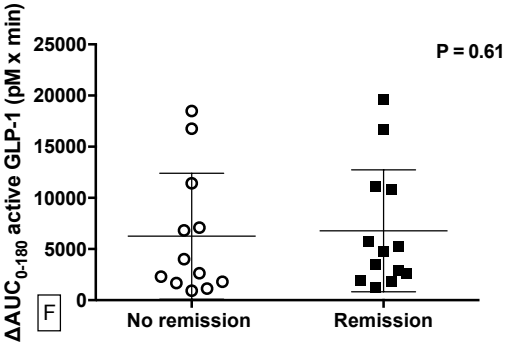
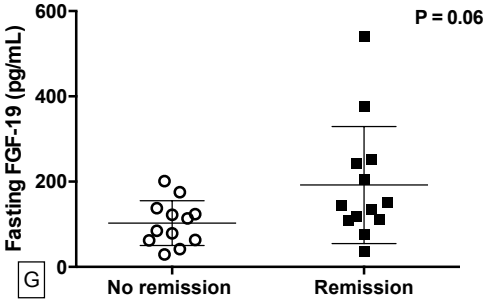
100000
50000
0
P = 0.4
B
No remission
Remission

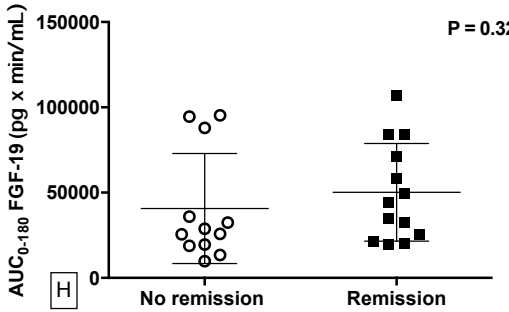

ΔAUC0-180 PYY (pg x min/mL)
150000
100000
50000
0
P = 0.8
C
No remission
Remission

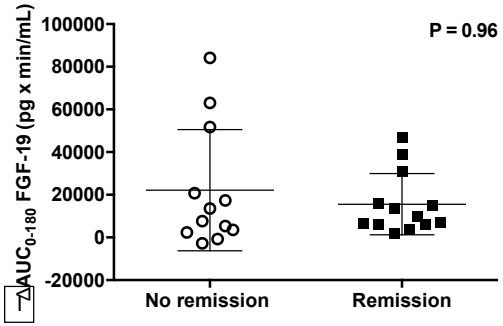

Fasting active GLP-1 (pM)
100
80
60
40
20
0
-20
P = 0.46
D
No remission
Remission


AUC0-180 active GLP-1 (pM x min)
30000
20000
10000
0
P = 0.69
E
No remission
Remission


ΔAUC0-180 active GLP-1 (pM x min)
25000
20000
15000
10000
5000
0
P = 0.61
F
No remission
Remission


Fasting FGF-19 (pg/mL)
600
400
200
0
P = 0.06
G
No remission
Remission


AUC0-180 FGF-19 (pg x min/mL)
150000
100000
50000
0
P = 0.32
H
No remission
Remission


ΔAUC0-180 FGF-19 (pg x min/mL)
100000
80000
60000
40000
20000
0
-20000
P = 0.96
I
No remission
Remission

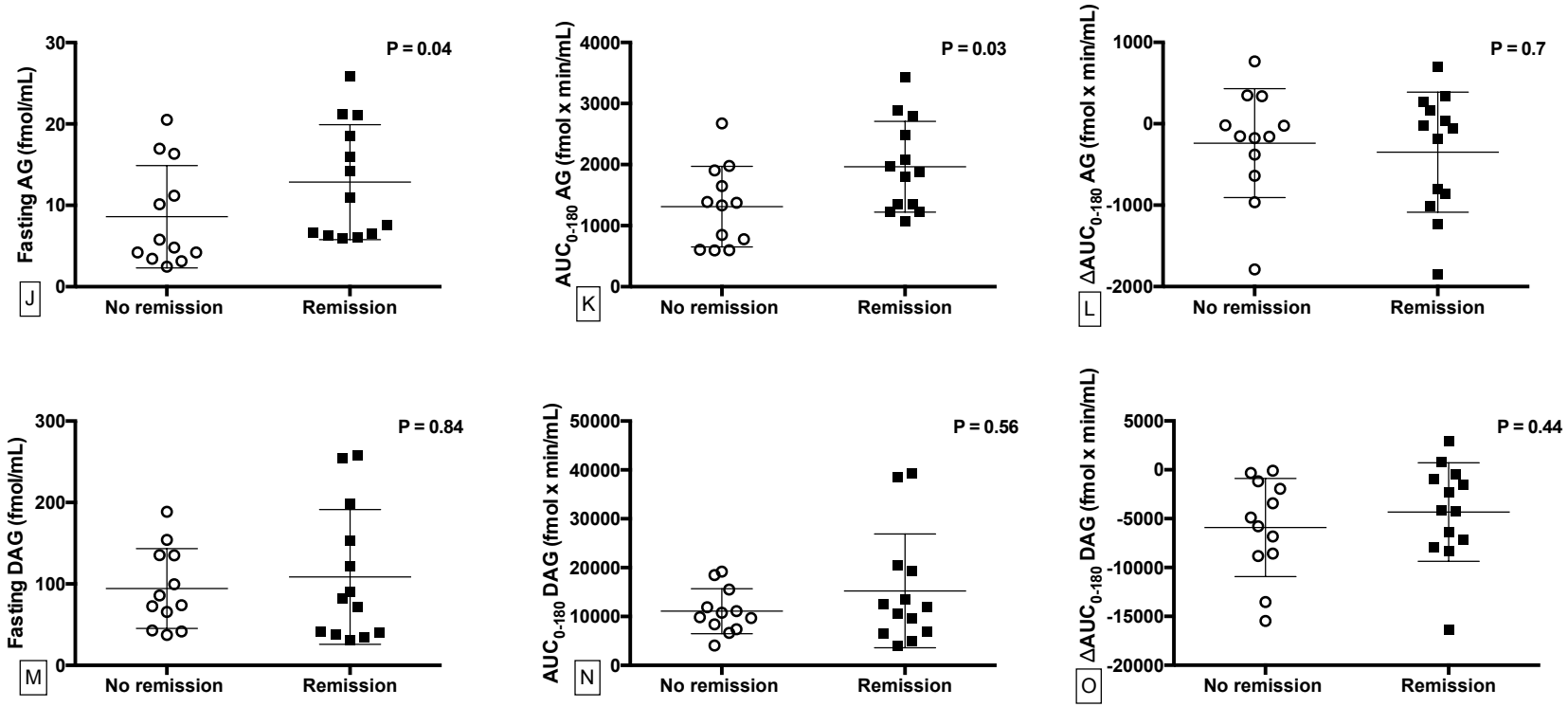


Figure 4.5 The comparisons of gut hormone profiles between non-remitters and remitters: (A) fasting PYY, (B) $AUC_{0\text{-}180}$ PYY, (C) $\Delta AUC_{0\text{-}180}$ PYY, (D) fasting active GLP-1, (E) $AUC_{0\text{-}180}$ active GLP-1, (F) $\Delta AUC_{0\text{-}180}$ active GLP-1, (G) fasting FGF-19, (H) $AUC_{0\text{-}180}$ FGF-19, (I) $\Delta AUC_{0\text{-}180}$ GLP-1, (J) fasting AG, (K) $AUC_{0\text{-}180}$ AG, (L) $\Delta AUC_{0\text{-}180}$ AG, (M) fasting DAG, (N) $AUC_{0\text{-}180}$ DAG and (O) $\Delta AUC_{0\text{-}180}$ DAG

FGF-19

The pattern of FGF-19 responses to the MMTT in remission and no remission subjects showed a slightly divergent path (Figure 4.4E). In non-remitters, after meal ingestion, the levels of FGF-19 gradually rose to reach the peak at 90 minutes, and then slowly declined until 180 minutes. In remitters, the hormone marginally reduced from 0 – 30 minutes, and then seemed to be plateau until 60 minutes. Following this FGF-19 levels increased to peak at 120 minutes, and then dropped until 180 minutes. Despite the inconsistent pattern, there was no statistically significant difference in hormone levels at any time-points between groups during the MMTT.

The fasting FGF-19 levels in remission group was marginally significantly greater than no remission (145.2 [110, 247.2] pg/mL vs 99.1 [62.7, 134.2] pg/mL, P = 0.06, Table 4.5, Figure 4.5G). $AUC_{0\text{-}180}$ FGF-19 in remitters was non-significantly higher than non-remitters (44,141 [23,429, 77,434] pg x min/mL vs 27,282 [18,971, 74,979] pg x min/mL, P = 0.32, Table 4.5, Figure 4.5H). Interestingly, the postprandial suppression of FGF-19 from time 0 – 60 minutes ($\Delta AUC_{0\text{-}60}$ FGF-19) in remitters were significantly greater than non-remitters (-954 [-4,190, -263] vs. 112 [-571, 3,322], P = 0.01), whilst the $\Delta AUC_{0\text{-}180}$ FGF-19 was comparable between groups (10,600 [2,618, 44,035] pg x min/mL in non-remitters vs 9,672 [6,071, 23,539] pg x min/mL in remitters, P = 0.96, Table 4.5, Figure 4.5I).

AG

Subjects with diabetes remission had a statistically significant higher fasting AG than no remission (11 [6.5, 19.9] fmol/mL vs 5.3 [3.6, 15.1] fmol/mL, P = 0.04, Table 4.5, Figure 4.5J). Up to 60 minutes after the test-meal ingestion, the AG levels considerably dropped, and then slowly rose until 180 minutes. In remitters, the hormone levels were significantly greater than non-remitters at 60-150 minutes (Figure 4.4C). The $AUC_{0\text{-}180}$ AG in remitters was also statistically significantly more than non-remitters (1,966 $\pm$ 743 fmol x min/mL vs 1,311 $\pm$ 660 fmol x min/mL, P = 0.03, Table 4.5, Figure 4.5K); however, the significant difference in $\Delta AUC_{0\text{-}180}$ AG between groups was not seen (-238 $\pm$ 668 fmol x min/mL in non-remitters vs -348 $\pm$ 737 fmol x min/mL in remitters, P = 0.7, Table 4.5, Figure 4.5L).

DAG

There was no statistically significant difference between groups in fasting levels of DAG (80 [49, 135] fmol/mL in non-remitters vs 82 [39, 176] in remitters, P = 0.84, Table 4.5, Figure 4.5M). In terms of nutrient-stimulated plasma concentrations of DAG, a similar pattern to AG was observed. The levels of DAG during the MMTT in remitters seemed to be greater than non-remitters at all time-points; nonetheless, it did not achieve statistical significance (Figure 4.4D). Regarding the AUC, no significant difference in both $AUC_{0\text{-}180}$ DAG and $\Delta AUC_{0\text{-}180}$ DAG between groups was observed ($AUC_{0\text{-}180}$ DAG: 10,313 [7,658, 14,629] fmol x min/mL in non-remitters vs 12,010 [6,656, 19,910] fmol x min/mL in remitters, P = 0.56; $\Delta AUC_{0\text{-}180}$ DAG -5,906 $\pm$ 5,013 fmol x min/mL in non-remitters vs -4,321 $\pm$ 5,040 fmol x min/mL in remitters, P = 0.44, Table 4.5, Figure 4.5N-O).

AG:DAG

The fasting AG:DAG and $AUC_{0\text{-}60}$ AG:DAG in non-remitters were significantly lower than remitters (0.09 $\pm$ 0.04 vs 0.14 $\pm$ 0.05, P = 0.007 for fasting AG:DAG and 0.07 [0.06, 0.12] vs. 0.13 [0.08, .16], P = 0.04 for $AUC_{0\text{-}60}$ AG:DAG, Table 4.5). This can be explained by the greater levels of fasting AG and AUC AG in remitters than non-remitters, since the levels of fasting DAG, AUC DAG and ΔAUC DAG were comparable between groups. There was no significant difference between groups in the rest of parameters Table 4.5.

4.3.3.6 MRI parameters at study

The hepatic and pancreatic fat content was similar in both groups (hepatic fat: 4.27 [3.23, 4.58]% in non-remitters vs 4.57 [3.64, 6.2]% in remitters, P = 0.31; pancreatic fat: 8.04 [7.58, 10.21]% in non-remitters vs 8.6 [7.52, 11.71]% in remitters, P = 0.75, Table 4.6, Figure 4.6A-B). The area of total fat and subcutaneous adipose tissue (SAT) was also statistically indistinguishable between groups (total fat area: 894.8 ± 296.2 cm^2 in non-remitters vs 888.6 ± 336 cm^2 in remitters, P = 0.96; SAT area: 530.2 ± 205.1 cm^2 in non-remitters vs 667.5 ± 307.9 cm^2 in remitters, P = 0.19, Table 4.6, Figure 4.6C-D).

Interestingly, the MRI scan revealed that patients who achieved diabetes remission possessed significantly less area of visceral adipose tissue (VAT) and the ratio of VAT area

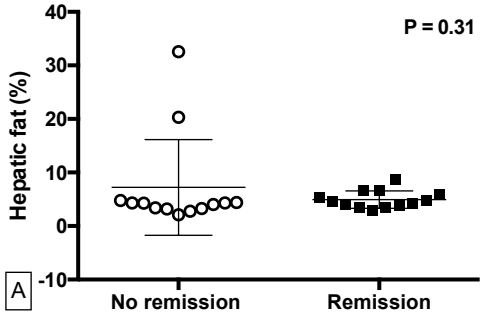
to SAT area (VAT:SAT ratio) than patients without remission (VAT area: 195.6 [143.4, 284.2] cm^2 in remitters vs 314.5 [225.7, 451.6] cm^2 in non-remitters, P = 0.04; VAT:SAT ratio: 0.29 [0.21, 0.48] in remitters vs 0.54 [0.37, 1.16] in non-remitters, P = 0.02, Table 4.6, Figure 4.6E-F).

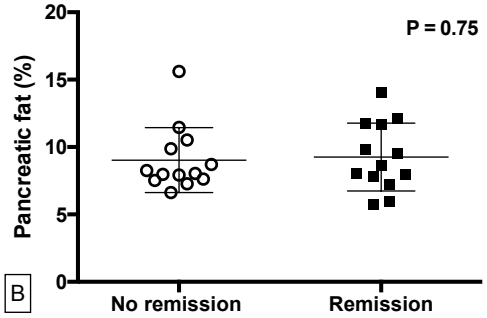
There was no statistically significant difference between remitters and non-remitters in the rest of parameters including fat mass (FM) index (17.7 ± 4.6 kg/m^2 in non-remitters vs 17.6 ± 4.6 kg/m^2 in remitters, P = 0.99), fat free mass (FFM) index (29.3 ± 4.5 kg/m^2 in non-remitters vs 29.1 ± 3.5 kg/m^2 in remitters, P = 0.89), skeletal muscle (SM) index (90.5 ± 15.5 cm^2/m^2 in non-remitters vs 89.7 ± 12.3 cm^2/m^2 in remitters, P = 0.88) and SM fat fraction (23.7 ± 4.3% in non-remitters vs 23.9 ± 4.8% in remitters, P = 0.91) (Table 4.6, Figure 4.6G-J).

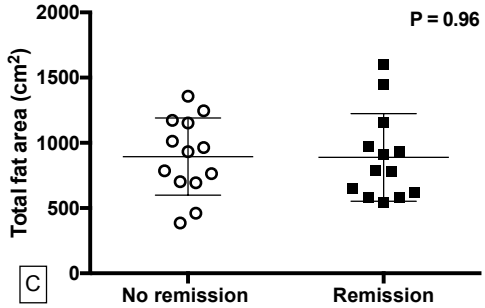
Table 4.6 MRI parameters at study

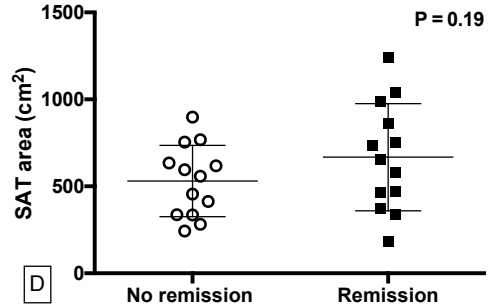
	No remission (n = 13)	**Remission (n = 13)**	**P-value**
Hepatic fat, %	4.27 (3.23, 4.58)	4.57 (3.64, 6.2)	0.31
Pancreatic fat, %	8.04 (7.58, 10.21)	8.6 (7.52, 11.71)	0.75
Total fat area, cm^2	894.8 ± 296.2	888.6 ± 336	0.96
SAT area, cm^2	530.2 ± 205.1	667.5 ± 307.9	0.19
VAT area, cm^2	314.5 (225.7, 451.6)	195.6 (143.4, 284.2)	0.04
VAT:SAT ratio	0.54 (0.37, 1.16)	0.29 (0.21, 0.48)	0.02
FM index, kg/m^2	17.7 ± 4.6	17.6 ± 4.6	0.99
FFM index, kg/m^2	29.3 ± 4.5	29.1 ± 3.5	0.89
SM index, cm^2/m^2	90.5 ± 15.5	89.7 ± 12.3	0.88
SM fat fraction, %	23.7 ± 4.3	23.9 ± 4.8	0.91

Data were expressed as mean ±SD, median (P25, P75); SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VAT:SAT ratio, the ratio of VAT area to SAT area; FM, fat mass; FFM, fat free mass; SM, skeletal muscle; FM index was calculated by fat mass/ height $(m)^2$; FFM index was calculated by fat free mass/ height $(m)^2$; SM index was calculated by skeletal muscle area/ height $(m)^2$


P = 0.31
Hepatic fat (%)
40
30
20
10
0
-10
A
No remission
Remission


P = 0.75
Pancreatic fat (%)
20
15
10
5
0
B
No remission
Remission


P = 0.96
Total fat area (cm²)
2000
1500
1000
500
0
C
No remission
Remission


P = 0.19
SAT area (cm²)
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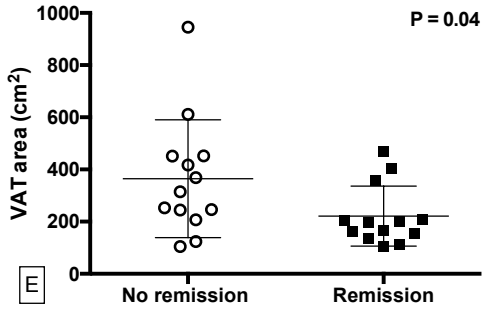
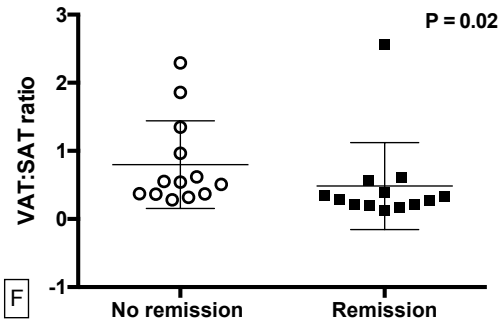
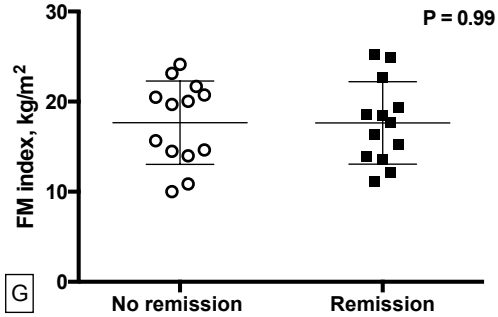
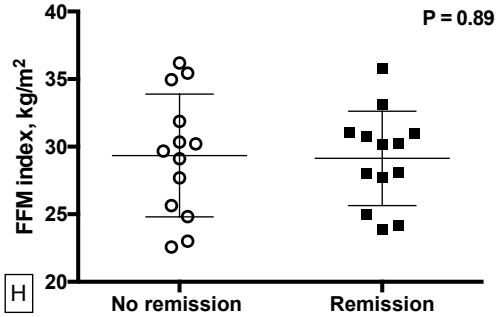
1000
500
0
D
No remission
Remission


P = 0.04
VAT area (cm²)
1000
800
600
400
200
0
E
No remission
Remission


P = 0.02
VAT:SAT ratio
3
2
1
0
-1
F
No remission
Remission


P = 0.99
FM index, kg/m²
30
20
10
0
G
No remission
Remission


P = 0.89
FFM index, kg/m²
40
35
30
25
20
H
No remission
Remission

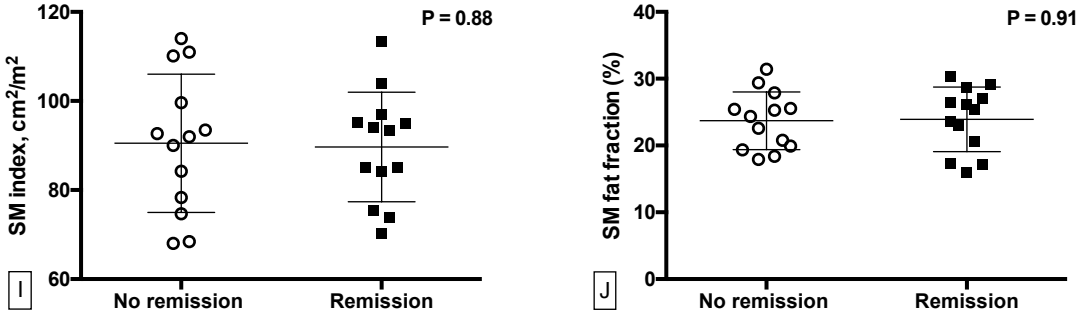


Figure 4.6 The comparisons of MRI parameters between groups: (A) Hepatic fat, (B) Pancreatic fat, (C) SAT area, (D) VAT area, (E) Total fat area, (F) VAT:SAT ratio, (G) FM index, (H) FFM index, (I) SM index and (J) SM fat fraction

4.3.3.7 BIA parameters at study

All parameters relevant to body composition that were measured by the BIA did not show statistically significant difference between groups. The median of FM index in non-remitters was 13.1 (9.4, 17) kg/m^2 whereas it was 14.1 (8.6, 15.4) kg/m^2 in remitters (P = 0.73, Table 4.7, Figure 4.7A). The mean FFM index was 21.6 ± 2.7 kg/m^2 and 20.8 ± 2.7 kg/m^2 in non-remitters and remitters, respectively (P = 0.45, Table 4.7, Figure 4.7B). The mean muscle mass index was 20.5 ± 2.6 kg/m^2 in non-remitters and 19.7 ± 2.6 kg/m^2 in remitters (P = 0.45, Table 4.7, Figure 4.7C). The average visceral fat rating was 16.3 ± 5.2 in non-remitters and 14.7 ± 6.4 in remitters (P = 0.49, Table 4.7, Figure 4.7D). The basal metabolic rate (BMR) was also similar in both groups (7,481 ± 1,350 kJ in non-remitters vs 7,346 ± 1,696 kJ in remitters, P = 0.82, Table 4.7, Figure 4.7E).

Table 4.7 BIA parameters at study

	No remission (n = 13)	**Remission (n = 13)**	**P-value**
FM index, kg/m^2	13.1 (9.4, 17)	14.1 (8.6, 15.4)	0.73
FFM index, kg/m^2	21.6 ± 2.7	20.8 ± 2.7	0.45
Muscle mass index, kg/m^2	20.5 ± 2.6	19.7 ± 2.6	0.45
Visceral fat rating	16.3 ± 5.2	14.7 ± 6.4	0.49
BMR, kJ	7,481 ± 1,350	7,346 ± 1,696	0.82

Data were expressed as mean ±SD, median (P25, P75);

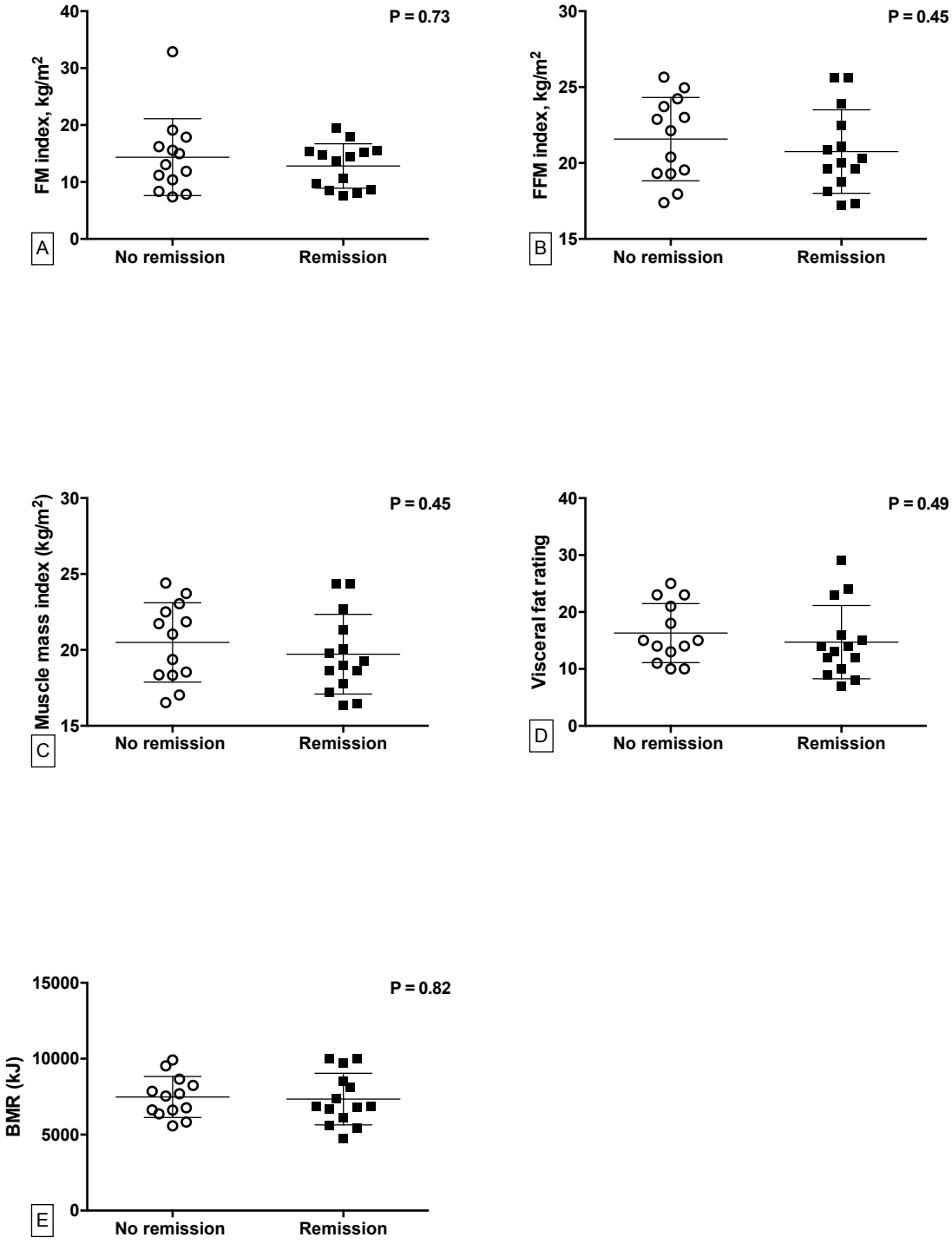


Figure 4.7 The comparisons of BIA parameters between groups: (A) Fat mass (FM) index, (B) fat-free mass (FFM) index, (C) muscle mass index, (D) visceral fat rating and (E) Basal metabolic rate (BMR)

4.3.4 Factors associated with diabetes remission

In order to examine whether insulin sensitivity and β-cell function indices, gut hormones and body composition measured by MRI and BIA are associated with diabetes remission, a logistic regression was performed. $AUC_{0\text{-}30}$ insulin was statistically significantly associated with diabetes remission (P = 0.03), and HOMA-β also showed a marginally significant association (P = 0.08, Table 4.8). With regard to gut hormone parameters, fasting FGF-19 and $\Delta AUC_{0\text{-}60}$ FGF-19 marginally correlated with diabetes remission (P = 0.08 for fasting FGF-19 and P = 0.09 for $\Delta AUC_{0\text{-}60}$ FGF-19, Table 4.8). $AUC_{0\text{-}180}$ AG and fasting AG:DAG had a significant association with the remission (P = 0.047 and P = 0.02, respectively, Table 4.8). In terms of body composition, only VAT area measured by MRI negatively correlated with T2D remission, albeit the association was just marginal (P = 0.08, Table 4.8).

Table 4.8 Factors associated with diabetes remission by logistic regression analysis

	OR	95% CI	P-value
Insulin sensitivity and β-cell function indices			
Fasting insulin, pM	1	0.97, 1.04	1.00
AUC_{0-30} insulin, pM x min	1	1, 1	0.03
AUC_{0-180} insulin, pM x min	1	1, 1	0.29
1/ fasting insulin, pM^{-1}	2.11	0.18, 25	0.55
HOMA-IR	0.5	0.22, 1.17	0.11
HOMA-β	1.04	1, 1.09	0.08
QUICKI	116	4.6×10^{-4}, 2.9×10^{7}	0.45
Gut hormones profiles			
Fasting PYY, pg/mL	0.99	0.97, 1	0.14
AUC_{0-60} PYY, pg x min/mL	1	1, 1	0.64
AUC_{0-180} PYY, pg x min/mL	1	1, 1	0.39
ΔAUC_{0-60} PYY, pg x min/mL	1	1, 1	0.93
ΔAUC_{0-180} PYY, pg x min/mL	1	1, 1	0.77
Fasting GLP-1, pM	1.03	0.95, 1.12	0.46
AUC_{0-60} GLP-1, pM x min	1	1, 1	0.69
AUC_{0-180} GLP-1, pM x min	1	1, 1	0.62
ΔAUC_{0-60} GLP-1, pM x min	1	1, 1	0.8
ΔAUC_{0-180} GLP-1, pM x min	1	1, 1	0.82
Fasting FGF-19, pg/mL	1.01	1, 1.03	0.08
AUC_{0-60} FGF-19, pg x min/mL	1	1, 1	0.61
AUC_{0-180} FGF-19, pg x min/mL	1	1, 1	0.43
ΔAUC_{0-60} FGF-19, pg x min/mL	1	1, 1	0.09
ΔAUC_{0-180} FGF-19, pg x min/mL	1	1, 1	0.45
Fasting AG, fmol/mL	1.11	0.97, 1.26	0.13
AUC_{0-60} AG, fmol x min/mL	1	1, 1	0.08
AUC_{0-180} AG, fmol x min/mL	1	1, 1	0.047
ΔAUC_{0-60} AG, fmol x min/mL	1	1, 1	0.33

$\Delta AUC_{0\text{-}180}$ AG, fmol x min/mL	1	1, 1	0.69
Fasting DAG, fmol/mL	1	0.99, 1.02	0.59
$AUC_{0\text{-}60}$ DAG, fmol x min/mL	1	1, 1	0.47
$AUC_{0\text{-}180}$ DAG, fmol x min/mL	1	1, 1	0.27
$\Delta AUC_{0\text{-}60}$ DAG, fmol x min/mL	1	1, 1	0.94
$\Delta AUC_{0\text{-}180}$ DAG, fmol x min/mL	1	1, 1	0.42
Fasting AG:DAG	3.6×10^{12}	90, 1.4×10^{23}	0.02
$AUC_{0\text{-}60}$ AG:DAG	1.3×10^{5}	0.02, 1×10^{12}	0.15
$AUC_{0\text{-}180}$ AG:DAG	2.2×10^{3}	0.02, 2.8×10^{8}	0.2
$\Delta AUC_{0\text{-}60}$ AG:DAG	2.81	0.04, 210	0.64
$\Delta AUC_{0\text{-}180}$ AG:DAG	1.35	0.45, 4.08	0.6
MRI parameters			
Hepatic fat, %	0.93	0.79, 1.1	0.4
Pancreatic fat, %	1.04	0.75, 1.44	0.81
Total fat area, cm^2	1	1, 1	0.96
SAT area, cm^2	1	1, 1.01	0.19
VAT area, cm^2	0.99	0.99, 1	0.08
VAT:SAT ratio	0.41	0.09, 1.8	0.24
FM index, kg/m^2	1	0.84, 1.19	0.99
FFM index, kg/m^2	0.99	0.81, 1.2	0.89
SM index, cm^2/m^2	1	0.94, 1.05	0.88
SM fat fraction, %	1.01	0.85, 1.2	0.91
BIA parameters			
FM index, kg/m^2	1.03	0.83, 1.27	0.79
FFM index, kg/m^2	0.92	0.69, 1.23	0.56
Muscle mass index, kg/m^2	0.91	0.67, 1.24	0.55
Visceral fat rating	0.97	0.84, 1.12	0.69
BMR, kJ	1	1, 1	0.83

4.3.5 Metabolomics study

4.3.5.1 Remitters vs. non-remitters

Two-hundred and forty-nine metabolic features were studied, including 228 lipid features, 10 amino acids, 4 glycolysis related metabolites, 4 ketone bodies, creatinine, albumin and glycoprotein acetyls (GlycA). From those, one-hundred forty-one were selected for Multivariate Analysis based on sPLS-DA. Variables highly dependent on other variables, were excluded to avoid multicollinearity issues in the model. Samples were projected in the spaced spanned by the 2 principal Latent Structures obtained by sPLS-DA (Figure 4.8). There was a distinction in the metabolic profiles between remitters and non-remitters.

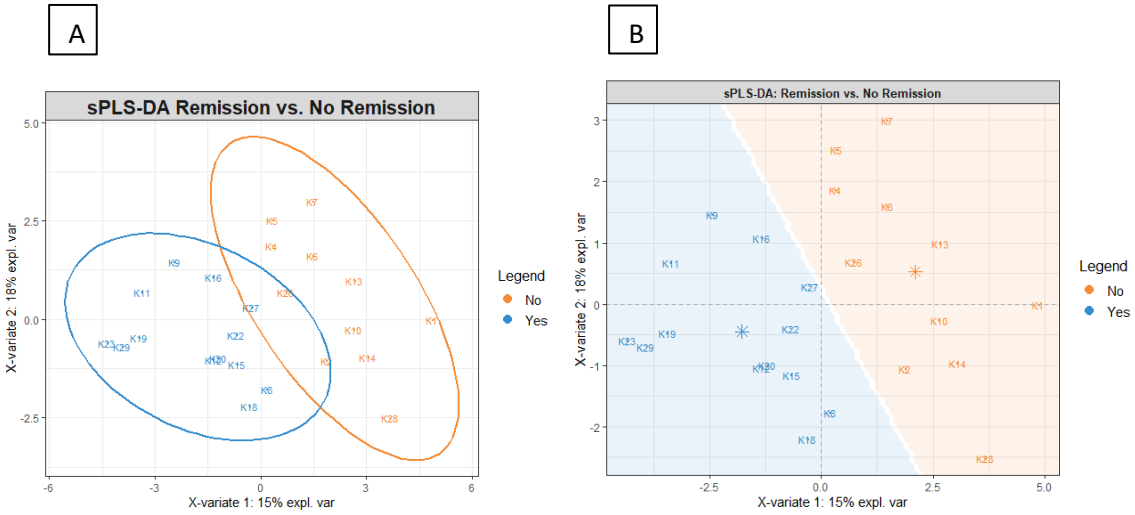


Figure 4.8 A, Sample projection in the spaced spanned by the two first Latent Structures obtained by sPLS-DA. Variables with component loading ≥ |0.1| were included (comp 1: 42; comp 2: 32). Ellipses represent the 95% Confidence Interval. B, Sample projection in the spaced spanned by the two first Latent Structures obtained by sPLS-DA. Variables with component loading ≥ |0.1| were included (comp 1: 42; comp 2: 32). The background represents the "area of influence" where a sample is more likely to be classified either as "remitters" or "non-remitters". Areas were calculated based on the Mahalonobis distance to the group's centroid.

The most significant metabolites in component 1 included size of VLDL particles, isoleucine, leucine, valine, glucose, lactate, β-hydroxybutyrate, and glycoprotein acetyls (Table 4.9). The component 2 was comprised of the size of LDL particles, degree of unsaturation, alanine, tyrosine, glucose, citrate, albumin, and triglyceride in small HDL (Table 4.9). These most discriminant variables in component 1 and 2 were confirmed by the PERMANOVA statistics at P = 0.001 (Table 4.10).

Table 4.9 Most relevant variables in the sPLS-DA method (Component loading ≥ |0.1|). These variables have the most discriminative power in the model. VLDL, very low density lipoprotein; Ile, isoleucine; Leu, leucine; Val, valine; GlycA, glycoprotein acetyls; LDL, low density lipoprotein; Ala, alanine; Tyr, tyrosine; S_HDL_TG, triglyceride in small high density lipoprotein

Variables	Component 1	Variables	Component 2
VLDL_size	0.187821	LDL_size	0.341486
Ile	0.428503	Unsaturation	0.141002
Leu	0.410028	Ala	0.160683
Val	0.426999	Tyr	-0.10813
Glucose	0.517607	Glucose	0.215299
Lactate	0.238909	Citrate	0.736279
β-OHbutyrate	0.115317	Albumin	-0.41638
GlycA	0.172622	S_HDL_TG	-0.13076

Table 4.10 PERMANOVA statistics for sPLS-DA (remission vs. no remission). The False Discovery Rate was controlled using the Benjamini-Hochberg method. Results were considered significant when P-adjusted < 0.05.

Factors	df	Sum of Sqs	R^2	F	P-adjusted
T2D remission	1	95.45	0.512	23.07	0.001
Residual	22	91.03	0.488		
Total	23	186.48	1		

Figure 4.9 shows sample clustering according to their likelihood (complete linkage). The heatmap represents the Euclidean distance for each variable, comparing to the most central sample (the sample closest to the origin of the space spanned by the two first LSs obtained by sPLS-DA). Patients with T2D Remission presented higher values of post-operative HDL during a MMTT.

Figure 4.10 displays overview of enriched metabolite sets and Figure 4.11 shows pathway analysis. Generally, discriminant metabolites are involved in glucose-alanine cycle, Warburg effect, BCAAs degradation, and transfer of acetyl groups into mitochondria.

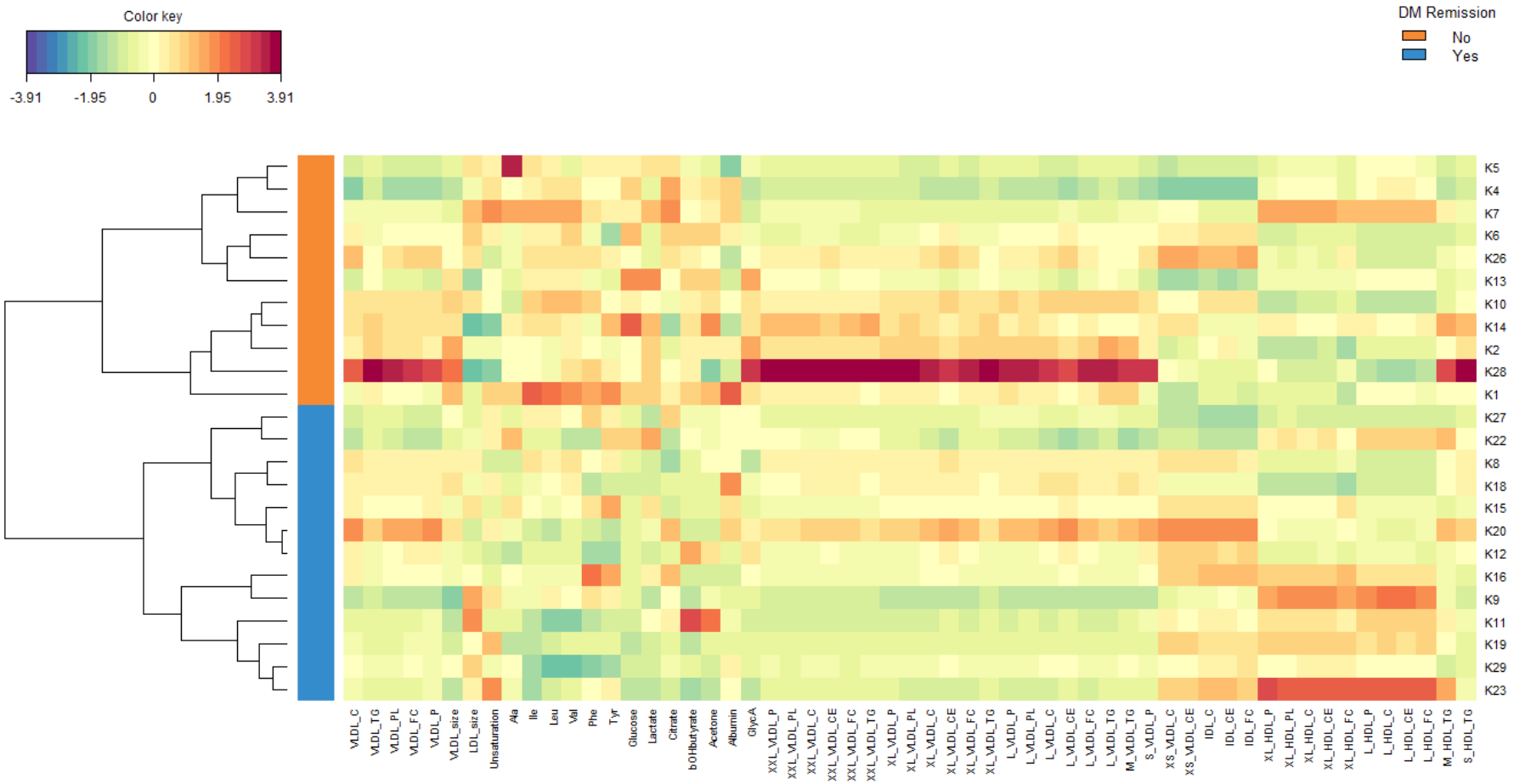


Figure 4.9 Sample clustering according to their likelihood (complete linkage). The heatmap represents the Euclidean distance for each variable, comparing to the most central sample (the sample closest to the origin of the space spanned by the two first Latent Structures obtained by sPLS-DA).

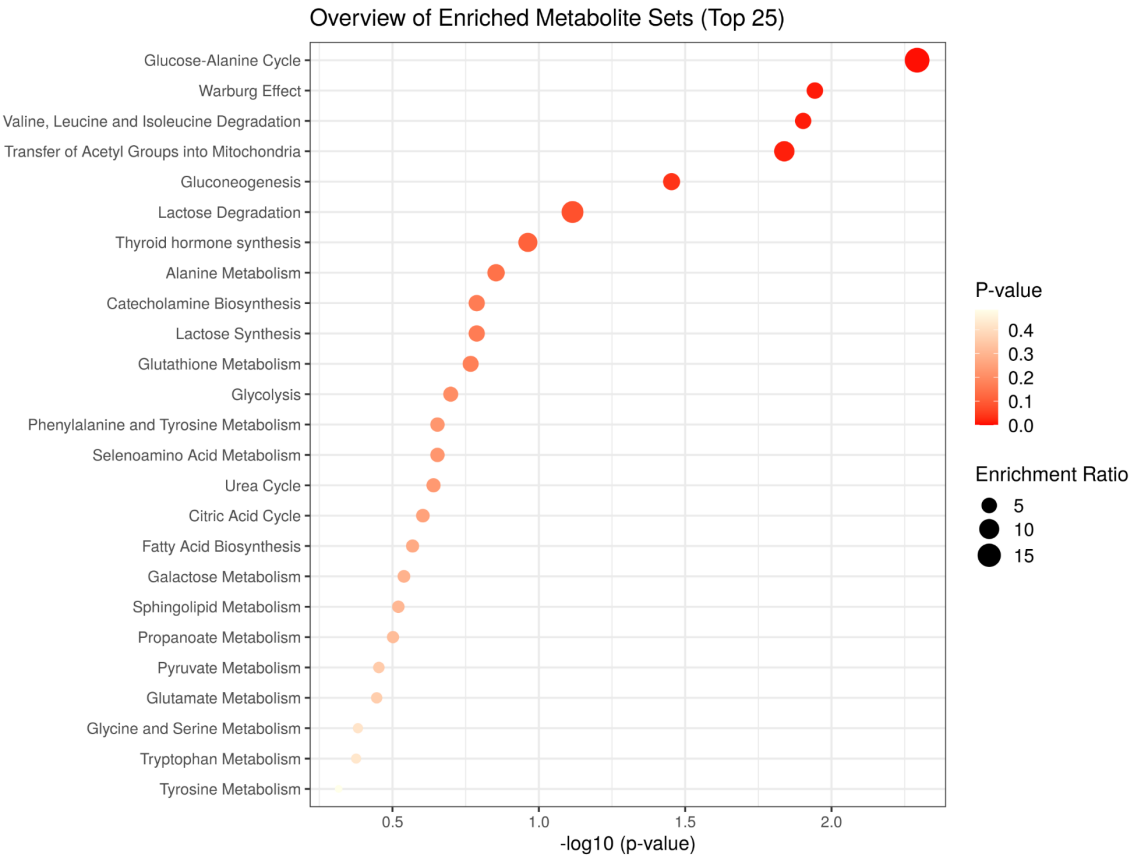


Figure 4.10 Enrichment analysis of metabolic pathways related to diabetes remission, obtained with MetaboAnalyst 5.0. The most discriminant variables in the sPLS-DA projection (component loading > |0.1|) were compared against the SMPDB of human metabolites to estimate the metabolic pathways more related to diabetes remission. Significance was considered when $p < 0.1$

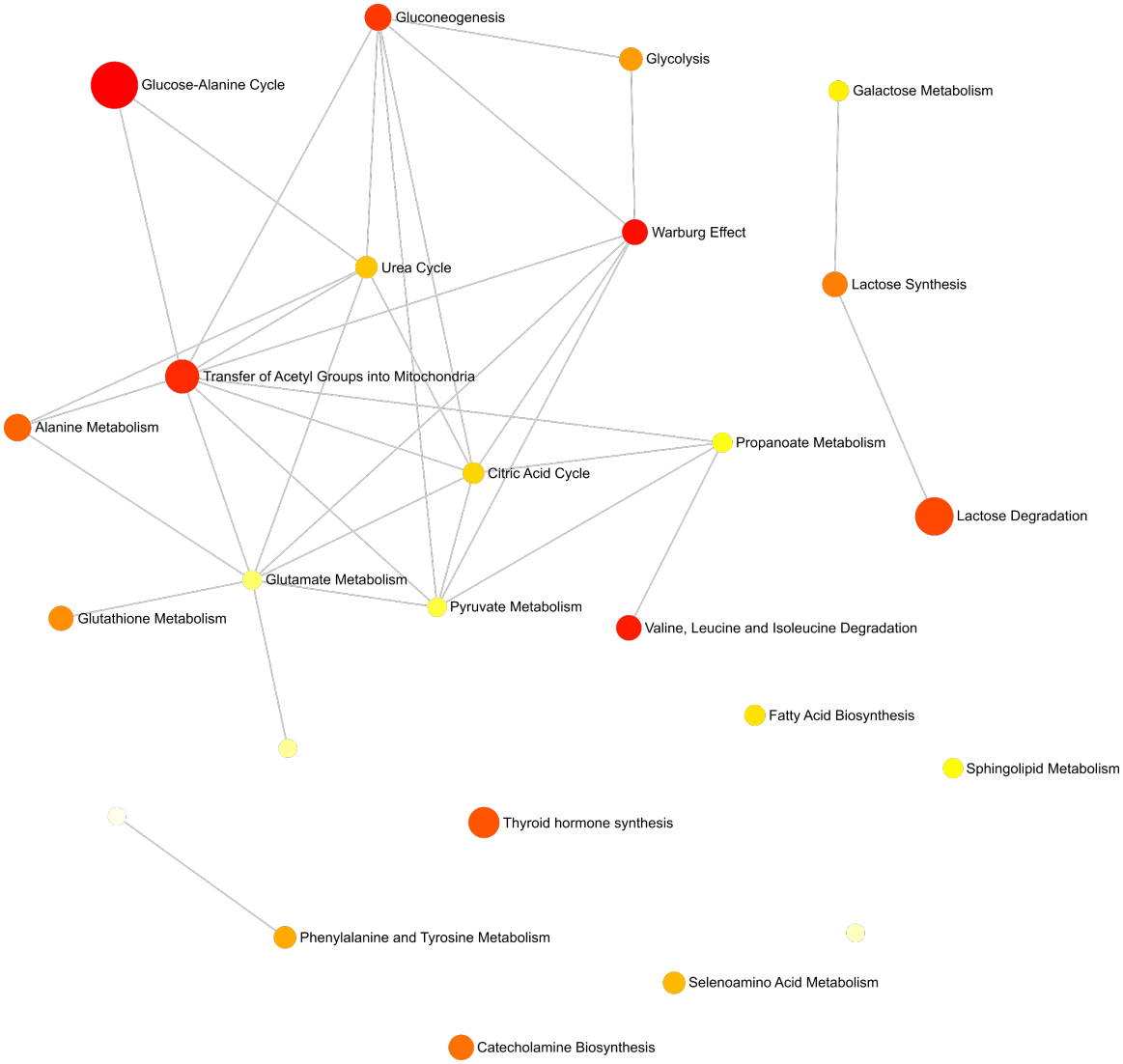


Figure 4.11 Network representation of the Enrichment Analysis of relevant metabolites related to diabetes remission, using MetaboAnalyst 5.0. This representation further displays the interactions between the significantly affected metabolic pathways (red circles) and other metabolic pathways.

The univariate analysis after adjusted for multiple hypothesis by the Benjamini-Hochberg FDR revealed that the $AUC_{0\text{-}180}$ of leucine, isoleucine, and valine in non-remitters were statistically significantly greater than remitters (leucine: 32 $\pm$ 4 mmol x min /L vs. 25 $\pm$ 4 mmol x min/L, P = 0.02; isoleucine: 16 $\pm$ 2 mmol x min/L vs. 13 $\pm$ 2 mmol x min/L, P = 0.02; valine: 54 $\pm$ 5 mmol x min/L vs. 45 $\pm$ 6 mmol x min/L, P = 0.02) (Table 4.11). As expected, the levels of glucose in non-remitters were also statistically significantly higher than remitters (1,938 $\pm$ 446 mmol x min/L vs. 1,191 $\pm$ 267 mmol x min/L, P <0.01) (Table 4.11).

We therefore subsequently performed the ROC analysis in order to determine the best cut-off value of total BCAAs for diabetes remission (Table 4.12, Figure 4.12). The AUC of ROC curve was 0.937 with 95% confidence interval at 0.85 – 1.03. The best cut-off value at $\geq$91.5 mmol x min/L gives sensitivity of 91%, specificity of 85% for being non-remitters (Table 4.12).

Table 4.11 Univariate analysis comparing metabolites between remitters and non-remitters, *adjusted for multiple hypothesis controlling the False Discovery Rate with the Benjamini-Hochberg method.

Metabolites	Non-remitters AUC_{0-180} (mean ± SD)	Remitters AUC_{0-180} (mean ± SD)	P-value	Adjusted P-value*
Glucose (mmol x min/l)	1938 ± 446	1191 ± 267	0.00	0.01
Isoleucine (mmol x min/l)	16.5 ± 2.3	12.7 ± 2.2	0.00	0.02
Valine (mmol x min/l)	53.9 ± 5.1	44.5 ± 6	0.00	0.02
Leucine (mmol x min/l)	31.8 ± 4.1	25.2 ± 4.1	0.00	0.02
Lactate (mmol x min/l)	324 ± 71	245 ± 69	0.01	0.33
Average diameter for VLDL particles (nm x min)	7225 ± 300	6969 ± 210	0.02	0.45
Glycoprotein acetyls (mmol x min/l)	157 ± 25	138 ± 12	0.03	0.45
3-Hydroxybutyrate (mmol x min/l)	12.8 ± 2	8.5 ± 6.5	0.05	0.45
Free cholesterol in very large HDL (mmol x min/l)	3.8 ± 0.7	4.4 ± 0.9	0.07	0.45
Cholesterol in very large HDL (mmol x min/l)	11.8 ± 3.3	15.1 ± 5	0.07	0.45
Triglycerides in large VLDL (mmol x min/l)	39.2 ± 23	25.9 ± 11.1	0.08	0.45
Triglycerides in very large VLDL (mmol x min/l)	31.7 ± 24.9	18 ± 9.1	0.08	0.45
Cholesteryl esters in very large HDL (mmol x min/l)	8 ± 2.7	10.7 ± 4.1	0.08	0.45
Phospholipids in very large HDL (mmol x min/l)	10.6 ± 5.1	15.2 ± 6.9	0.08	0.45
Cholesteryl esters in very small VLDL (mmol x min/l)	16.5 ± 5.5	20.1 ± 4.4	0.08	0.45
Acetone (mmol x min/l)	3.8 ± 0.9	3.1 ± 0.9	0.09	0.45
Triglycerides in chylomicrons and extremely large VLDL (mmol x min/l)	57.4 ± 56	28.2 ± 18.9	0.09	0.45
Phospholipids in chylomicrons and extremely large VLDL (mmol x min/l)	12.9 ± 11.8	6.6 ± 4.5	0.09	0.45
Phenylalanine (mmol x min/l)	10.2 ± 0.7	9.4 ± 1.5	0.09	0.45
Triglycerides in VLDL (mmol x min/l)	219 ± 139	146 ± 54	0.09	0.45
Concentration of chylomicrons and extremely large VLDL particles (mmol x min/l)	0 ± 0	0 ± 0	0.09	0.45
Free cholesterol in chylomicrons and extremely large VLDL (mmol x min/l)	8.16 ± 6.92	4.51 ± 2.78	0.09	0.45
Concentration of very large VLDL particles (mmol x min/l)	0 ± 0	0 ± 0	0.10	0.45
Cholesterol in very small VLDL (mmol x min/l)	24.4 ± 7.7	29.1 ± 6.2	0.11	0.45
Cholesterol in chylomicrons and extremely large VLDL (mmol x min/l)	16.1 ± 14	9 ± 5.8	0.11	0.45
Concentration of very large HDL particles (mmol x min/l)	0.03 ± 0.01	0.04 ± 0.01	0.11	0.45
Alanine (mmol x min/l)	83.4 ± 24.5	70.2 ± 13.3	0.11	0.45

Phospholipids in very large VLDL (mmol x min/l)	10.3 ± 7.9	6.3 ± 3.7	0.11	0.45
Concentration of large VLDL particles (mmol x min/l)	0 ± 0	0 ± 0	0.12	0.45
Citrate (mmol x min/l)	15.8 ± 3.3	13.7 ± 3	0.12	0.45
Free cholesterol in large HDL (mmol x min/l)	8.5 ± 4.3	12.4 ± 6.7	0.12	0.45
Cholesteryl esters in chylomicrons and extremely large VLDL (mmol x min/l)	7.9 ± 7.1	4.4 ± 3	0.12	0.45
Cholesteryl esters in IDL (mmol x min/l)	85.4 ± 21.4	99. 5 ± 21.3	0.12	0.45
Cholesterol in IDL (mmol x min/l)	116.1 ± 28.5	134.7 ± 28.4	0.12	0.45
Cholesterol in large HDL (mmol x min/l)	38.3 ± 20.1	54.9 ± 29.1	0.12	0.45
Free cholesterol in HDL (mmol x min/l)	43.5 ± 7.5	50.9 ± 14.7	0.13	0.45
Free cholesterol in very large VLDL (mmol x min/l)	5.9 ± 4.2	3.8 ± 2. 1	0.13	0.45
Cholesteryl esters in large HDL (mmol x min/l)	29.8 ± 15.9	42.6 ± 22.4	0.13	0.45
Phospholipids in large VLDL (mmol x min/l)	15.1 ± 10	10.1 ± 5.3	0.13	0.45
Concentration of large HDL particles (mmol x min/l)	0.18 ± 0.09	0.26 ± 0.14	0.13	0.45
Free cholesterol in IDL (mmol x min/l)	30.7 ± 7.4	35.3 ± 7.2	0.14	0.45
Free cholesterol in large VLDL (mmol x min/l)	10.2 ± 6.2	7.05 ± 3.5	0.14	0.45
Triglycerides in medium VLDL (mmol x min/l)	52.2 ± 23.9	40.3 ± 13.4	0.14	0.45
Phospholipids in IDL (mmol x min/l)	40.8 ± 8.9	46.1 ± 8.3	0.14	0.45
Phospholipids in large HDL (mmol x min/l)	43.1 ± 19.7	59 ± 29.8	0.14	0.45
Acetoacetate (mmol x min/l)	8.55 ± 2.65	6.6 ± 3.53	0.15	0.45
Average diameter for HDL particles (nm x min)	1719 ± 32	1739 ± 35	0.17	0.47
HDL cholesterol (mmol x min/l)	202 ± 38	233 ± 65	0.17	0.47
Glutamine (mmol x min/l)	94.4 ± 11.8	101.4 ± 9.8	0.17	0.47
Triglycerides in small VLDL (mmol x min/l)	28.3 ± 12.1	23.2 ± 4.6	0.17	0.47
Cholesterol in very large VLDL (mmol x min/l)	11.35 ± 7.43	7.94 ± 4.37	0.18	0.47
Triglycerides in small LDL (mmol x min/l)	3.12 ± 1.82	2.39 ± 0.49	0.18	0.47
Sphingomyelins (mmol x min/l)	68.3 ± 5.2	72.7 ± 9.4	0.18	0.47
Cholesteryl esters in HDL (mmol x min/l)	158.5 ± 31	182.5 ± 50.1	0.18	0.47
Triglycerides in large HDL (mmol x min/l)	4.06 ± 1.68	5.04 ± 1.81	0.19	0.48
Phospholipids in HDL (mmol x min/l)	244.4 ± 39.4	274.9 ± 69.8	0.19	0.49
Free cholesterol in very small VLDL (mmol x min/l)	7.91 ± 2.24	8.99 ± 1.76	0.20	0.49
Free cholesterol in medium HDL (mmol x min/l)	12.7 ± 3	15 ± 5.2	0.21	0.51
Monounsaturated fatty acids (mmol x min/l)	616.8 ± 284.8	501.4 ± 83.8	0.22	0.52
Cholesterol in large VLDL (mmol x min/l)	18.9 ± 10.7	14.4 ± 7.1	0.22	0.52
Apolipoprotein A1 (g x min/l)	235 ± 28	256 ± 51	0.23	0.53
Glycine (mmol x min/l)	20.5 ± 9.7	25.2 ± 8.5	0.23	0.53
Average diameter for LDL particles (nm x min)	4289 ± 22	4299 ± 15	0.24	0.54
Cholesterol in medium HDL (mmol x min/l)	76.7 ± 16.5	87.6 ± 26.1	0.25	0.54
Cholesteryl esters in medium HDL (mmol x min/l)	64 ± 13.6	72.6 ± 20.9	0.26	0.55
Concentration of medium HDL particles (mmol x min/l)	0.6 ± 0.12	0.68 ± 0.2	0.26	0.55

Cholesteryl esters in very large VLDL (mmol x min/l)	5.47 ± 3.31	4.14 ± 2.28	0.26	0.55
Triglycerides in small HDL (mmol x min/l)	9.78 ± 3.52	8.58 ± 1.37	0.27	0.56
Pyruvate (mmol x min/l)	13.9 ± 5.7	10.9 ± 5.8	0.28	0.57
Phospholipids in VLDL (mmol x min/l)	87.9 ± 46	71.5 ± 25.7	0.28	0.57
Free cholesterol in large LDL (mmol x min/l)	42.2 ± 10.5	46.9 ± 10.2	0.29	0.57
Phosphatidylcholines (mmol x min/l)	341 ± 44.1	365.3 ± 62.7	0.29	0.57
Phospholipids in medium HDL (mmol x min/l)	78 ± 12.8	85.8 ± 21.8	0.31	0.58
Concentration of IDL particles (mmol x min/l)	0.05 ± 0.01	0.05 ± 0.01	0.31	0.58
Concentration of HDL particles (mmol x min/l)	2.47 ± 0.25	2.63 ± 0.46	0.31	0.58
Concentration of very small VLDL particles (mmol x min/l)	0.01 ± 0.00	0.01 ± 0.00	0.32	0.59
Triglycerides in medium LDL (mmol x min/l)	5.66 ± 2.57	4.92 ± 0.74	0.33	0.61
Total cholines (mmol x min/l)	410.8 ± 43.6	432.9 ± 64.2	0.34	0.61
Free cholesterol in VLDL (mmol x min/l)	52.2 ± 25.8	43.9 ± 16	0.35	0.61
Phospholipids in very small VLDL (mmol x min/l)	14.8 ± 4.3	16.2 ± 2.9	0.35	0.61
Free cholesterol in small HDL (mmol x min/l)	18.4 ± 1.4	19.1 ± 2.4	0.38	0.66
Cholesteryl esters in large VLDL (mmol x min/l)	8.79 ± 4.63	7.31 ± 3.59	0.39	0.67
Omega-6 fatty acids (mmol x min/l)	786.3 ± 116.7	753.8 ± 85.9	0.44	0.74
Linoleic acid (mmol x min/l)	613.4 ± 128.4	578.8 ± 87.6	0.44	0.74
Saturated fatty acids (mmol x min/l)	714.8 ± 239	658.9 ± 92.4	0.44	0.74
Free cholesterol in LDL (mmol x min/l)	67.8 ± 16. 8	72.9 ± 16.1	0.46	0.75
Polyunsaturated fatty acids (mmol x min/l)	865.7 ± 142.2	828 ± 111.8	0.47	0.77
Concentration of medium VLDL particles (mmol x min/l)	0.01 ± 0.00	0.01 ± 0.00	0.49	0.78
Concentration of small VLDL particles (mmol x min/l)	0.01 ± 0.00	0.01 ± 0.00	0.49	0.78
Phosphoglycerides (mmol x min/l)	372 ± 49.3	388.2 ± 63.8	0.50	0.78
Cholesteryl esters in medium VLDL (mmol x min/l)	11.5 ± 6.1	13.1 ± 5.5	0.52	0.81
Phospholipids in large LDL (mmol x min/l)	53.2 ± 11.7	56 ± 11	0.55	0.82
Docosahexaenoic acid (mmol x min/l)	29.1 ± 10.4	32.2 ± 14	0.55	0.82
Creatinine (µmol x min/l)	12,978 ± 2,844	12,326 ± 2,167	0.55	0.82
Tyrosine (mmol x min/l)	15 ± 1.7	14.5 ± 2.1	0.55	0.82
Triglycerides in LDL (mmol x min/l)	23.7 ± 9.3	22.1 ± 3.1	0.56	0.82
Cholesterol in large LDL (mmol x min/l)	165.2 ± 39.6	174.2 ± 36.7	0.57	0.83
Concentration of VLDL particles (mmol x min/l)	0.02 ± 0.01	0.02 ± 0.01	0.58	0.83
Cholesteryl esters in small LDL (mmol x min/l)	21.7 ± 6.7	20.4 ± 5	0.59	0.83
Phospholipids in medium VLDL (mmol x min/l)	20.4 ± 9.6	18.5 ± 7.3	0.59	0.83
Concentration of small LDL particles (mmol x min/l)	0.03 ± 0.01	0.03 ± 0.01	0.61	0.86
VLDL cholesterol (mmol x min/l)	115.9 ± 52.9	106.8 ± 37.2	0.63	0.87
Cholesteryl esters in medium LDL (mmol x min/l)	48.7 ± 15.2	46.1 ± 12.1	0.64	0.87
Acetate (mmol x min/l)	7.25 ± 2.36	6.75 ± 2.80	0.64	0.87
Degree of unsaturation (degree x min)	234.8 ± 18.2	237.8 ± 13.7	0.65	0.88

Clinical LDL cholesterol (mmol x min/l)	370.4 ± 109.8	389 ± 101.5	0.67	0.89
Cholesterol in small LDL (mmol x min/l)	29.3 ± 8.2	28.1 ± 6.6	0.69	0.90
Phospholipids in small HDL (mmol x min/l)	112.9 ± 10.8	114.5 ± 14.3	0.69	0.90
Cholesteryl esters in large LDL (mmol x min/l)	123 ± 29.5	127.4 ± 26.6	0.70	0.90
Albumin (g x min/l)	6,647 ± 562	6,719 ± 354	0.70	0.90
Phospholipids in small LDL (mmol x min/l)	14.2 ± 3.2	13.7 ± 2.8	0.71	0.90
Concentration of medium LDL particles (mmol x min/l)	0.05 ± 0.01	0.05 ± 0.01	0.72	0.91
Remnant cholesterol (non-HDL, non-LDL -cholesterol) (mmol x min/l)	231.9 ± 73.6	241.5 ± 61.4	0.73	0.91
Omega-3 fatty acids (mmol x min/l)	79.4 ± 33.5	74.1 ± 41.1	0.74	0.91
Phospholipids in small VLDL (mmol x min/l)	14.5 ± 5.3	13.9 ± 4.1	0.75	0.91
Cholesterol in medium LDL (mmol x min/l)	66.6 ± 19.4	64.4 ± 16.4	0.76	0.91
Phospholipids in medium LDL (mmol x min/l)	25.3 ± 7.2	24.5 ± 5.6	0.77	0.91
Triglycerides in very large HDL (mmol x min/l)	1.17 ± 0.73	1.10 ± 0.27	0.77	0.91
Free cholesterol in medium VLDL (mmol x min/l)	11.8 ± 5.4	11.2 ± 4.5	0.77	0.91
Triglycerides in very small VLDL (mmol x min/l)	10.7 ± 4	10.3 ± 1.4	0.78	0.91
Cholesterol in small HDL (mmol x min/l)	75.1 ± 7	75.8 ± 7.6	0.81	0.94
Free cholesterol in medium LDL (mmol x min/l)	17.9 ± 4.7	18.3 ± 4.4	0.83	0.94
LDL cholesterol (mmol x min/l)	261.1 ± 65.5	266.7 ± 58.6	0.83	0.94
Cholesterol in medium VLDL (mmol x min/l)	23.4 ± 11.1	24.3 ± 9.9	0.83	0.94
Triglycerides in IDL (mmol x min/l)	14.7 ± 4.8	15.1 ± 2.1	0.83	0.94
Concentration of LDL particles (mmol x min/l)	0.19 ± 0.05	0.18 ± 0.04	0.85	0.95
Phospholipids in LDL (mmol x min/l)	92.7 ± 21.6	94.3 ± 19.4	0.85	0.95
Cholesteryl esters in small VLDL (mmol x min/l)	13.5 ± 5.3	13.8 ± 4.5	0.89	0.97
Triglycerides in medium HDL (mmol x min/l)	8.92 ± 2.92	9.04 ± 1.92	0.90	0.97
Histidine (mmol x min/l)	13.2 ± 1.5	13.1 ± 0.9	0.90	0.97
Cholesterol in small VLDL (mmol x min/l)	21.8 ± 8.4	22.1 ± 7.2	0.91	0.97
Triglycerides in large LDL (mmol x min/l)	14.9 ± 5	14.7 ± 2.1	0.92	0.97
Free cholesterol in small LDL (mmol x min/l)	7.66 ± 1.76	7.73 ± 1.75	0.92	0.97
Cholesteryl esters in VLDL (mmol x min/l)	63.7 ± 28	62.9 ± 21.4	0.94	0.99
Free cholesterol in small VLDL (mmol x min/l)	8.27 ± 3.09	8.35 ± 2.70	0.95	0.99
Triglycerides in HDL (mmol x min/l)	23.9 ± 8.4	23.8 ± 4.5	0.95	0.99
Concentration of small HDL particles (mmol x min/l)	1.65 ± 0.15	1.65 ± 0.15	0.98	1.00
Cholesteryl esters in LDL (mmol x min/l)	193.3 ± 50	193.8 ± 42.7	0.98	1.00
Concentration of large LDL particles (mmol x min/l)	0.11 ± 0.03	0.11 ± 0.02	0.99	1.00
Cholesteryl esters in small HDL (mmol x min/l)	56.7 ± 5.9	56.7 ± 5.4	1.00	1.00
Apolipoprotein B (g x min/l)	131.3 ± 35.7	131.3 ± 29.9	1.00	1.00

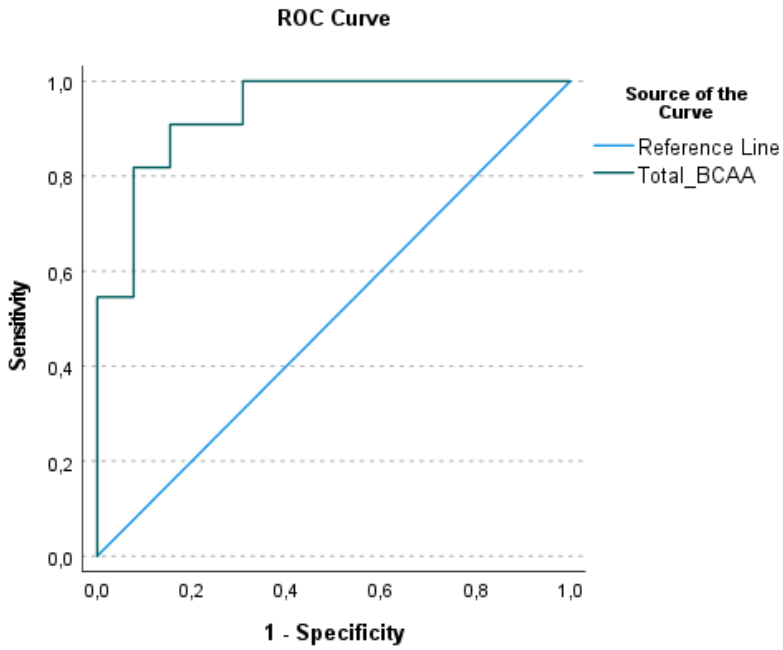


Figure 4.12 ROC curve determining the best cut-off value of $AUC_{0\text{-}180}$ total BCAAs for being non-remitters after bariatric surgery

Table 4.12 Cut-off values of total BCAAs for being non-remitters after bariatric surgery

Positive if ≥ (mmol x min/l)	Sensitivity	1 - specificity	Specificity
58.0	1.00	1.00	0.00
61.0	1.00	0.92	0.08
70.5	1.00	0.85	0.15
78.1	1.00	0.77	0.23
78.4	1.00	0.69	0.31
78.7	1.00	0.62	0.38
80.1	1.00	0.54	0.46
84.6	1.00	0.46	0.54
88.8	1.00	0.38	0.62
90.5	1.00	0.31	0.69
91.3	0.91	0.31	0.69
91.4	0.91	0.23	0.77
91.5	0.91	0.15	0.85
92.3	0.82	0.15	0.85
93.6	0.82	0.08	0.92
95.3	0.73	0.08	0.92
97.2	0.64	0.08	0.92
98.8	0.55	0.08	0.92
99.6	0.55	0.00	1.00
99.8	0.45	0.00	1.00
101.0	0.36	0.00	1.00
106.2	0.27	0.00	1.00
112.8	0.18	0.00	1.00
120.1	0.09	0.00	1.00
125.8	0.00	0.00	1.00

We further examined the association of the $AUC_{0\text{-}180}$ BCAAs with visceral adipose tissue, VAT:SAT ratio and insulin sensitivity indices, using a linear regression analysis. A marginally significant association was observed between the $AUC_{0\text{-}180}$ BCAAs and visceral adipose tissue (cm^2) (β = 0.03, 95%CI [-3 $x10^{-4}$, 0.06], P = 0.05, Figure 4.13A). The association was greater with VAT:SAT ratio (β = 9.9, 95%CI [1.1, 18.6], P = 0.03, Figure 4.13B). Among the insulin sensitivity indices including HOMA-β, HOMA-IR, AUC0-30 insulin and QUICKI, only HOMA-β showed a marginally negative correlation with the levels of $AUC_{0\text{-}180}$ BCAAs (β = -0.2, 95%CI [-0.3, 0.01], P = 0.07, Figure 4.13C). None of the rest parameters had an association with the circulating levels of $AUC_{0\text{-}180}$ BCAAs (Figure 4.13D-F).

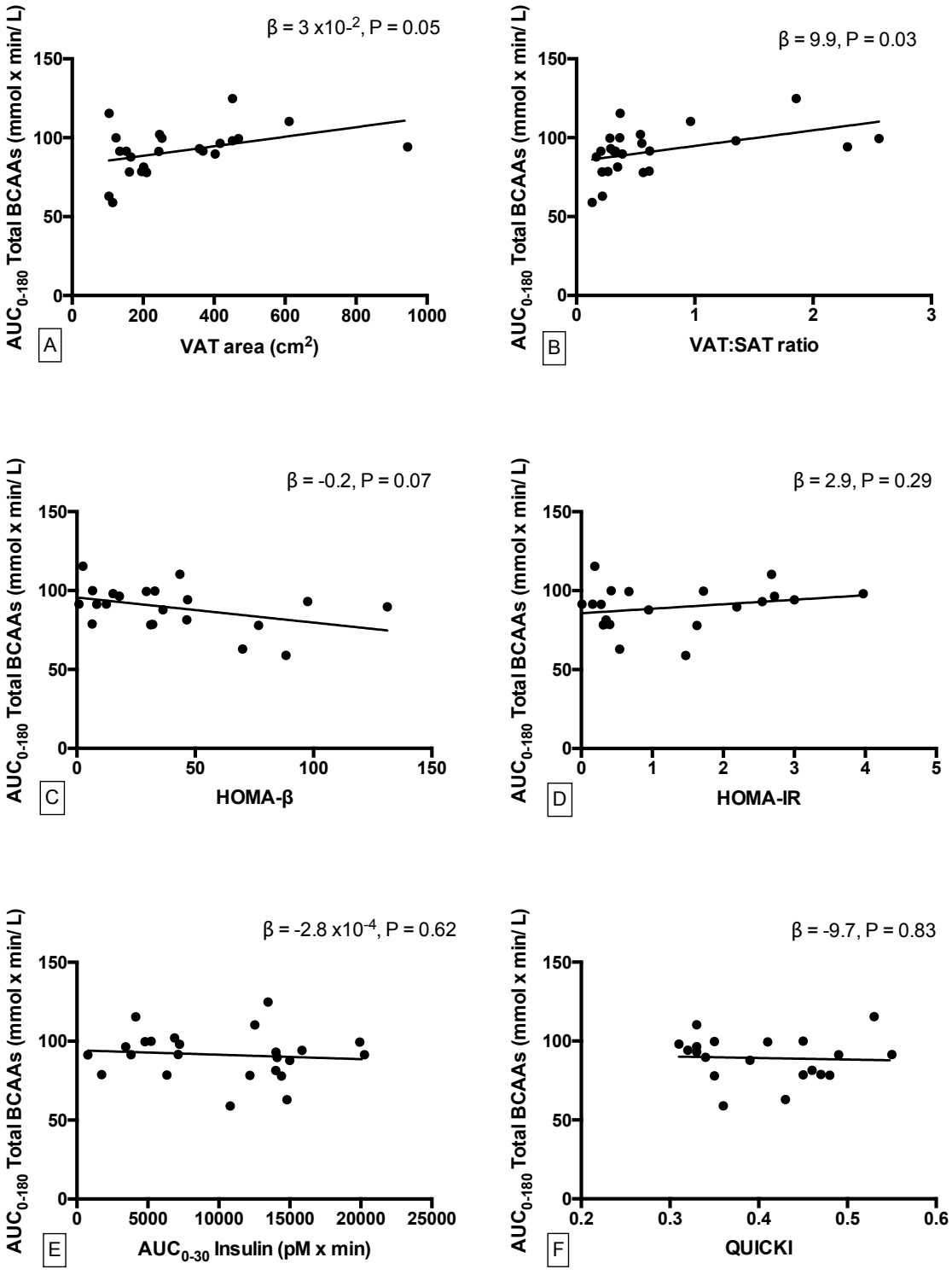


Figure 4.13 Association of $AUC_{0\text{-}180}$ BCAAs with visceral fat parameters and insulin sensitivity indices; A, visceral fat area (cm^2); B, VAT:SAT ratio; C, HOMA-β; D, HOMA-IR; E, $AUC_{0\text{-}30}$ insulin (pM x min); F, QUICKI

4.3.5.2 Complete remission versus partial remission versus no remission

According to the sample projection in the spaced spanned by the two first Latent Structures obtained by sPLS-DA, the segregation of metabolites between patients with no diabetes remission and complete remission was evident. However, the range of metabolites of patients with partial remission overlapped those of patients with complete remission and no remission (Figure 4.14).

A B

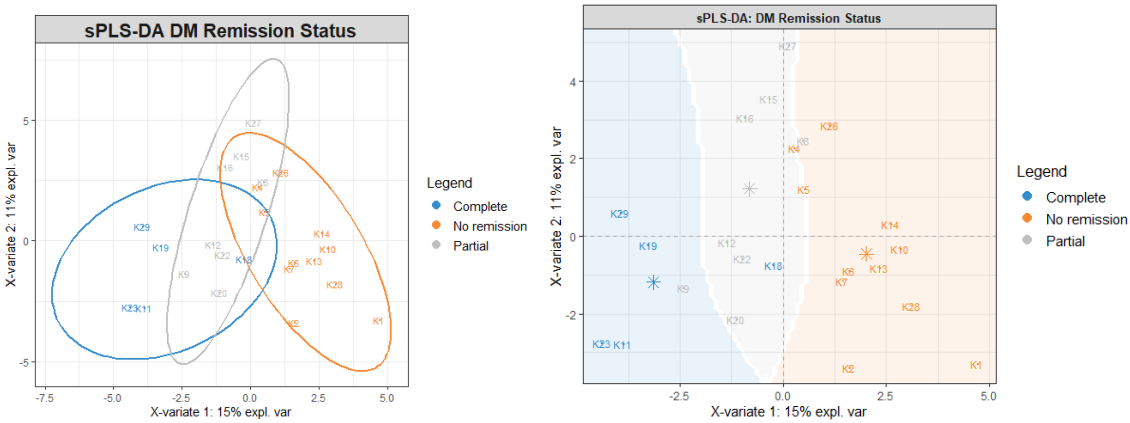


Figure 4.14 A, Sample projection in the spaced spanned by the two first Latent Structures obtained by sPLS-DA. Variables with component loading ≥ |0.1| were included (comp 1: 36; comp 2: 30). Ellipses represent the 95% Confidence Interval. B, Sample projection in the spaced spanned by the 2 principal Latent Structures obtained by sPLS-DA. Variables with component loading ≥ |0.1| were included (comp 1: 36; comp 2: 30). The background represents the "area of influence" where a sample is more likely to be classified either as "complete remission", "partial remission" or "no remission". Areas were calculated based on the Mahalonobis distance to the group's centroid.

Table 4.14 shows most discriminant variables in component 1 and 2 by the sPLS-DA method. Most significant metabolites in component 1 included VLDL diameter, isoleucine, leucine, valine, phenylalanine, glucose, lactate, and GlycA. HDL concentration, degree of unsaturation, omega-3 fatty acids, omega-6 fatty acids, polyunsaturated fatty acids (PUFA), linoleic acids (LA), docosahexaenoic acids (DHA), glutamine, phenylalanine, tyrosine, β-hydroxybutyrate, acetone, IDL concentration, small HDL concentration, cholesterol in small HDL, cholesteryl esters in small HDL were most discriminant in component 2. PERMANOVA statistics was utilised to confirm the separation of no remission, partial remission, and complete remission by the sPLS-DA method (Table 4.13).

Table 4.13 PERMANOVA statistics for sPLS-DA (complete remission vs. partial remission vs. no remission). The False Discovery Rate was controlled using the Benjamini-Hochberg method. Results were considered significant when P-adjusted < 0.05.

Factors	df	Sum of Sqs	R^2	F	P-adjusted
T2D remission	2	109.53	0.587	14.95	0.001
Residual	21	76.95	0.413		
Total	23	186.48	1		

Figure 4.15 shows sample clustering according to their likelihood (complete linkage). The heatmap represents the Euclidean distance for each variable, comparing to the most central sample (the sample closest to the origin of the space spanned by the 2 components obtained by sPLS-DA). During a MMTT, as the T2D remission status progress, the levels of VLDL and LDL decreased, whereas the levels of HDL increased.

Figure 4.16 displays overview of enriched metabolite sets and Figure 4.17 shows pathway analysis. Generally, discriminant metabolites are involved in α-linolenic acid and linoleic acid metabolism, Warburg effect, and BCAAs degradation.

Table 4.14 Most relevant variables in the sPLS-DA method (Component loading ≥ |0.1|). These variables have the most discriminative power in the model. VLDL, very low density lipoprotein; Ile, isoleucine; Leu, leucine; Val, valine; Phe, phenylalanine; GlycA, glycoprotein acetyls; HDL_P, concentration of high density lipoprotein; PUFA, polyunsaturated fatty acids; LA, linoleic acids; DHA, docosahexaenoic acids; Gln, glutamine; Tyr, tyrosine; IDL_P, concentration of intermediate density lipoprotein; S_HDL_P, concentration of small high density lipoprotein; S_HDL_C, cholesterol in small high density lipoprotein; S_HDL_CE, cholesteryl esters in small high density lipoprotein

Variables	Component 1	Variables	Component 2
VLDL_size	0.142857	HDL_P	-0,10364
Ile	0.469549	Unsaturation	-0,24948
Leu	0.437919	Omega_3	-0,20387
Val	0.423811	Omega_6	-0,29304
Phe	0.119818	PUFA	-0,31942
Glucose	0.488463	LA	-0,19691
Lactate	0.225319	DHA	-0,23657
GlycA	0.188039	Gln	0,424408
		Phe	0,197642
		Tyr	0,328551
		β-hydroxybutyrate	-0,24319
		Acetone	-0,15646
		IDL_P	0,11443
		S_HDL_P	-0,22637
		S_HDL_C	-0,21222
		S_HDL_CE	-0,19253

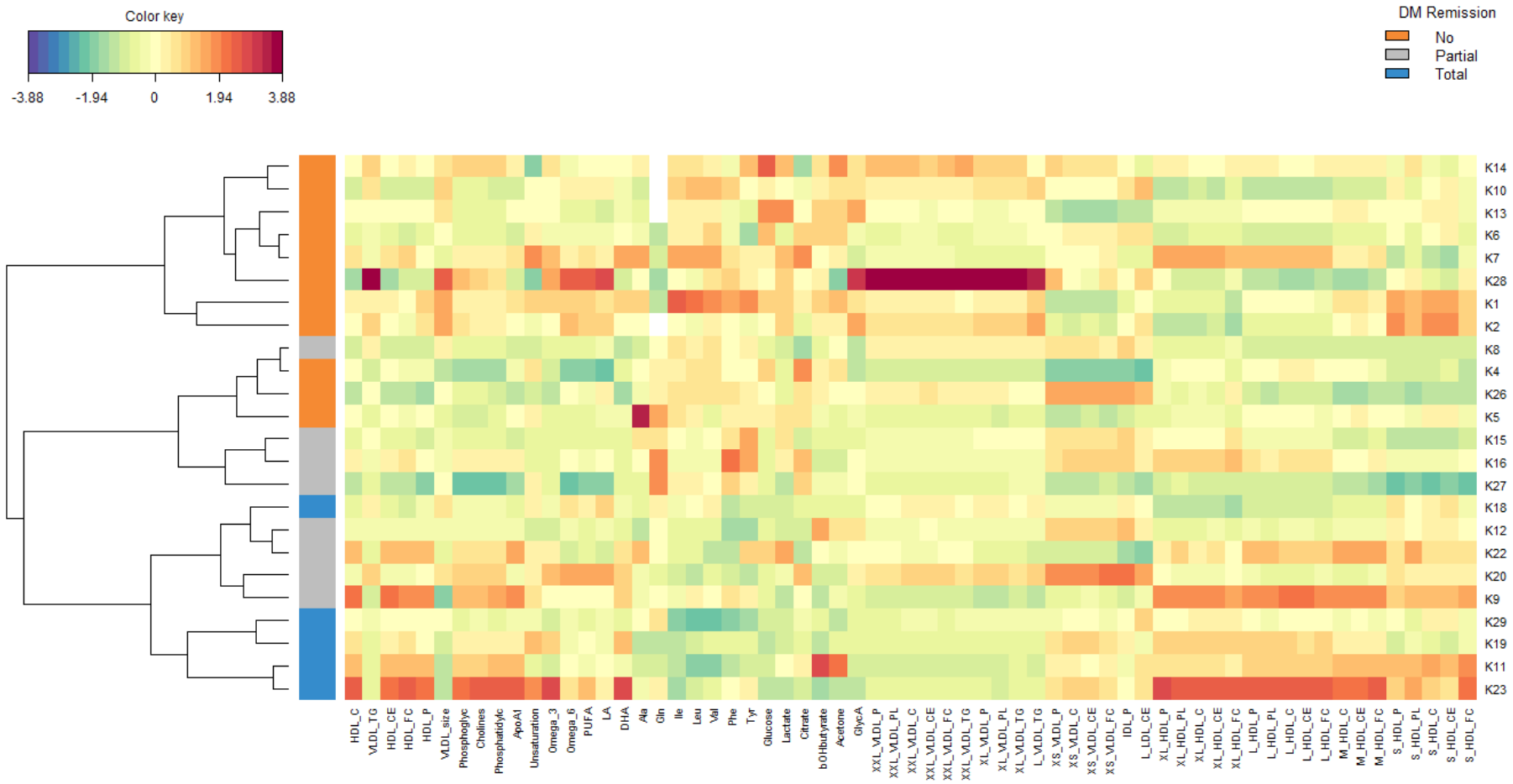


Figure 4.15 Sample clustering according to their likelihood (complete linkage). The heatmap represents the Euclidean distance for each variable, comparing to the most central sample (the sample closest to the origin of the space spanned by the two first Latent Structures obtained by sPLS-DA).

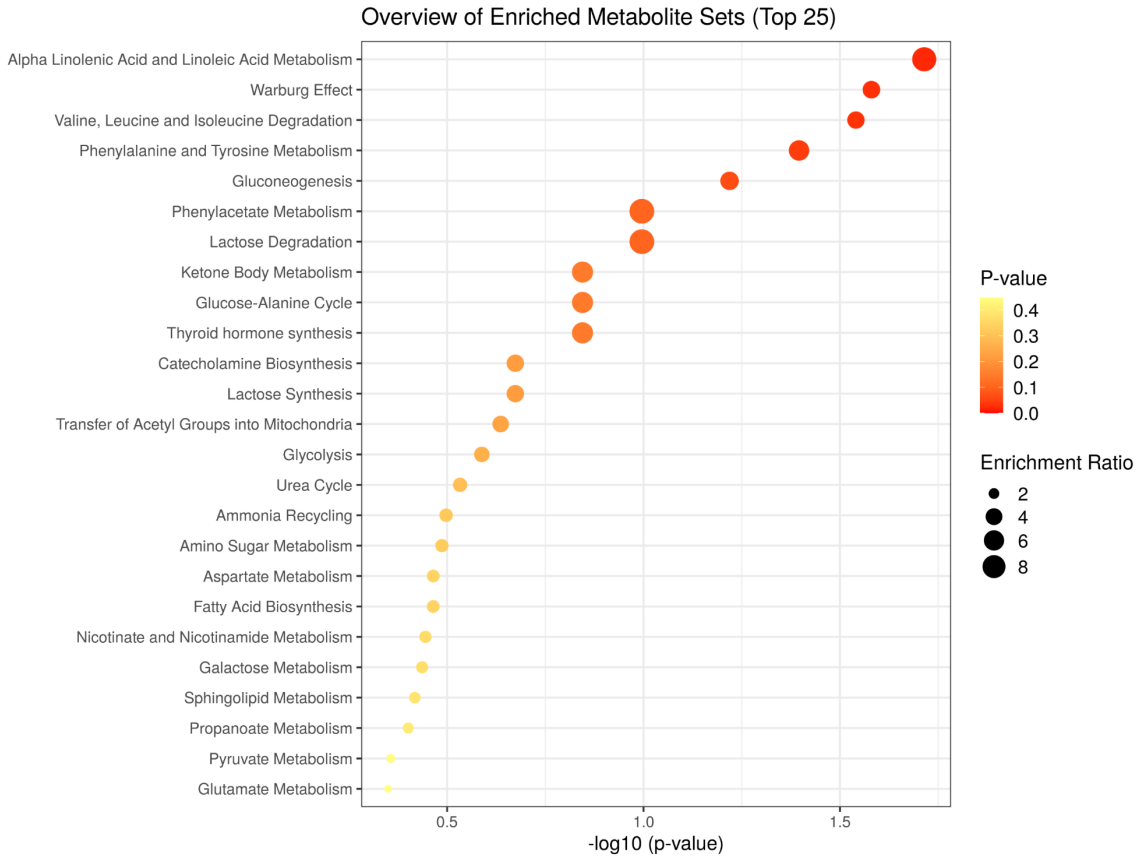


Figure 4.16 Enrichment analysis of metabolic pathways related to diabetes remission status (Total, Partial or No remission), obtained with MetaboAnalyst 5.0. The most discriminant variables in the sPLS-DA projection (component loading > |0.1|) were compared against the SMPDB of human metabolites to estimate the metabolic pathways more related to diabetes remission. Significance was considered when $p < 0.1$.

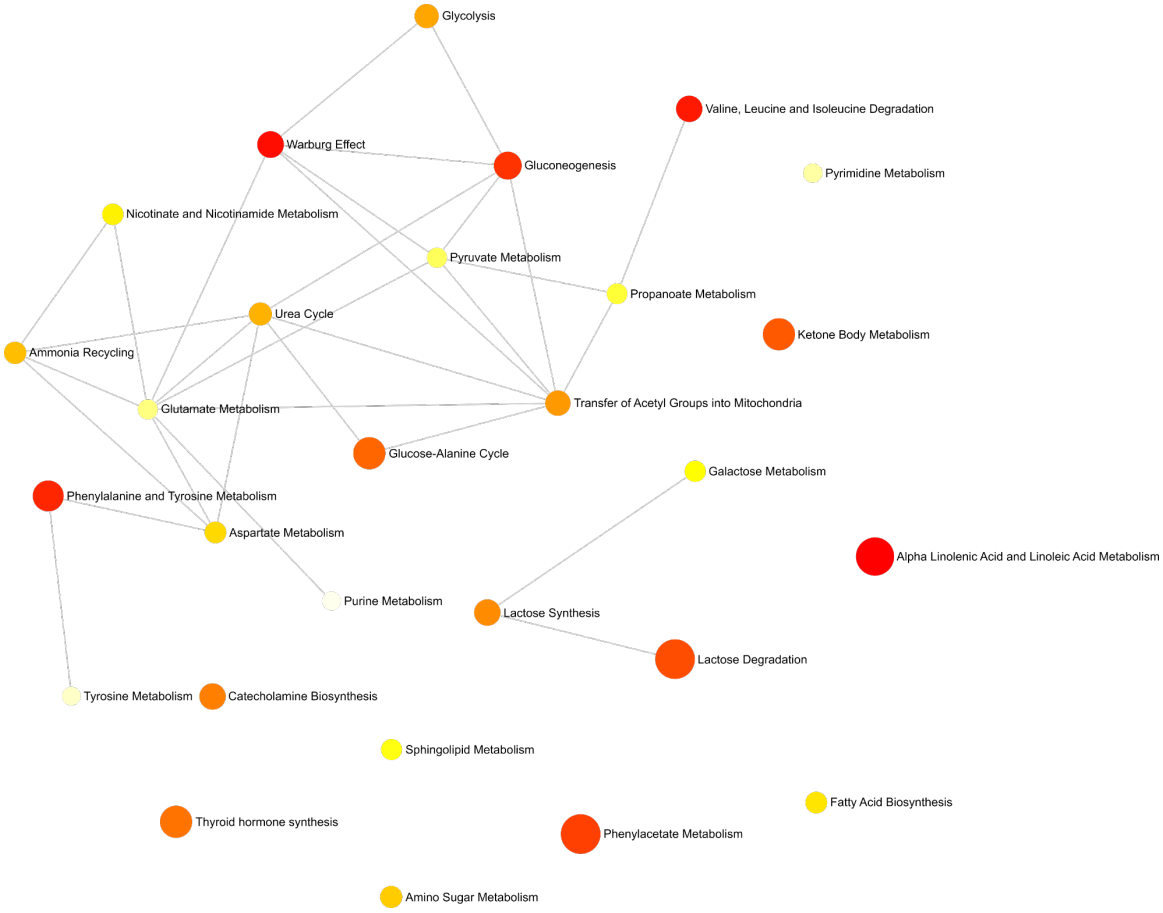


Figure 4.17 Network representation of the Enrichment Analysis of relevant metabolites related to diabetes remission status (Total, Partial or No remission), using MetaboAnalyst 5.0. This representation further displays the interactions between the significantly affected metabolic pathways (red circles) and other metabolic pathways.

One-way ANOVA was used to compare metabolites between the 3 groups (Table 4.15). The analysis adjusted for multiple hypothesis by Benjamini-Hochberg FDR demonstrated that the $AUC_{0\text{-}180}$ of leucine, isoleucine, and valine in non-remitters were statistically significantly greater than complete remitters (leucine: 32 ± 4 mmol x min /L vs. 22 ± 3 mmol x min/L, P = 0.01; isoleucine: 16 ± 2 mmol x min/L vs. 11 ± 2 mmol x min/L, P = 0.01; valine: 54 ± 5 mmol x min/L vs. 41 ± 8 mmol x min/L, P = 0.02) (Table 4.15). As expected, the levels of glucose in non-remitters were also statistically significantly higher than remitters (1,938 ± 446 mmol x min/L vs. 996 ± 92 mmol x min/L, P = 0.01) (Table 4.15). These findings are in line with non-remitters vs. remitters.

Table 4.15 One-way ANOVA comparing metabolites in complete remitters vs. partial remitters vs. non-remitters, *adjusted for multiple hypothesis the Benjamini-Hochberg FDR

Variables	NR	PR	CR	ANOVA		NR vs. PR		NR vs. CR		PR vs. CR	
	AUC	AUC	AUC	P	P*	P	P*	P	P*	P	P*
Isoleucine (mmol/l)	16,45 ± 2,29	13,86 ± 1,60	10,72 ± 1,59	0.00	0.01	0.01	0.53	0.00	0.01	0.01	0.53
Leucine (mmol/l)	31,80 ± 4,10	27,07 ± 2,76	22,07 ± 4,16	0.00	0.01	0.01	0.53	0.00	0.01	0.03	0.83
Phenylalanine (mmol/l)	10,18 ± 0,74	9,85 ± 1,58	8,56 ± 0,91	0.04	0.74	0.53	0.99	0.01	0.53	0.06	0.83
Valine (mmol/l)	53,91 ± 5,08	46,54 ± 3,74	41,25 ± 7,75	0.00	0.02	0.01	0.51	0.00	0.03	0.10	0.83
Tyrosine (mmol/l)	14,99 ± 1,71	15,20 ± 2,31	13,40 ± 1,21	0.22	0.74	0.81	0.99	0.13	0.83	0.10	0.83
Glucose (mmol/l)	1938,04 ± 445,99	1313,25 ± 270,41	996,08 ± 91,93	0.00	0.01	0.00	0.07	0.00	0.01	0.12	0.83
Free cholesterol in small HDL (mmol/l)	18,38 ± 1,41	18,49 ± 2,31	20,12 ± 2,40	0.25	0.74	0.91	0.99	0.11	0.83	0.16	0.83
Docosahexaenoic acid (mmol/l)	29,13 ± 10,43	28,32 ± 9,89	38,49 ± 18,44	0.30	0.74	0.89	0.99	0.17	0.83	0.16	0.83
Degree of unsaturation (degree)	234,80 ± 18,18	232,96 ± 9,73	245,44 ± 16,68	0.35	0.76	0.80	0.99	0.22	0.83	0.17	0.83
Concentration of HDL particles (mmol/l)	2,47 ± 0,25	2,52 ± 0,47	2,80 ± 0,42	0.26	0.74	0.76	0.99	0.11	0.83	0.20	0.83
Glycoprotein acetyls (mmol/l)	157,06 ± 25,06	143,58 ± 12,85	130,13 ± 5,41	0.04	0.74	0.14	0.83	0.02	0.55	0.23	0.83
Cholesterol in small HDL (mmol/l)	75,09 ± 6,98	73,90 ± 8,09	78,91 ± 6,13	0.48	0.84	0.73	0.99	0.34	0.87	0.24	0.83
Free cholesterol in HDL (mmol/l)	43,45 ± 7,49	47,82 ± 13,83	55,88 ± 16,14	0.17	0.74	0.44	0.94	0.06	0.83	0.25	0.83
HDL cholesterol (mmol/l)	201,92 ± 38,03	219,43 ± 64,95	255,88 ± 64,36	0.20	0.74	0.49	0.99	0.08	0.83	0.25	0.83
Apolipoprotein A1 (g/l)	234,94 ± 28,04	245,31 ± 51,27	273,50 ± 50,78	0.25	0.74	0.60	0.99	0.10	0.83	0.25	0.83
Cholesteryl esters in HDL (mmol/l)	158,47 ± 31,02	171,61 ± 51,20	200,00 ± 48,26	0.21	0.74	0.51	0.99	0.08	0.83	0.25	0.83
Free cholesterol in medium HDL (mmol/l)	12,74 ± 2,95	13,92 ± 5,22	16,79 ± 5,24	0.24	0.74	0.56	0.99	0.09	0.83	0.25	0.83
Phosphatidylcholines (mmol/l)	340,95 ± 44,10	351,29 ± 61,36	387,66 ± 64,76	0.30	0.74	0.69	0.99	0.13	0.83	0.26	0.83
Cholesterol in medium HDL (mmol/l)	76,71 ± 16,47	82,09 ± 27,22	96,37 ± 24,23	0.28	0.74	0.61	0.99	0.11	0.83	0.27	0.83
Concentration of small HDL particles (mmol/l)	1,65 ± 0,15	1,62 ± 0,16	1,71 ± 0,13	0.54	0.90	0.58	0.99	0.48	0.99	0.27	0.83
Cholesteryl esters in medium HDL (mmol/l)	63,97 ± 13,58	68,17 ± 22,03	79,58 ± 19,01	0.29	0.74	0.62	0.99	0.12	0.83	0.27	0.83
Concentration of medium HDL particles (mmol/l)	0,60 ± 0,12	0,64 ± 0,20	0,74 ± 0,19	0.29	0.74	0.62	0.99	0.12	0.83	0.28	0.83
Total cholines (mmol/l)	410,77 ± 43,60	419,38 ± 65,06	454,50 ± 63,43	0.35	0.76	0.74	0.99	0.16	0.83	0.28	0.83

Concentration of large HDL particles (mmol/l)	0,18 ± 0,09	0,23 ± 0,13	0,30 ± 0,15	0.19	0.74	0.39	0.89	0.07	0.83	0.29	0.85
Polyunsaturated fatty acids (mmol/l)	865,69 ± 142,22	798,43 ± 118,55	875,27 ± 91,38	0.45	0.84	0.26	0.83	0.89	0.99	0.30	0.86
Cholesteryl esters in small HDL (mmol/l)	56,71 ± 5,92	55,41 ± 5,85	58,79 ± 4,42	0.58	0.95	0.63	0.99	0.50	0.99	0.31	0.87
Phospholipids in HDL (mmol/l)	244,41 ± 39,35	261,73 ± 69,59	296,07 ± 72,29	0.28	0.74	0.53	0.99	0.11	0.83	0.31	0.87
Cholesteryl esters in large HDL (mmol/l)	29,81 ± 15,89	38,12 ± 21,60	49,73 ± 24,24	0.19	0.74	0.37	0.87	0.07	0.83	0.31	0.87
Cholesterol in large HDL (mmol/l)	38,34 ± 20,06	49,21 ± 27,72	64,09 ± 32,02	0.19	0.74	0.37	0.87	0.07	0.83	0.31	0.87
Phosphoglycerides (mmol/l)	372,00 ± 49,33	375,20 ± 63,59	409,01 ± 65,35	0.48	0.84	0.91	0.99	0.25	0.83	0.32	0.87
Glutamine (mmol/l)	94,40 ± 11,84	103,75 ± 10,33	97,64 ± 8,41	0.24	0.74	0.10	0.83	0.60	0.99	0.32	0.87
Free cholesterol in large HDL (mmol/l)	8,53 ± 4,26	11,09 ± 6,14	14,37 ± 7,79	0.18	0.74	0.35	0.87	0.07	0.83	0.33	0.87
Phospholipids in large HDL (mmol/l)	43,05 ± 19,74	53,42 ± 28,39	67,99 ± 32,99	0.22	0.74	0.40	0.90	0.09	0.83	0.33	0.87
Phospholipids in medium HDL (mmol/l)	77,96 ± 12,84	81,87 ± 22,77	92,10 ± 20,82	0.37	0.76	0.65	0.99	0.17	0.83	0.34	0.87
Omega-6 fatty acids (mmol/l)	786,26 ± 116,68	732,06 ± 99,31	788,68 ± 49,32	0.47	0.84	0.26	0.83	0.97	0.99	0.34	0.87
Cholesteryl esters in very large HDL (mmol/l)	7,98 ± 2,74	9,91 ± 3,60	11,90 ± 5,05	0.14	0.74	0.26	0.83	0.05	0.83	0.34	0.87
Concentration of very large HDL particles (mmol/l)	0,03 ± 0,01	0,04 ± 0,01	0,05 ± 0,02	0.18	0.74	0.32	0.87	0.07	0.83	0.35	0.87
Sphingomyelins (mmol/l)	68,30 ± 5,18	71,09 ± 9,49	75,32 ± 9,70	0.27	0.74	0.45	0.95	0.11	0.83	0.35	0.87
Lactate (mmol/l)	323,68 ± 70,92	259,59 ± 81,45	221,92 ± 39,08	0.03	0.74	0.06	0.83	0.01	0.53	0.36	0.87
Triglycerides in large HDL (mmol/l)	4,06 ± 1,68	4,68 ± 1,27	5,62 ± 2,51	0.28	0.74	0.46	0.96	0.11	0.83	0.36	0.87
Omega-3 fatty acids (mmol/l)	79,43 ± 33,50	66,36 ± 28,68	86,59 ± 57,46	0.62	0.95	0.47	0.96	0.73	0.99	0.36	0.87
Cholesterol in very large HDL (mmol/l)	11,79 ± 3,30	14,24 ± 4,25	16,50 ± 6,28	0.14	0.74	0.24	0.83	0.06	0.83	0.37	0.87
Phospholipids in small HDL (mmol/l)	112,85 ± 10,82	112,38 ± 15,36	119,08 ± 12,78	0.62	0.95	0.94	0.99	0.38	0.88	0.37	0.87
Average diameter for LDL particles (nm)	4289,44 ± 22,19	4294,85 ± 14,81	4304,63 ± 15,41	0.34	0.75	0.54	0.99	0.15	0.83	0.37	0.87
Average diameter for HDL particles (nm)	1719,06 ± 32,32	1732,42 ± 33,64	1749,43 ± 39,57	0.27	0.74	0.41	0.92	0.12	0.83	0.39	0.90
Phospholipids in very large HDL (mmol/l)	10,55 ± 5,12	14,07 ± 5,70	16,91 ± 8,82	0.16	0.74	0.23	0.83	0.07	0.83	0.43	0.94
Glycine (mmol/l)	20,51 ± 9,68	23,56 ± 10,04	27,71 ± 4,98	0.36	0.76	0.49	0.99	0.16	0.83	0.43	0.94
Cholesteryl esters in large LDL (mmol/l)	122,95 ± 29,54	122,48 ± 32,30	135,21 ± 13,31	0.68	1.00	0.97	0.99	0.43	0.94	0.44	0.94
Cholesterol in large LDL (mmol/l)	165,18 ± 39,59	167,75 ± 44,86	184,57 ± 17,63	0.64	0.95	0.89	0.99	0.36	0.87	0.45	0.95
Linoleic acid (mmol/l)	613,44 ± 128,39	560,72 ± 100,71	607,80 ± 59,62	0.57	0.93	0.31	0.87	0.92	0.99	0.46	0.96
Alanine (mmol/l)	83,35 ± 24,52	73,41 ± 15,05	65,14 ± 9,15	0.22	0.74	0.28	0.84	0.10	0.83	0.46	0.96

Free cholesterol in large LDL (mmol/l)	42,24 ± 10,48	45,27 ± 12,62	49,37 ± 4,55	0.46	0.84	0.54	0.99	0.22	0.83	0.50	0.99
Pyruvate (mmol/l)	13,89 ± 5,73	11,76 ± 6,80	9,47 ± 3,77	0.45	0.84	0.49	0.99	0.21	0.83	0.50	0.99
Cholesteryl esters in chylomicrons and extremely large VLDL (mmol/l)	7,93 ± 7,08	5,17 ± 3,36	3,28 ± 2,18	0.25	0.74	0.28	0.83	0.12	0.83	0.54	0.99
Acetate (mmol/l)	7,25 ± 2,36	7,11 ± 3,03	6,17 ± 2,59	0.75	1.00	0.91	0.99	0.46	0.96	0.54	0.99
Albumin (g/l)	6646,55 ± 561,60	6656,09 ± 316,03	6820,30 ± 425,45	0.77	1.00	0.97	0.99	0.50	0.99	0.54	0.99
Citrate (mmol/l)	15,80 ± 3,25	14,15 ± 3,66	13,06 ± 1,49	0.25	0.74	0.27	0.83	0.12	0.83	0.55	0.99
Triglycerides in chylomicrons and extremely large VLDL (mmol/l)	57,40 ± 56,01	33,56 ± 20,54	19,50 ± 13,45	0.21	0.74	0.22	0.83	0.10	0.83	0.55	0.99
Cholesterol in chylomicrons and extremely large VLDL (mmol/l)	16,08 ± 13,98	10,32 ± 6,37	6,77 ± 4,38	0.24	0.74	0.25	0.83	0.11	0.83	0.56	0.99
Free cholesterol in LDL (mmol/l)	67,77 ± 16,78	70,71 ± 20,13	76,29 ± 6,66	0.64	0.95	0.71	0.99	0.35	0.87	0.56	0.99
Phospholipids in chylomicrons and extremely large VLDL (mmol/l)	12,85 ± 11,78	7,70 ± 4,90	4,83 ± 3,58	0.21	0.74	0.22	0.83	0.10	0.83	0.57	0.99
Free cholesterol in very large HDL (mmol/l)	3,80 ± 0,69	4,33 ± 0,70	4,60 ± 1,25	0.18	0.74	0.19	0.83	0.09	0.83	0.57	0.99
Free cholesterol in chylomicrons and extremely large VLDL (mmol/l)	8,16 ± 6,92	5,16 ± 3,04	3,49 ± 2,20	0.22	0.74	0.23	0.83	0.11	0.83	0.58	0.99
Concentration of chylomicrons and extremely large VLDL particles (mmol/l)	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0.21	0.74	0.22	0.83	0.11	0.83	0.58	0.99
LDL cholesterol (mmol/l)	261,11 ± 65,54	259,02 ± 73,51	278,91 ± 23,39	0.84	1.00	0.94	0.99	0.61	0.99	0.58	0.99
Cholesteryl esters in LDL (mmol/l)	193,34 ± 49,95	188,31 ± 53,51	202,61 ± 17,08	0.87	1.00	0.82	0.99	0.72	0.99	0.60	0.99
Phospholipids in very large VLDL (mmol/l)	10,28 ± 7,85	6,91 ± 4,20	5,21 ± 2,84	0.26	0.74	0.25	0.83	0.14	0.83	0.63	0.99
Free cholesterol in very large VLDL (mmol/l)	5,89 ± 4,19	4,15 ± 2,41	3,24 ± 1,62	0.29	0.74	0.27	0.83	0.15	0.83	0.63	0.99
Free cholesterol in medium LDL (mmol/l)	17,87 ± 4,70	17,79 ± 5,44	19,06 ± 2,08	0.87	1.00	0.97	0.99	0.64	0.99	0.63	0.99
Cholesterol in very large VLDL (mmol/l)	11,35 ± 7,43	8,56 ± 5,10	6,95 ± 3,12	0.37	0.76	0.33	0.87	0.19	0.83	0.65	0.99
Histidine (mmol/l)	13,20 ± 1,49	13,02 ± 0,90	13,34 ± 1,01	0.89	1.00	0.75	0.99	0.84	0.99	0.65	0.99
Concentration of very large VLDL particles (mmol/l)	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0.25	0.74	0.22	0.83	0.14	0.83	0.66	0.99
Phospholipids in IDL (mmol/l)	40,79 ± 8,90	45,31 ± 10,17	47,47 ± 4,92	0.32	0.74	0.28	0.83	0.17	0.83	0.67	0.99
Triglycerides in very large VLDL (mmol/l)	31,70 ± 24,91	19,74 ± 10,31	15,24 ± 6,81	0.20	0.74	0.18	0.83	0.11	0.83	0.67	0.99
Cholesteryl esters in very large VLDL (mmol/l)	5,47 ± 3,31	4,41 ± 2,72	3,72 ± 1,51	0.49	0.86	0.43	0.94	0.27	0.83	0.67	0.99
Phospholipids in large VLDL (mmol/l)	15,13 ± 10,04	10,77 ± 6,07	8,99 ± 4,17	0.30	0.74	0.25	0.83	0.17	0.83	0.70	0.99

Phospholipids in large LDL (mmol/l)	53,21 ± 11,71	55,07 ± 14,09	57,59 ± 3,00	0.78	1.00	0.73	0.99	0.49	0.99	0.71	0.99
Triglycerides in VLDL (mmol/l)	219,44 ± 139,19	154,39 ± 62,12	132,02 ± 40,69	0.23	0.74	0.19	0.83	0.13	0.83	0.71	0.99
Free cholesterol in large VLDL (mmol/l)	10,15 ± 6,17	7,46 ± 4,04	6,39 ± 2,82	0.32	0.74	0.26	0.83	0.18	0.83	0.71	0.99
Concentration of IDL particles (mmol/l)	0,05 ± 0,01	0,05 ± 0,02	0,05 ± 0,00	0.56	0.93	0.29	0.85	0.60	0.99	0.71	0.99
Phospholipids in VLDL (mmol/l)	87,90 ± 45,99	74,49 ± 31,54	66,66 ± 13,75	0.53	0.90	0.45	0.95	0.30	0.86	0.71	0.99
Free cholesterol in VLDL (mmol/l)	52,19 ± 25,77	45,60 ± 19,79	41,25 ± 8,35	0.61	0.95	0.52	0.99	0.36	0.87	0.73	0.99
Average diameter for VLDL particles (nm)	7225,10 ± 299,59	6989,12 ± 202,73	6936,72 ± 242,08	0.07	0.74	0.06	0.83	0.05	0.83	0.73	0.99
Cholesteryl esters in small VLDL (mmol/l)	13,49 ± 5,30	14,17 ± 5,77	13,16 ± 1,63	0.93	1.00	0.78	0.99	0.90	0.99	0.73	0.99
Cholesterol in large VLDL (mmol/l)	18,94 ± 10,69	15,03 ± 8,32	13,28 ± 5,27	0.46	0.84	0.37	0.87	0.26	0.83	0.74	0.99
Concentration of large VLDL particles (mmol/l)	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0.28	0.74	0.22	0.83	0.17	0.83	0.74	0.99
VLDL cholesterol (mmol/l)	115,85 ± 52,89	110,14 ± 47,09	101,47 ± 14,88	0.84	1.00	0.79	0.99	0.57	0.99	0.74	0.99
Acetoacetate (mmol/l)	8,55 ± 2,65	6,83 ± 3,42	6,23 ± 4,07	0.34	0.75	0.26	0.83	0.20	0.83	0.74	0.99
Clinical LDL cholesterol (mmol/l)	370,44 ± 109,83	381,29 ± 129,92	401,36 ± 32,14	0.87	1.00	0.83	0.99	0.60	0.99	0.75	0.99
Free cholesterol in IDL (mmol/l)	30,68 ± 7,36	34,74 ± 8,96	36,12 ± 3,47	0.32	0.74	0.25	0.83	0.19	0.83	0.75	0.99
Cholesterol in IDL (mmol/l)	116,06 ± 28,54	132,68 ± 35,45	137,99 ± 13,99	0.30	0.74	0.23	0.83	0.18	0.83	0.75	0.99
Cholesteryl esters in IDL (mmol/l)	85,37 ± 21,37	97,94 ± 26,58	101,87 ± 10,72	0.30	0.74	0.23	0.83	0.18	0.83	0.76	0.99
Cholesteryl esters in VLDL (mmol/l)	63,67 ± 27,95	64,54 ± 27,41	60,21 ± 6,62	0.95	1.00	0.94	0.99	0.80	0.99	0.77	0.99
3-Hydroxybutyrate (mmol/l)	12,79 ± 2,01	8,21 ± 4,91	9,08 ± 9,22	0.15	0.74	0.07	0.83	0.19	0.83	0.77	0.99
Triglycerides in medium HDL (mmol/l)	8,92 ± 2,92	8,89 ± 1,86	9,29 ± 2,21	0.95	1.00	0.98	0.99	0.78	0.99	0.78	0.99
Cholesteryl esters in large VLDL (mmol/l)	8,79 ± 4,63	7,57 ± 4,30	6,89 ± 2,46	0.67	0.98	0.54	0.99	0.41	0.92	0.78	0.99
Triglycerides in large VLDL (mmol/l)	39,23 ± 22,99	26,96 ± 12,69	24,17 ± 9,07	0.21	0.74	0.16	0.83	0.13	0.83	0.79	0.99
Concentration of large LDL particles (mmol/l)	0,11 ± 0,03	0,11 ± 0,03	0,11 ± 0,01	0.96	1.00	0.89	0.99	0.87	0.99	0.79	0.99
Phospholipids in LDL (mmol/l)	92,70 ± 21,60	93,03 ± 24,92	96,27 ± 6,07	0.95	1.00	0.97	0.99	0.75	0.99	0.79	0.99
Cholesterol in small VLDL (mmol/l)	21,76 ± 8,36	22,60 ± 9,22	21,38 ± 2,41	0.96	1.00	0.82	0.99	0.93	0.99	0.79	0.99
Triglycerides in HDL (mmol/l)	23,93 ± 8,36	23,37 ± 4,23	24,40 ± 5,32	0.96	1.00	0.86	0.99	0.90	0.99	0.79	0.99
Concentration of VLDL particles (mmol/l)	0,02 ± 0,01	0,02 ± 0,01	0,02 ± 0,00	0.83	1.00	0.72	0.99	0.56	0.99	0.79	0.99
Triglycerides in small LDL (mmol/l)	3,12 ± 1,82	2,47 ± 0,60	2,27 ± 0,25	0.40	0.79	0.29	0.85	0.24	0.83	0.79	0.99
Cholesterol in medium LDL (mmol/l)	66,60 ± 19,38	63,36 ± 20,80	65,97 ± 6,36	0.93	1.00	0.71	0.99	0.95	0.99	0.80	0.99

Triglycerides in small HDL (mmol/l)	9,78 ± 3,52	8,72 ± 1,59	8,35 ± 1,07	0.54	0.90	0.40	0.90	0.33	0.87	0.81	0.99
Concentration of very small VLDL particles (mmol/l)	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0.60	0.95	0.33	0.87	0.55	0.99	0.82	0.99
Concentration of small VLDL particles (mmol/l)	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0.77	1.00	0.62	0.99	0.52	0.99	0.83	0.99
Free cholesterol in small LDL (mmol/l)	7,66 ± 1,76	7,65 ± 2,20	7,86 ± 0,81	0.97	1.00	0.99	0.99	0.84	0.99	0.84	0.99
Acetone (mmol/l)	3,76 ± 0,94	3,06 ± 0,51	3,16 ± 1,35	0.24	0.74	0.12	0.83	0.25	0.83	0.84	0.99
Phospholipids in medium LDL (mmol/l)	25,31 ± 7,15	24,24 ± 7,50	24,99 ± 2,67	0.94	1.00	0.73	0.99	0.93	0.99	0.84	0.99
Monounsaturated fatty acids (mmol/l)	616,82 ± 284,81	509,23 ± 106,85	488,91 ± 27,46	0.40	0.79	0.27	0.83	0.26	0.83	0.86	0.99
Cholesteryl esters in medium LDL (mmol/l)	48,73 ± 15,20	45,57 ± 15,45	46,91 ± 4,59	0.89	1.00	0.63	0.99	0.81	0.99	0.87	0.99
Phospholipids in small VLDL (mmol/l)	14,48 ± 5,28	14,04 ± 5,26	13,60 ± 1,52	0.94	1.00	0.85	0.99	0.74	0.99	0.87	0.99
Free cholesterol in medium VLDL (mmol/l)	11,82 ± 5,41	11,40 ± 5,67	10,96 ± 1,86	0.95	1.00	0.86	0.99	0.75	0.99	0.88	0.99
Phospholipids in very small VLDL (mmol/l)	14,75 ± 4,28	16,29 ± 3,52	15,97 ± 1,92	0.64	0.95	0.38	0.88	0.55	0.99	0.88	0.99
Triglycerides in very large HDL (mmol/l)	1,17 ± 0,73	1,09 ± 0,25	1,13 ± 0,33	0.94	1.00	0.73	0.99	0.89	0.99	0.88	0.99
Concentration of medium LDL particles (mmol/l)	0,05 ± 0,01	0,05 ± 0,02	0,04 ± 0,00	0.93	1.00	0.81	0.99	0.72	0.99	0.88	0.99
Phospholipids in medium VLDL (mmol/l)	20,41 ± 9,58	18,79 ± 9,20	18,07 ± 3,39	0.86	1.00	0.69	0.99	0.62	0.99	0.89	0.99
Concentration of medium VLDL particles (mmol/l)	0,01 ± 0,00	0,01 ± 0,00	0,00 ± 0,00	0.78	1.00	0.59	0.99	0.54	0.99	0.89	0.99
Free cholesterol in small VLDL (mmol/l)	8,27 ± 3,09	8,43 ± 3,47	8,21 ± 0,84	0.99	1.00	0.91	0.99	0.97	0.99	0.90	0.99
Concentration of small LDL particles (mmol/l)	0,03 ± 0,01	0,03 ± 0,01	0,03 ± 0,00	0.88	1.00	0.71	0.99	0.65	0.99	0.90	0.99
Cholesterol in medium VLDL (mmol/l)	23,35 ± 11,13	24,57 ± 12,73	23,84 ± 3,39	0.97	1.00	0.81	0.99	0.93	0.99	0.91	0.99
Triglycerides in small VLDL (mmol/l)	28,25 ± 12,12	23,38 ± 5,39	22,79 ± 3,30	0.40	0.79	0.26	0.83	0.28	0.83	0.91	0.99
Triglycerides in very small VLDL (mmol/l)	10,71 ± 4,01	10,41 ± 1,29	10,23 ± 1,70	0.95	1.00	0.83	0.99	0.77	0.99	0.92	0.99
Cholesterol in small LDL (mmol/l)	29,32 ± 8,16	27,91 ± 8,46	28,36 ± 2,59	0.92	1.00	0.69	0.99	0.82	0.99	0.92	0.99
Concentration of LDL particles (mmol/l)	0,19 ± 0,05	0,18 ± 0,05	0,19 ± 0,01	0.98	1.00	0.84	0.99	0.94	0.99	0.92	0.99
Cholesteryl esters in medium VLDL (mmol/l)	11,53 ± 6,05	13,17 ± 7,10	12,88 ± 1,62	0.82	1.00	0.56	0.99	0.68	0.99	0.93	0.99
Remnant cholesterol (non-HDL, non-LDL -cholesterol) (mmol/l)	231,91 ± 73,63	242,82 ± 79,78	239,46 ± 12,07	0.94	1.00	0.74	0.99	0.84	0.99	0.93	0.99
Cholesteryl esters in very small VLDL (mmol/l)	16,46 ± 5,49	20,05 ± 5,34	20,28 ± 3,06	0.23	0.74	0.14	0.83	0.18	0.83	0.94	0.99
Cholesteryl esters in small LDL (mmol/l)	21,66 ± 6,65	20,26 ± 6,33	20,50 ± 1,97	0.86	1.00	0.62	0.99	0.72	0.99	0.94	0.99
Triglycerides in IDL (mmol/l)	14,71 ± 4,76	15,10 ± 1,69	14,97 ± 2,96	0.97	1.00	0.82	0.99	0.90	0.99	0.95	0.99
Triglycerides in medium LDL (mmol/l)	5,66 ± 2,57	4,94 ± 0,86	4,89 ± 0,58	0.63	0.95	0.42	0.93	0.45	0.95	0.96	0.99

Cholesterol in very small VLDL (mmol/l)	24,37 ± 7,66	29,06 ± 7,49	29,25 ± 4,00	0.28	0.74	0.17	0.83	0.21	0.83	0.96	0.99
Creatinine (µmol/l)	12977,55 ± 2843,89	12354,35 ± 1485,92	12276,98 ± 3350,35	0.84	1.00	0.62	0.99	0.65	0.99	0.96	0.99
Triglycerides in LDL (mmol/l)	23,68 ± 9,30	22,12 ± 3,30	21,93 ± 3,11	0.84	1.00	0.63	0.99	0.64	0.99	0.96	0.99
Free cholesterol in very small VLDL (mmol/l)	7,91 ± 2,24	9,01 ± 2,17	8,97 ± 0,99	0.44	0.84	0.26	0.83	0.34	0.87	0.97	0.99
Apolipoprotein B (g/l)	131,27 ± 35,67	131,52 ± 38,74	130,90 ± 7,45	1.00	1.00	0.99	0.99	0.98	0.99	0.97	0.99
Triglycerides in large LDL (mmol/l)	14,89 ± 4,97	14,71 ± 2,00	14,78 ± 2,57	0.99	1.00	0.92	0.99	0.95	0.99	0.97	0.99
Triglycerides in medium VLDL (mmol/l)	52,15 ± 23,85	40,34 ± 15,70	40,10 ± 10,28	0.34	0.75	0.20	0.83	0.26	0.83	0.98	0.99
Phospholipids in small LDL (mmol/l)	14,18 ± 3,21	13,73 ± 3,55	13,69 ± 1,22	0.93	1.00	0.75	0.99	0.77	0.99	0.98	0.99
Saturated fatty acids (mmol/l)	714,81 ± 239,01	659,50 ± 107,29	658,02 ± 73,82	0.75	1.00	0.51	0.99	0.56	0.99	0.99	0.99

4.4 Discussion

This study investigated whether there were differences in insulin sensitivity and β-cell function indices, gut hormones, body composition measured by MRI and BIA, and systemic metabolomics between subjects who did and did not return to normal glycaemic control after bariatric surgery at long-term (≥ 5 years). Individuals with diabetes remission had significantly greater β-cell function and levels of plasma AG and FGF-19 but lower area of VAT, VAT:SAT ratio and levels of leucine, isoleucine, and valine than those without remission.

Insulin sensitivity and β-cell function indices, gut hormones and body composition

In order to measure β-cell function in this study, the HOMA-β was used and owing to the absence of postprandial plasma glucose levels measured, we applied to use the $AUC_{0\text{-}30}$ insulin to also reflect the ability of β-cell function since the first-phase insulin secretion occurring at 30 minutes post-meal (Bacha et al., 2008). In addition, according to the graph of $AUC_{0\text{-}180}$ insulin, there was a steep rise in insulin secretion at 15 and 30 minutes as well as a significant difference in insulin levels at 30 minutes between groups. As a result, the greater levels of $AUC_{0\text{-}30}$ insulin and HOMA-β in the responders could indicate that there is a restoration of normal β-cell function in patients who returned to non-diabetic glucose homeostasis, and this could be the key determinant for T2D remission after bariatric surgery.

We then examined further to identify factors restoring normal β-cell function and bring about diabetes remission after bariatric surgery. The changes in gut hormones, bile acids metabolism and gut microbiome have been proposed to be the mechanisms in addition to weight loss (Batterham and Cummings, 2016). Suppression of AG is one of the mechanisms propounded to underpin the diabetes remission after bariatric surgery (Cummings, 2009, Yada et al., 2014). A number of studies have shown that AG possesses disadvantages on glucose homeostasis via direct effects on pancreatic α- and β-cells to raise glucagon secretion, and to hinder glucose-stimulated insulin release (Tong et al., 2010, Dezaki et al., 2006). Furthermore, it brings about the increase in hepatic glucose production as well as the decrease in skeletal and adipose-tissue insulin sensitivity (Alamri

et al., 2016, Tong et al., 2014). Nonetheless, Dezaki and colleagues described that the AG's attenuated glucose-induced insulin release is to determine physiological secretion of insulin and to prevent hypoglycaemia during fasting (Dezaki et al., 2008).

AG also contributes to various health benefits including a potent growth hormone secretagogue, modulating glucose homeostasis, controlling eating behaviour and energy balance, effects on positive cardiovascular functions, and more importantly a cytoprotective property for regulating cell proliferation and survival (Mao et al., 2014, Sovetkina et al., 2020). Yang et al. have demonstrated that ghrelin gene products namely AG, DAG, and obestatin protect β-cells from apoptosis that is induced by hyperglycaemic conditions. These ghrelin gene products, in particular AG, inhibit intracellular Ca^{2+} influx which leads to cell injury and apoptosis (Yang et al., 2014). This beneficial effect was allegedly mediated via unknown receptors other than growth hormone secretagogue receptor (GHS-R).

The levels of plasma ghrelin have been reported to be negatively associated with insulin resistance and positively associated with insulin sensitivity in adolescents and at a population-based level, even though the causal relationship has not yet been elucidated (Ikezaki et al., 2002, Poykko et al., 2003). Two studies suggested that hyperinsulinemic state potentially downregulated the levels of ghrelin (Saad et al., 2002, Lucidi et al., 2002). Our finding of the higher levels of AG and AG:DAG in remitters where they have greater β-cell function parameters highlights the idea of AG's advantages particularly on β-cell protection and survival as well as its role on determining physiological secretion of insulin at the long term after bariatric surgery.

We found that the levels of fasting FGF-19 in remitters were marginally higher than non-remitters in line with a study by Gerhard and colleagues (Gerhard et al., 2013) revealing that the fasting levels of FGF-19 increased after RYGB in T2D remitters than non-remitters. This supports the beneficial role of FGF-19 on glucose homeostasis, which contributes to reducing hepatic glucose output, increasing glucose uptake by skeletal muscles and adipose tissue, decreasing food intake and increasing energy expenditure (Batterham and Cummings, 2016). Considering that the levels of FGF-19 typically surge at 90 – 120 minutes following the post-prandial rise of bile acids (Lundasen et al., 2006), our finding of the

postprandial suppression of FGF-19 from time 0 – 60 minutes during a MMTT ($\Delta AUC_{0\text{-}60}$ FGF-19) in remitters greater than non-remitters might not be clinically meaningful. The AUCs accounting for time 0 to 90 – 120 minutes during a MMTT could be better proxies for postprandial FGF-19 levels.

The concept of elevating incretin hormones contributing to the improvement in glucose homeostasis and diabetes remission after bariatric surgery has been widely accepted (Hopkins et al., 2020, Koliaki et al., 2017). Nannipieri et al. have shown that in patients with diabetes remission after RYGB and SG, increased GLP-1 response was the key associated with improvement of β-cell function at 15 days and 1 year post-surgery, and that determined diabetes remission. PYY, glucagon, ghrelin, amylin and pancreatic polypeptide were not different between groups (Nannipieri et al., 2013). Casajoana et al. also showed that the increase in AUC GLP-1 from pre-op to 1 month was predictive for diabetes remission (Casajoana et al., 2017). In contrast, some evidence postulate that PYY is a key determinant of diabetes remission after the surgery (Guida et al., 2019). In the present study, there was no significant difference in GLP-1 and PYY between remitters and non-remitters.

The discrepancy in these findings could be due to differences in 1) the time of gut hormone measurements as in the majority of studies the gut hormones were examined in the first year after bariatric surgery, whereas it is 5 to 7-year post-surgery in the present study 2) types of bariatric surgery and 3) study design. However, studies comparing the levels of these gut hormones between remitters and non-remitters, especially in the long-term after bariatric surgery is scarce. The GLP-1 and PYY levels measured in our study were not statistically significantly different between groups, indicating that these hormones may not be the key mechanism underlying the recovery of β-cell function, particularly in the long-term.

The state of chronic energy excess leading to raised hepatic and pancreatic fat contents has been postulated to be a part of the pathogenesis of T2D (Shibata et al., 2007, Steven et al., 2016, Taylor, 2013, McGarry, 2002). An increased intracellular diacylglycerol in the liver stimulates the action of a protein kinase C isoform PKCepsilon (an inhibitor of the insulin signalling pathway), thus developing hepatic insulin resistance. It also increases

hepatic glucose production (Samuel et al., 2010, Samuel et al., 2004). Moreover, the chronic exposure of β-cells to excess fatty acids or TG diminishes the β-cell function (McGarry, 2002, Unger, 1995) as well as brings about the loss of complete β-cell differentiation (Bensellam et al., 2018, Brereton et al., 2014), hence triggering T2D.

In our cohort where PWL was identical in both groups the percentage of fat contents in liver and pancreas was also similar between groups. These findings are in agreement with a previous study by Taylor and colleagues, which they found that following a weight-loss intervention provided in the Diabetes Remission Clinical Trial (DiRECT), the hepatic and pancreatic fat significantly reduced relating to degree of weight loss and was equal between remitters and non-remitters. Only the improvement in β-cell function that was observed in the remitters (Taylor et al., 2018) in line with our finding that the HOMA-β and $AUC_{0\text{-}30}$ insulin which represent the β-cell function in remitters were greater than non-remitters. This supports that the remission of human T2D is essentially dependent on the intrinsic capacity for β-cell recovery (Taylor et al., 2018). Furthermore, a previous genetic study in the UK Biobank showed that pancreatic fat had no impact on developing T2D (Liu et al., 2020).

Patients without diabetes remission in this study possessed visceral fat area approximately 1.5 times as much as patients with remission. They also had almost 2-fold as much VAT:SAT ratio as the remitters. These findings are in line with the fundamentals of visceral adipose tissue contributing to cardiometabolic diseases, in particular T2D. The mechanisms of excessive visceral fat on various health outcomes, particularly T2D, include: first, the hyperlytic property of VAT releases excessive amount of free fatty acids and glycerol to liver via the portal vein, leading to decreased hepatic extraction of insulin (aggravating hyperinsulinemia), increased hepatic gluconeogenesis and increased TG-rich lipoprotein production; second, a release of inflammatory cytokines and a reduction in adiponectin (an anti-inflammatory, anti-atherogenic, and anti-diabetic protein) production; third, the accompanied ectopic fat deposition (liver, pancreas, heart, and skeletal muscles) in which they locally damage their own organs (Neeland et al., 2018, Neeland et al., 2019).

A recent multivariate genome-wide association study of metabolic biomarkers and percentage of body adiposity has revealed that adiposity genetic variants with unfavourable metabolic outcomes including T2D, hypertension, cardiovascular diseases, non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS) were associated with higher VAT, hepatic and pancreatic fat, whereas adiposity genetic variants with favourable metabolic effects were associated with lower hepatic fat but had no correlation with VAT and pancreatic fat (Martin et al., 2021). They concluded that hepatic fat was a key determinant for developing T2D not VAT. However, our findings highlight the important role of VAT on T2D remission over hepatic fat. Further experiments are necessary to determine the difference in mechanisms of VAT and hepatic fat on the establishment and remission of T2D.

Of note, the difference in VAT parameters were only observed from the MRI in our study. There was no statistically significant difference in visceral fat rating between the two groups from the BIA. This could be due to the fact that the visceral fat level measured by the BIA has a significantly strong correlation with total abdominal adipose tissue measured by the MRI, rather than the VAT (Browning et al., 2010).

Metabolomics study

This study also aims to identify metabolites related to diabetes remission post-bariatric surgery. Therefore, we analysed 2 groups of patients, who had previously undergone bariatric surgery, with and without T2D remission. In addition, we also examined the difference in metabolites between patients with complete remission versus partial remission versus no remission of T2D. Regarding the sPLS-DA analysis and the sample clustering, it is noticeable that there was an evident discrimination between patients with and without T2D remission post-surgery based on the identified metabolites. In addition, the metabolites in patients with partial remission overlapped with those with complete remission and no remission probably due to the nature of the borderline group.

The most significant metabolites discriminating remitters vs. non-remitters analysed by sPLS-DA method include size of VLDL and LDL particles, TG content in small HDL, degree of fatty acids unsaturation, the levels of Isoleucine, Leucine, Valine, Alanine, Tyrosine,

albumin, glucose, lactate, β-hydroxybutyrate, citrate, and glycoprotein acetyls (P = 0.001). According to the heatmap, patients with T2D Remission presented higher values of post-operative HDL during a MMTT. Nonetheless, the univariate analysis comparing metabolites between the 2 groups revealed that only the $AUC_{0\text{-}180}$ of leucine, isoleucine, and valine in non-remitters were statistically significantly greater than remitters, and the cut-off value of $AUC_{0\text{-}180}$ of total BCAAs at ≥91.5 mmol x min/L gives sensitivity of 91%, specificity of 85% for being T2D non-remitters.

In terms of metabolites differentiating complete vs. partial vs. non-remitters, VLDL diameter, HDL, small HDL and IDL concentrations, the amount of cholesterol and cholesteryl esters in small HDL, the levels of isoleucine, leucine, valine, phenylalanine, tyrosine, glutamine, glucose, lactate, β-hydroxybutyrate, acetone, degree of fatty acids unsaturation, the levels of omega-3 fatty acids, omega-6 fatty acids, PUFA, LA, DHA and GlycA were found from the sPLS-DA method (P = 0.001). During a MMTT, as the T2D remission status progress, there was a decrease in the levels of VLDL and LDL, whereas an increase in the levels of HDL were observed from the heatmap. When comparing metabolites between the 3 groups using One-way ANOVA, only the $AUC_{0\text{-}180}$ of leucine, isoleucine, and valine in non-remitters were statistically significantly greater than complete remitters in line with the previous comparison between remitters and non-remitters.

The detected metabolic profile after bariatric surgery between remitters and non-remitters provides more insights into the diabetes remission mechanisms and potentially lead to novel treatment strategies. The most discriminant detected metabolites for remitters vs. non-remitters and complete remitters vs. partial remitters vs. non-remitters can be divided into 4 categories: 1) amino acids 2) energy metabolism related metabolites 3) lipoproteins 4) metabolites related to inflammation.

Amino acids: The top metabolites determining clustering were the $AUC_{0\text{-}180}$ of leucine, isoleucine and valine which in T2D non-remitters the value was significantly greater than T2D remitters (p=0.02). Interestingly, there is mounting evidence indicating that the three BCAAs, isoleucine, leucine and valine, are causally linked with insulin resistance, impaired glucose metabolism and are a strong predictor of insulin sensitivity in both adults (Shaham

et al., 2008) and children (McCormack et al., 2013). Metabolomic studies in healthy subjects with normal insulin sensitivity revealed that the alteration in BCAA metabolism occurred decade or more before the development of diabetes (Wang et al., 2011, Liu et al., 2017, Guasch-Ferre et al., 2016). A large-scale human genetic and metabolomic study showed that polymorphisms of the PPM1K gene which encodes an activator of the branched-chain alpha-ketoacid dehydrogenase (BCKD) responsible for the rate-limiting step in BCAA catabolism escalated the risk of insulin resistance, confirming a causal role of BCAA metabolism on developing T2D (Lotta et al., 2016).

In addition, supplementing BCAA to high-fat diet in transgenic mice results in impaired glucose tolerance and insulin resistance (Newgard et al., 2009), whereas dietary BCAA deprivation in mice improves hepatic insulin sensitivity under insulin-resistant conditions, likely via decreased mTOR/S6K1 signaling (Xiao et al., 2011). Importantly, oral ingestion of valine in humans results in insulin resistance via accumulation of its metabolite, 3-hydroxyisobutyrate (3-HIB), which increases fatty acid uptake in muscles and suppression of fibroblast growth factor 21 (FGF21) which enhances insulin sensitivity (Harris et al., 2017).

In the attempts of enlightening the contribution of BCAAs to insulin resistance, the reduction in gene expression of enzymes responsible for BCAAs catabolism and oxidation in adipose tissue and liver were discovered (She et al., 2007, Herman et al., 2010, Neinast et al., 2019a). Hypoxia, endoplasmic reticulum stress and inflammation were proposed to be the causes of this suppression in cell-culture studies (Burrill et al., 2015). Alternatively, or, in addition, White and Newgard postulate that upregulated systemic BCAAs are due to disturbances in glycolysis and interferences in lipid oxidation, resulting in deficient BCAA catabolism and oxidation involving transcriptional suppression of BCAA catabolising enzymes (White and Newgard, 2019). Further, Lynch et al. suggests that BCAAs themselves do not cause insulin resistance, per se, but rather that the accumulation of toxic metabolic intermediates, generated by the impairment of BCAAs catabolism, potentially causes β-cell mitochondrial dysfunction and subsequent apoptosis (Lynch and Adams, 2014).

The rise in circulating levels of BCAAs and the shift of BCAAs into skeletal muscles, causing lipotoxicity and insulin resistance (Neinast et al., 2019b) via: first, activation of chronic phosphorylation of mTORC1/S6K1 kinase and c-Jun amino-terminal kinase (JNK), a stress kinase, which subsequently inhibit downstream serine/ threonine phosphorylation of insulin receptors signaling cascade; and second, the overload of intermediates of BCAA catabolism impairs mitochondrial fatty acid oxidation and promotes the deposition of mitochondrial acylcarnitines, which subsequently impinges insulin signalling (Lackey et al., 2013, Herman et al., 2010, Newgard, 2012, Newgard et al., 2009, Lee et al., 2016, Vangipurapu et al., 2019, Boucher et al., 2014). In addition, emerging evidence have shown a connection between BCAAs and ectopic fat accumulation via: first, BCAA overload leads to impaired normal mitochondrial fatty acid oxidation, causing the accumulation of fatty acyl-CoAs (White et al., 2016); second, a catabolic intermediate of the valine (3-hydroxyisobutyrate, 3-HIB) acts as a paracrine manner to stimulate transendothelial fatty acid transport, activate muscle fatty acid uptake in vivo and promote lipid deposition in skeletal muscles (Jang et al., 2016), thus insulin resistance.

Nevertheless, the causal role of BCAAs on insulin resistance and diabetes has been challenged by Mendelian randomisation studies demonstrating that HOMA-IR was causally associated with greater levels of circulating BCAAs and the genetic risk score of BCAAs did not relate to the levels of fasting insulin or HOMA-IR (Mahendran et al., 2017). Furthermore, another Mendelian randomisation study of 53 genetic variants associated with insulin resistance showed that 1-SD genetically increased insulin resistance was associated with higher levels of circulating BCAAs, highlighting a causal role of insulin resistance on BCAAs (Wang et al., 2017). In addition, as insulin possesses anti-proteolytic effect and insulin resistance condition results in impaired mitochondrial fuel oxidation leading to decreased amino acids clearance, the high circulating levels of BCAAs potentially results from the insulin resistance, not the cause. Yao and colleagues (Yao et al., 2019) revealed that at pre-SG, the suppression of insulin on BCAAs during the hyperinsulinaemic-euglycemic clamp in patients with obesity was smaller than lean controls, and this improved post-surgery. In contrast, the plasma concentrations of acylcarnitines which reflect BCAA catabolism were not significantly different between patients with obesity and lean controls at pre-SG and did not change following SG. This

suggests that the elevated levels of BCAAs in obesity is caused by the impairment in insulin-mediated proteolysis suppression, rather than reduced catabolism. Hence, the elevations of BCAAs resulting in insulin resistance and diabetes remains unclear.

It is generally known that visceral adiposity, ectopic adipose tissue accumulating around intra-abdominal organs, is hormonally active, and possesses unique metabolic characteristics. It is associated with a variety of medical conditions such as metabolic syndrome, T2D, cardiovascular diseases and several types of cancer (Shuster et al., 2012). Of note, a number of evidence have demonstrated a clear link between BCAAs and VAT by measuring gene expression (mRNA levels) in subcutaneous adipose tissue (SAT) and VAT for BCAA catabolism enzymes namely the branched-chain α-ketoacid dehydrogenase (BCKDH) complex and branched- chain aminotransferase (BCAT). The levels of mRNA for BCKDH and BCAT in SAT were not significantly different between lean subjects and patients with morbid obesity (Pakiet et al., 2020), but the mRNA levels in VAT for BCKDH and BCAT in subjects with morbid obesity were significantly lower than lean controls (Pakiet et al., 2020, Boulet et al., 2015, Su et al., 2015). Furthermore, the gene expression of BCAA catabolism enzymes in VAT in patients with morbid obesity and insulin resistance were lower than overweight individuals without insulin resistance (Serralde-Zuniga et al., 2014). These findings highlight the predominant downregulation of BCAA catabolism in VAT in patients with obesity and insulin resistance. Importantly, Su et al. reported an upregulation of BCAT and BCKDH in VAT and SAT of patients with morbid obesity following gastric bypass surgery (Su et al., 2015). These findings support the relationship between VAT and BCAAs levels observed in our study.

Of note, according to the univariate analysis, only $AUC_{0\text{-}180}$ of BCAAs were statistically significantly different between patients with remission vs. without remission and between complete remitters vs. non-remitters. The greater circulating levels of leucine, isoleucine, and valine correlated with non-remission of T2D. We further examined the cut-off values indicating less likelihood of having diabetes remission, and at the combination of AUC of valine, leucine and isoleucine $\geq$91.5 mmol x min/l represents sensitivity of 91% and specificity of 85%. Our results suggest that the metabolism of BCAAs play a key role in diabetes remission.

Other amino acids including alanine, glutamine, tyrosine and phenylalanine were also able to discriminate patients with and without diabetes remission according to the sPLS-DA method. We found that the circulating levels of alanine were decreasing as the diabetes remission progress (the levels in no remission > partial remission > complete remission). This is consistent with previous studies revealing that the levels of alanine in patients with diabetes remission were significantly lower than non-remitters (Narath et al., 2016), and many studies have established a positive correlation between the levels of alanine and increased risk of T2D (Chen et al., 2019, Tillin et al., 2015, Stancakova et al., 2012, Ferrannini et al., 2013). The mechanism of elevated systemic alanine associated with T2D is not well understood. It is thought to be a result of the escalating levels of alanine aminotransferase (ALT), a crucial enzyme of the glucose-alanine level, which correlates with increasing hepatic fat responsible for insulin resistance and diabetes (Sattar et al., 2004, Vozarova et al., 2002).

On the other hand, the levels of glutamine were greater in remitters than non-remitters in agreement with a number of studies demonstrating that the levels of glutamine were inversely associated with T2D (Chen et al., 2019, Stancakova et al., 2012, Cheng et al., 2012, Ferrannini et al., 2013). Glutamine benefits glycaemia by enhancement of 1) secretion of GLP-1 2) externalisation of glucose transporter type 4 (GLUT 4) 3) transcription of insulin-dependent enzymes 4) insulin release by the pancreatic β-cells 5) insulin sensitivity in adipose tissue (Greenfield et al., 2009, Bakalar et al., 2006, Li et al., 2004). In rodents, administration of glutamine improved glucose tolerance and reduced blood pressure (Cheng et al., 2012). Interestingly, glutamine supplementation led to increased glucose tolerance in 24 adults (Greenfield et al., 2009).

Phenylalanine which can be converted into tyrosine have also been reported to be associated with reduced insulin secretion and sensitivity, thus insulin resistance and diabetes (Vangipurapu et al., 2019, Palmer et al., 2015). This is in line with our findings that the circulating levels of phenylalanine and tyrosine were greater in non-remitters than remitters. The mechanisms explaining this are still not well established.

Energy metabolism related metabolites: Another finding from our study is that the AUC_{0-180} of lactate in non-remitters was greater than that in remitters. Lactate is a product from

glycolysis, and high concentrations of lactate have been reported to be connected with insulin resistance and T2D (Higuchi et al., 2020, Lovejoy et al., 1992, Reaven et al., 1988). A diminished mitochondrial oxidative capacity and an attenuated lactate transport ability for lactate transferring between skeletal muscle and circulation through monocarboxylate transporter (MCT)-1 proteins result in developing insulin resistance and diabetes (Pagel-Langenickel et al., 2010, Juel et al., 2004, Higuchi et al., 2020, Avogaro et al., 1996).

β-hydroxybutyrate and acetone are ketone bodies primarily involve in energy transferring from adipose tissues. They are produced by the liver from fatty acids during fasting, carbohydrate restrictive diet and exercise. By converting into acetyl-CoA, they can then enter the TCA cycle in the mitochondria and generate energy. Moreover, ketone bodies are also substrates for gluconeogenesis. The real mechanisms of β-hydroxybutyrate and acetone contributing to diabetes is still lacking. The effects of downstream metabolites of β-hydroxybutyric acid on functions of many mitochondrial enzymes were reported to link to pathogenesis of T2D (Samczuk et al., 2018), and this could be an explanation of our findings that there were higher levels of circulating β-hydroxybutyrate and acetone in non-remitters than remitters.

In terms of fatty acids, we found that the degree of unsaturation, the levels of omega-3 fatty acids, omega-6 fatty acids, PUFA, LA and DHA were able to discriminate individuals with and without diabetes remission by the sPLS-DA method. Previous evidence demonstrated that there was a significant association of longer chain and unsaturation of TGs and phospholipids with decreased T2D risk (Rhee et al., 2011). Aurora and colleagues observed that at pre-bariatric surgery the levels of TG with long-chain fatty acids in patients with diabetes remission was greater than patients without remission; however, the significant difference between groups was not seen after bariatric surgery (Arora et al., 2015). In addition, the beneficial effects of PUFA (mainly omega-3 fatty acids: DHA and EPA) on blood pressure, dyslipidaemia, and inflammation which links to T2D have been confirmed by many epidemiological studies and meta-analyses (Pinti et al., 2019, Ellulu et al., 2016, Gouaref et al., 2020).

Since mitochondrial function primarily involves in production of ATP, fatty acid oxidation, and cellular metabolism regulation, the discrimination of amino acids, fatty acids, lactate

and ketone bodies between diabetes remitters and non-remitters could indicate that the mitochondrial function has an impact on diabetes remission. Studies showed that there is a connection between diabetes and mitochondrial function (Pinti et al., 2019). Nonetheless, it is still unclear whether decreased mitochondrial density or mitochondrial dysfunction leads to insulin resistance or whether mitochondrial dysfunction is a consequence of insulin resistance and subsequent T2D (Dankel et al., 2011, Samczuk et al., 2018).

Lipoproteins: During a MMTT in our study, a notable finding was that as the T2D remission status progressed in participants, VLDL and LDL levels decreased, whereas HDL levels increased. Also, the average diameter of VLDL and TG content in small HDL in non-remitters were greater than remitters, whereas the size of LDL and the cholesterol ester content in HDL in remitters were higher than non-remitters. These findings are in line with a number of studies which have demonstrated that insulin resistance and T2D are associated with a distinct dyslipidaemia profile (Mora et al., 2010, Ahola-Olli et al., 2019). Diabetic dyslipoproteinaemia is typically characterised by increase in production of large VLDLs, increase number of glycated LDLs, small, dense LDLs, oxidised LDLs and increase in catabolism of HDLs (Verges, 2015). Similarly, in a prospective study of women, Mora et al. (2010) found that larger VLDL concurrent with smaller LDL and HDL particle size and concentration (as measured by nuclear magnetic resonance) were significantly associated with incident T2D (Mora et al., 2010).

In addition, there is a pervasive alteration in the composition of lipoprotein particles, mainly increase in relative proportion of TG in VLDL, LDL and HDL in insulin resistance and T2D (Ahola-Olli et al., 2019). The aberrations of lipoprotein composition result from increase in free fatty acids which are substrates for VLDL production as well as increased CETP activity which transfers TG from TG-rich lipoproteins to LDLs and HDLs (Verges, 2015). These lipid alterations reflect that the persistence of insulin resistance is one of the factors preventing individuals from the remission of diabetes after bariatric surgery. Abnormal metabolism of HDL was also discovered as the earliest signal of T2D, preceding high levels of BCAAs and GlyA, and this occurred decades before the diagnosis of T2D (Bell et al., 2020).

Of note, it has also been suggested that HDL has an anti-inflammatory function. Even though it did not reach statistical significance in our present study, the attenuated concentration of HDL particles in non-remitters (2.47± 0.25 mmol/l) compared to partial remitters (2.52 ± 0.47) and complete remitters (2.80 ± 0.42) could provide a potential explanation between the difference in remitters and non-remitters. As there is in an abundance of studies recognising the intimate link between inflammation and T2D (Tsalamandris et al., 2019, Boni-Schnetzler et al., 2008), the diminished levels of anti-inflammatory HDL and enhanced levels of pro-inflammatory VLDL and LDL could plausibly contribute to T2D persistence in non-remitters.

Inflammation: In line with this hypothesis, our findings reveal that non-remitters have a significantly greater level of GlyA compared to complete remitters. Glycoprotein acetyls is emerging as a novel inflammation-responsive signal in nuclear magnetic resonance of circulating glycoproteins such as haptoglobin, α1-antitrypsin and α1-acid glycoprotein (orosomucoid) (Otvos et al., 2015, Bell et al., 1987, Ritchie et al., 2015). These glycoproteins are acute-phase proteins produced by liver and neutrophils (Ritchie et al., 2015, Connelly et al., 2017) and are associated with chronic inflammation involving circulating levels of C-reactive protein (CRP), tumour necrosis factor (TNF)-α, interleukin (IL)-6, etc. Therefore, this may reflect that in our study, non-remitters have elevated systemic inflammation burden. The role of inflammation in the pathophysiology of diabetes is well-established; for example, pro-inflammatory cytokines destroy the function of pancreatic β cells (Tsalamandris et al., 2019). Furthermore, in rodents, there is evidence of pancreatic islet inflammation caused by macrophages infiltration which are attracted by islet-derived chemokines, generated in metabolic stress and under the regulation of IL-1β, and this is reversed by IL-1 receptor antagonist (Boni-Schnetzler et al., 2008). This therefore may provide a plausible mechanistic explanation to the persistence of diabetes in non-remitters after bariatric surgery in our study.

Notably, GlycA has been reported to be independently associated with T2D, cardiovascular diseases, certain cancers, severe infections, chronic inflammatory conditions, non-alcoholic fatty liver disease and all-cause mortality (Kettunen et al., 2018, Connelly et al., 2017). Other cohort studies with metabolomics measurements have also

identified that GlycA concentrations are associated with diminished insulin secretion (Fizelova et al., 2017).

Metabolomics study targeting the difference between patients with and without T2D remission after bariatric surgery is currently scarce. Aurora et al. (Arora et al., 2015) compared global metabolomics and lipidomic at pre-RYGB, post-RYGB 4 and 42 days between 7 patients with diabetes remission and 7 patients without the remission. They found that at pre-surgery patients with T2D remission possessed the levels of TCA cycle intermediates and TG with long-chain fatty acids greater than patients without diabetes remission. Luo et al. (Luo et al., 2016) also compared metabolites at pre-RYGB, post-RYGB 6 and 12 months between 23 T2D remitters and 12 non-remitters. They revealed that the reduction in BCAAs levels positively correlated with down regulation of fasting C-peptide, fasting blood glucose and postprandial blood glucose post-surgery in remitters, whilst none of these associations were seen in non-remitters. Furthermore, most free fatty acids significantly decreased in remitters post-operatively, whereas only a few free fatty acids reduced in individuals without the remission. These findings are in agreement with our results highlighting the advantages of bariatric surgery on the improvement of amino acids metabolism, especially BCAAs, and the capacity of mitochondrial function, in particular TCA cycle and fatty-acid oxidation, which may further enhance glucose homeostasis.

There are several limitations of this study. First, the lack of sample size calculation and small sample size potentially lead to the under power to detect significant differences between groups. Second, although the differences in the duration of diabetes and glycaemic control at the time of surgery were not statistically significant between remitters and non-remitters, it is undeniable that it might be clinically meaningful. They could be additional factors determining diabetes remission in this study. Severity of T2D may well be another reason determining the remission since the number of patients with antidiabetic agents at the time of surgery in non-remitters were greater than remitters. Lastly, the present study cannot prove the causal relationship of different factors between groups with the remission of T2D. The different findings between remitters and non-remitters could be either mechanisms underlying the remission or results from good and poor glycaemic control. Therefore, further study examining this is now warranted.

4.5 Conclusion

This study has demonstrated that the improvement in β-cell function is the key factor underlying T2D remission post bariatric surgery. In addition to the post-operative weight loss, the reduction in visceral fat and the increase in AG and FGF-19 potentially enhance the β-cell function, which may contribute to the remission of T2D. The metabolomics analysis highlights the role of BCAA metabolism and its link with VAT, mitochondrial function (particularly TCA cycle and fatty-acid oxidation), and inflammation on the diabetes remission. Further research should focus on how the mitochondrial function, the reduction in VAT, the increase in AG and FGF-19, and circulating levels of BCAAs connect to the improvement in β-cell function, thus diabetes remission after bariatric surgery. Elucidating whether elevated BCAAs are indeed a cause or consequence of T2D is also of value to further investigate. A pharmacological approach targeting ghrelin and FGF-19 systems, and restoration of BCAA catabolic activity may be of worthy investigation as a potential T2D treatment for non-remitters post-bariatric surgery.

Chapter 5

The role of genetics in predicting weight loss after LRGB and LSG

Chapter 5 The role of genetics in predicting weight loss after LRGB and LSG

Genome-wide association study and genetic risk scores analyses in this chapter was performed by Kusuma Chaiyasoot and Dr. Wei Gan, a research associate in the Wellcome Centre for Human Genetics, University of Oxford. Kusuma Chaiyasoot was also responsible for patient recruitment, DNA sample preparation, data collection, data analysis and critical discussion.

5.1 Introduction

Studies undertaken in twins and close relatives demonstrate that genetic factors explain a significant portion of the variation in weight loss after gastric bypass surgery (e.g. the intra-class correlation coefficient is estimated to be up to 70%) (Rinella et al., 2013). Two previous groups have examined the genetic influence on weight loss after bariatric surgery using a genome-wide association study (GWAS) approach. GWAS studies of excess body weight loss (Rinella et al., 2013) and percentage weight loss (PWL) (Hatoum et al., 2013) after gastric bypass surgery have identified variants at two loci that associated with excess body weight loss (EBWL) or PWL but not at a GWAS significant threshold level. Importantly, studies undertaken in people undergoing sleeve gastrectomy which is now the most common operation undertaken globally is scarce (Angrisani et al., 2015).

In complex diseases, particularly obesity and T2D, their phenotypes are affected by many genetic variants. Genetic risk score (GRS), also called polygenic risk score (PRS), or genome-wide score, is a number that aggregates the estimated effect of numerous trait-associated genetic variants on a phenotype of interest. Nowadays, it has been used to predict a given trait, stipulating a person's genetic predisposition to that phenotype.

Bandstein and colleagues have revealed that GRSs consisting of BMI-associated single nucleotide polymorphisms (SNPs) (*MC4R, TMEM160, PTBP2, NUDT3, TFAP2B, ZNF608, MAP2K5, GNPDA2,* and *MTCH2*) and waist-hip ratio (WHR) associated variants (*HOXC13, LYPLAL1*, and *DNM3-PIGC*) significantly correlated with 2-year weight loss after RYGB (Bandstein et al., 2016). De Toro-Martin et al. showed that 2 PRSs of 186 and 11 SNPs

associated with BMI has a significant impact on 4-year weight reduction following biliopancreatic diversion with duodenal switch (BPD-DS) (de Toro-Martin et al., 2018). Additionally, Katsareli et al. demonstrated that a GRS of BMI- and WHR-associated variants designated a 4.6% reduction of 12-month percentage of excess weight loss (PEWL), calculated using the following formula: (postoperative weight loss)/ (preoperative excess weight) × 100, per score unit, and a 3% decrease of 24-month PEWL per score unit (Katsareli et al., 2020).

We aimed to identify genetics factors that are associated with weight loss after LRYGB and LSG. Hence, the objectives of this study are:

1. To analyse the correlation of GRSs of genetic variants previously reported to be associated with anthropometric traits (BMI and WHR) with PWL after LRGB and LSG.
2. To identify SNPs associated with 1-year PWL following LRGB and LSG using the GWAS approach.

5.2 Materials and Methods

5.2.1 Subjects

2,129 participants with severe obesity from six bariatric centres; 1) the University College London Hospitals (UCLH) Bariatric Centre for Weight Management and Metabolic Surgery, London, UK, 2) Department of Medicine, Imperial College London, London, UK, 3) St Richard's Hospital, Chichester, UK 4) Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal, 5) Department of General Surgery, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira, Portugal 6) the University Hospital of Pisa (UHP), Pisa, Italy, in Europe were recruited between April 2005 and April 2018. Only individuals of European ancestry and those who underwent bariatric surgery; laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LSG), were included in the analysis. The inclusion criteria were:

1. BMI ≥40 or ≥35 kg/m^2 with obesity associated co-morbidities
2. Aged 18-65 years
3. Scheduled to undergo primary bariatric surgery

The exclusion criteria were:

1. Incomplete clinical data
2. Several conditions that affects their weight pre- or post-surgery (e.g. cancer, acute kidney disease, unknown cause of death within 1 years, psychiatric disorders)
3. Pregnancy within the time period of interest after the procedure
4. Weight loss medication
5. Conversion of surgical procedures within the time period of interest

Demographic, anthropometric and detailed clinical data were electronically collected in the bariatric database for each participant. Longitudinal BW and clinical data were recorded before and after the surgery.

5.2.2 Surgical procedures

Both sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB) were performed laparoscopically. An antecolic-antegastric Roux-en-Y was constructed with a 30-mL gastric pouch, 120-cm alimentary limb and 80-cm biliopancreatic limb for the LRYGB. LSG was created by removal of approximately 80% of stomach along the greater curvature, using stapling commenced 5 cm from the pylorus. Consequently, a long narrow tubular stomach was created.

5.2.3 Anthropometric measurement

Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (metre). Body weight loss was expressed as percentage weight loss (PWL) since it is less influenced by baseline BMI, using the formula: PWL = ([baseline body weight – body weight at each study visit]/ baseline body weight) x 100.

5.2.4 Genotyping and quality control

Genomic DNA was extracted from peripheral blood or saliva samples using the QIAamp DNA Blood Midi Kit (Qiagen, UK) according to the manufacturer's instructions. Genotyping was performed using Illumina HumanCoreExome-24 BeadChip genotyping arrays (Illumina Inc., San Diego, CA) at the Wellcome Trust Sanger Institute (Hinxton, UK) and the Oxford Genomics Centre (Headington, UK). Quality control filters were applied at the individual level and single nucleotide polymorphism (SNP) levels. At the individual level, we removed the samples that met any of the following criteria (Anderson et al., 2010):

1. Call rates <96% (n=11)
2. With sex mismatches between the reported and genetically inferred (n = 13)
3. With excessive heterozygosity and population outliers (non-Europeans, n = 316) detected by using principal component analysis.

At the SNP level, we excluded the SNPs in Y and mitochondrial chromosomes, and the SNPs (n = 23,260) if they:

1. Had call rate <99%
2. Had Hardy-Weinberg equilibrium (HWE) P $<10^{-4}$
3. Minor allele frequency (MAF) <1%
4. Met the exclusion criteria according to the Oxford exome chip QC protocol
5. Had significant difference of call rate across the two genotyping centres

There were 1,789 samples and 497,016 genotyped SNPs passed QC. The samples that passed all QC criteria were then used to impute for the missing SNPs from the 1000 Genomes project phase 3 reference panel using IMPUTE (version 2.3.2) if the correlation coefficient (R^2) >0.8.

5.2.5 Genetic risk scores (GRSs)

Two GRSs were constructed from:

1. **GRS-BMI**: 941 BMI related variants identified from a meta-analysis of GWAS for height and BMI in ~ 700,000 individuals of European ancestry (Yengo et al., 2018)
2. **GRS-WHRadjBMI**: 49 loci associated with WHR adjusted for BMI (WHRadjBMI), identified in 224,459 individuals of the GIANT consortium (Shungin et al., 2015).

The scores were then weighted by variant-specific coefficients from the corresponding GIANT GWAS studies. The standardised GRS was created by using the formula: GRS × total number of the risk alleles/ (2× sum of weights). Each point of the rescaled GRS thus corresponded to, on average, one additional risk allele.

5.2.6 GWAS

In order to identify genetic variants that are linked to differences in weight loss at 1 year following bariatric surgery we undertook a GWAS in participants who underwent either primary LRYGB or LSG. The association of 1-year PWL with 3,327,675 SNPs was tested.

5.2.7 Statistical analysis

Statistical analyses were performed using the programs PLINK 1.9 (Chang et al., 2015), EMMAX (Kang et al., 2010), BOLT-LMM (Loh et al., 2015), RAREMETAL (Feng et al., 2014) and R software environment (R version 3.2.2). Autosomal SNPs were analysed by linear mixed models implemented in BOLT-LMM to account for cryptic population structure and relatedness within this group in our genetic association tests. X-chromosome SNPs were analysed using SNPTEST (Marchini et al., 2007). The PWL was transformed by adjusting for age, gender, sample batch, type of operation, bariatric centres, duration of follow-up, and first 6 principal components and obtaining the residuals and finally inverse-normalising to assure a normally distributed phenotype. Association analyses were performed in patients who underwent LSG, patients who underwent LRYGB and the overall patients.

Multiple linear regression analysis was performed to examine the association between the 2 GRSs with PWL after LRYGB and LSG, adjusting for age, gender, type of operation, bariatric centres, sample batch, duration of follow-up, and first 6 principal components. Pre-surgical BMI was further included as covariate as secondary analysis.

5.3 Results –GRSs

5.3.1 Baseline characteristics

Of the 1,789 samples that passed the genotyping quality control, 1,401 subjects passed the clinical exclusion criteria and were included in the GRSs analysis. The mean age, BW and BMI were 46.2 ± 10.9 years, 128.9 ± 25.9 kg, and 46.7 ± 7.7 kg/m^2, respectively. The majority of subjects (59%) were from the UCLH, 17% from Italy, 13% from Chichester, 7% from Portugal, and 4% from Imperial College London. Seventy-seven percent were female, 30% had T2D, and 65% underwent LRYGB. The mean GRS-BMI of the whole study population was 18.11 ± 0.4, and the mean GRS-WHRadjBMI was 1.15 ± 0.11 (Table 5.1).

After divided subjects by type of surgery, subjects undergoing LSG had significantly greater BMI than LRYGB (47.2 ± 8.2 kg/m^2 vs 46.4 ± 7.4 kg/m^2, P = 0.01, Table 5.1). In addition, the GRS-WHRadjBMI in LSG was statistically significantly higher than LRYGB (1.52 ± 0.12 vs 1.51 ± 0.13, P = 0.03, Table 5.1).

5.3.2 Weight loss after bariatric surgery

Figure 5.1 exhibits BW, BMI, PWL and weight change velocity (WCV) after LRYGB and LSG up to 3 years. Apparently, rapid weight loss occurred during the first six months (PWL: at 6 weeks = 10.3 ± 3.5%, at 3 months = 16.6 ± 4.8%, at 6 months = 24 ± 6.2%, at 1 year = 29.9 ± 9%, at 2 years = 30.5 ± 10.4%, and at 3 years = 28.4 ± 10.3%, Table 5.2, Figure 5.1C). At 2 years, the maximal PWL (31%) was achieved. After this point, at 3 years, patients started to regain weight, and the WCV increased from -0.02 ± 0.13 kg/week at 2 years to 0.04 ± 0.1 kg/week (Table 5.2, Figure 5.1C, D). However, the lowest BW and BMI were observed at 3 years (88.1 ± 23.3 kg and 31.8 ± 6.9 kg/m^2, respectively), significantly reduced from baseline (BW: 128.9 ± 25.9 kg, and BMI: 46.7 ± 7.7 kg/m^2, (Table 5.2, Figure 5.1A, B).

Table 5.1 Baseline characteristics of GRSs analysis

	Total (n = 1,401)	**LRYGB (n = 907)**	**LSG (n = 494)**	**P-value**
Age ± SD, years	46.2 ± 10.9	46.1 ± 10.7	46.3 ± 11.3	0.56
Weight ± SD, kg	128.9 ± 25.9	127.3 ± 24.5	131.9 ± 28.1	0.41
BMI ± SD, kg/m^2	46.7 ± 7.7	46.4 ± 7.4	47.2 ± 8.2	0.01
Sites				
UCH, %	58.7	42.6	88.4	<0.001
Chichester, %	12.8	19.7	0	<0.001
Imperial, %	4.3	4.2	4.5	<0.001
Portugal, %	6.8	10.6	0	<0.001
Italy, %	17.4	22.9	7.1	<0.001
Gender				
Female, %	77.2	80.9	69.2	<0.001
Male, %	22.8	19.1	30.8	<0.001
Diabetes				
No, %	70.8	70.7	70.9	0.95
Yes, %	29.2	29.3	29.1	0.95
Genetic risk score (GRS)				
GRS BMI 97 ± SD	2.37 ± 0.16	2.37 ± 0.16	2.37 ± 0.16	0.86
GRS BMI 941 ± SD	18.11 ± 0.4	18.1 ± 0.36	18.1 ± 0.37	0.05
GRS WHRadjBMI 49 ± SD	1.15 ± 0.11	1.15 ± 0.11	1.16 ± 0.11	0.11
GRS WHRadjBMI 66 ± SD	1.51 ± 0.13	1.51 ± 0.13	1.52 ± 0.12	0.03

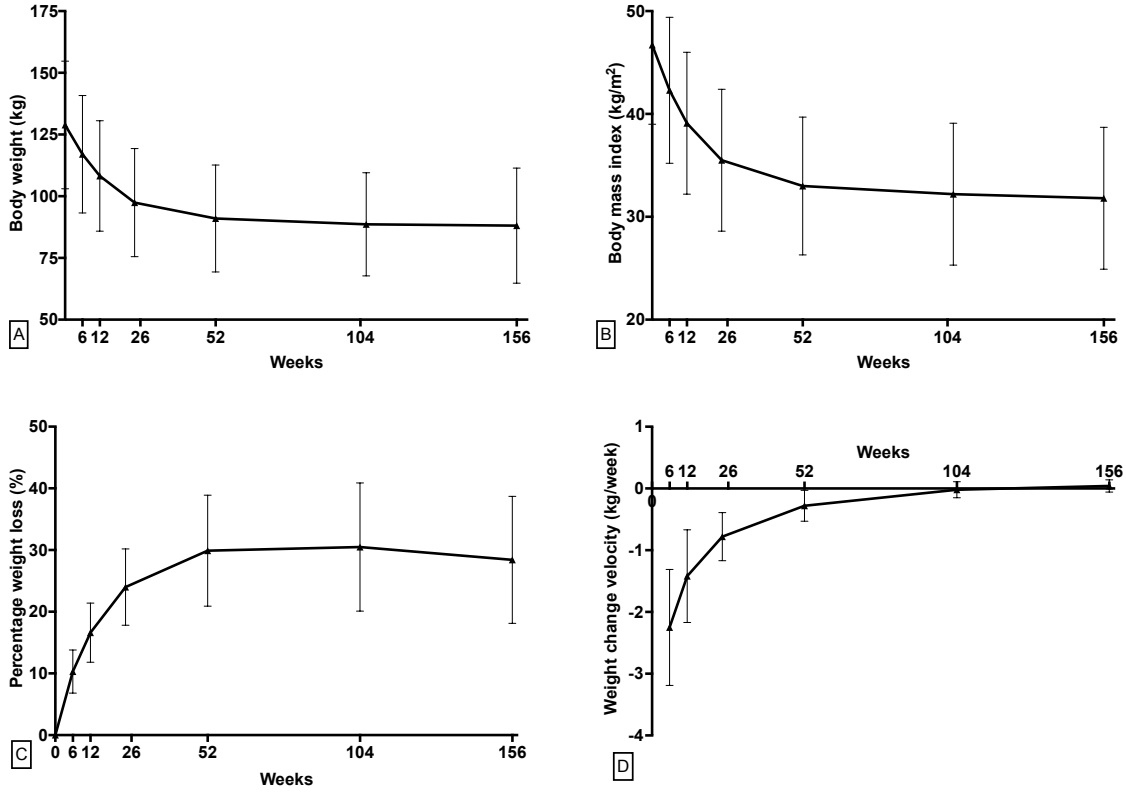


Figure 5.1 Weight loss after surgery. A, pre- and post-operative body weight (kg); B, body mass index (kg/m^2); C, percentage weight loss (%); D, weight change velocity (kg/week)

Table 5.2 BW, BMI, PWL, and WCV after surgery

Visits	Mean BW ± SD (kg)	Mean BMI ± SD (kg/m^2)	Mean PWL ± SD (%)	Mean WCV ± SD (kg/wk)	n
Baseline	128.9 ± 25.9	46.7 ± 7.7	0	0	1,401
6 weeks	117 ± 23.8	42.3 ± 7.1	10.3 ± 3.5	-2.25 ± 0.94	964
3 months	108.2 ± 22.4	39.1 ± 6.9	16.6 ± 4.8	-1.42 ± 0.75	820
6 months	97.4 ± 21.9	35.5 ± 6.9	24 ± 6.2	-0.78 ± 0.39	662
1 year	91 ± 21.7	33 ± 6.7	29.9 ± 9	-0.28 ± 0.25	821
2 years	88.6 ± 20.9	32.2 ± 6.9	30.5 ± 10.4	-0.02 ± 0.13	365
3 years	88.1 ± 23.3	31.8 ± 6.9	28.4 ± 10.3	0.04 ± 0.1	178

BW, body weight; BMI, body mass index; PWL, percentage weight loss; WCV, weight change velocity

5.3.3 Association between GRSs and baseline BMI

The association between GRS-BMI and baseline BMI was clearly evident with β-coefficient of 1.59, 95% confidence interval (CI) of 0.53 – 2.65, and P <0.01 (Table 5.3). The GRS-WHRadjBMI also significantly correlated with the baseline BMI but in the negative direction (β = -6.94, 95%CI [-10.41, -3.47], P <0.001, Table 5.3).

5.3.4 Association between GRSs and PWL after surgery

Following surgery, there was no association between GRS-BMI and PWL at 1, 2, and 3 years (β = -0.46, 95%CI [-2.01, 1.09], P = 0.56 for 1 year; β = -0.28, 95%CI [-3.06, 2.5], P = 0.85 for 2 years; and β = 0.03, 95%CI [-4.2, 4.26], P = 0.99, Table 5.3).

In contrast, the GRS-WHRadjBMI had a negative correlation with PWL at 2 and 3 years β = -10.97, 95%CI [-19.73, -2.21], P = 0.01 for 2 years; and β = -19.02, 95%CI [-32.5, -5.54], P = 0.01 for 3 years, Table 5.3).

We next stratified subjects by GRS-WHRadjBMI deciles. A distinct gradient with regards to weight loss was significantly observed at 3 years with the figures of the top decile significantly lower than the bottom decile (PWL: 24.2 ± 11.9% in the top decile vs. 33.6 ± 8.4% in the bottom decile, P = 0.007, Table 5.4, Figure 5.2). Subjects in the top decile experienced non-significantly greater weight regain than the bottom decile at 3 years (WCV: 0.08 [-0.01, 0.12] kg/week vs. 0.02 [-0.02, 0.06] kg/week, P = 0.13, Table 5.4). Nonetheless, the BW and BMI were not statistically significantly different between groups at 3 years (BW: 80 [68, 97.4] kg in the top decile vs. 78.5 [69.8, 92.6] kg in the bottom decile, P = 0.62; BMI: 29.7 [26.1, 31.6] kg/m^2 in the top decile vs. 30.9 [24.8, 35.1] kg/m^2 in the bottom decile, P = 0.81, Table 5.4).

Table 5.3 Association of GRSs with BMI at surgery and PWL after surgery

GRSs	Phenotype	β-coefficient	95% CI	P-value	n
BMI	BMI at surgery	1.59	0.53, 2.65	<0.01	1,401
	1-year PWL	-0.46	-2.01, 1.09	0.56	820
	2-year PWL	-0.28	-3.06, 2.5	0.85	364
	3-year PWL	0.03	-4.2, 4.26	0.99	177
WHRadjBMI	BMI at surgery	-6.94	-10.41, -3.47	<0.001	1,401
	1-year PWL	-3.11	-8.11, 1.89	0.22	820
	2-year PWL	-10.97	-19.73, -2.21	0.01	364
	3-year PWL	-19.02	-32.5, -5.54	0.01	177

GRSs, genetic risk scores; BMI, body mass index (kg/m2); WHRadjBMI, waist-hip-ratio adjusted for BMI; PWL, percentage weight loss (%); 95%, 95% confidence interval

Table 5.4 BW, BMI, PWL, and WCV of subjects stratified by top versus bottom deciles of GRS-WHRadjBMI

Visits	BW (kg)			BMI (kg/m²)			PWL (%)			WCV (kg/week)		
	$1^{th} – 10^{th}$ percentile	$91^{th} – 100^{th}$ percentile	P-value	$1^{th} – 10^{th}$ percentile	$91^{th} – 100^{th}$ percentile	P-value	$1^{th} – 10^{th}$ percentile	$91^{th} – 100^{th}$ percentile	P-value	$1^{th} – 10^{th}$ percentile	$91^{th} – 100^{th}$ percentile	P-value
Baseline	123.8	122	0.1	46.4	43.6	0.004	0	0	NA	0	0	NA
	(109.9, 140)	(105.2, 136.8)		(41.8, 51.7)	(39.7, 48.3)							
n	140	140		140	140							
1 year	87.4	86	0.94	32.4	31.5	0.64	29.7 ± 8.6	28.3 ± 9.6	0.29	-0.25	-0.17	0.38
	(74.2, 100.1)	(74.8, 101)		(28, 36.4)	(27.5, 36)					(-0.42, -0.09)	(-0.34, 0)	
n	90	83		90	83		90	83		57	31	
2 years	84	82.7	0.64	31.2	30.2	0.55	31.7 ± 10.1	28.1 ± 10	0.11	-0.01	0.02	0.71
	(69.7, 102.5)	(66.7, 97.6)		(27.4, 36.9)	(26.2, 34.1)					(-0.08, 0.05)	(-0.07, 0.08)	
n	49	37		49	37		49	37		38	33	
3 years	78.5	80	0.62	30.9	29.7	0.81	33.6 ± 8.4	24.2 ± 11.9	0.007	0.02	0.08	0.13
	(69.8, 92.6)	(68, 97.4)		(24.8, 35.1)	(26.1, 31.6)					(-0.02, 0.06)	(-0.01, 0.12)	
n	18	23		18	23		18	23		14	13	

BW, body weight; BMI, body mass index; PWL, percentage weight loss; WCV, weight change velocity

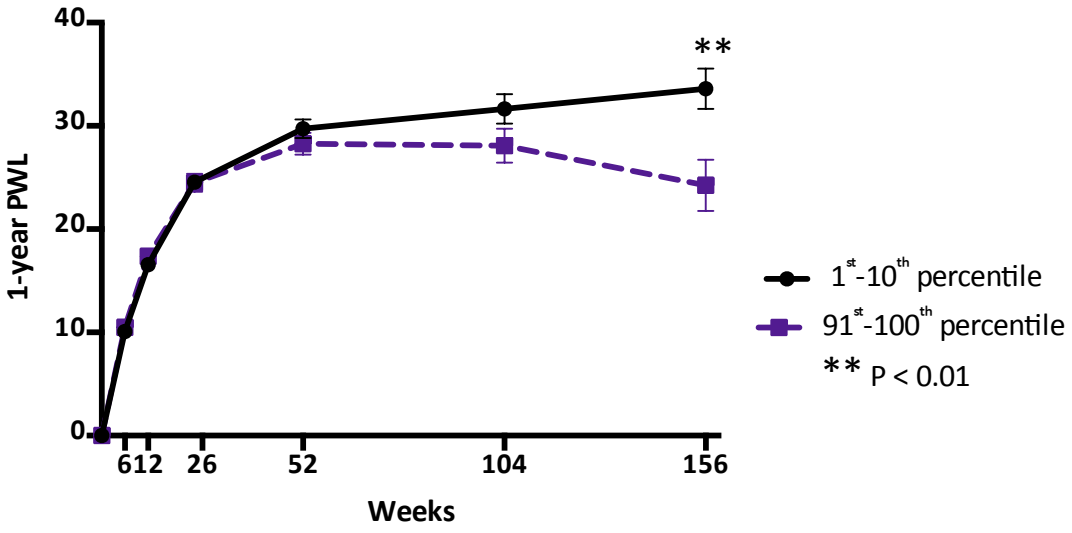


Figure 5.2 1-year PWL stratified by top versus bottom deciles of GRS of WHRadjBMI; PWL, percentage weight loss; GRS, genetic risk score; WHRadjBMI, waist-hip-ratio adjusted for BMI

5.4 Results –GWAS

5.4.1 Weight loss

Of the 1,789 samples that passed the genotyping quality control, 996 subjects passed the clinical exclusion criteria and were included in the GWAS. The mean of 1-year PWL of overall subjects was 29.9 ± 0.3% (Figure 5.3). It was 25.4 ± 0.5% in subjects undergoing LSG and 31.7 ± 0.3% in subjects with LRYGB.

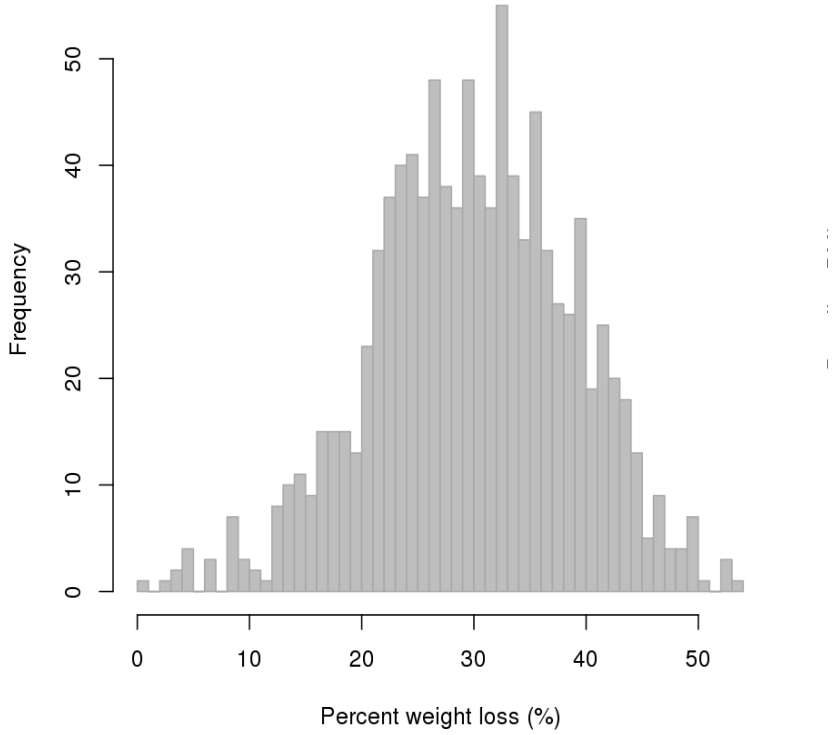


Figure 5.3 Distribution of percentage weight loss at 1 year after surgery

5.4.2 Genome-wide association study of 1-year PWL after surgery

In the GWAS of 1-year PWL, we tested the association of 1-year PWL with 3,327,675 genotyped and imputed SNPs that passed all QC criteria in 996 study samples with complete data. There was no genome-wide inflation due to population stratification as the genomic control factor was close to 1.0 (λ=0.99). SNPs in 35 independent loci showed significant association with 1-year PWL at P $<1\times10^{-4}$ (Table 5.5, Figure 5.4). Of these, variants in four loci (KLF3-rs1491199, MAMDC2-rs2975907, GSAP-rs740158, CASZ1-rs7555879) were significantly associated with the 1-year PWL at P$<5\times 10^{-6}$ (Figure 5.4 and Figure 5.5A-D). The minor allele frequency (MAF) of all of these variants are similar to those observed in European population from 1000 genomes project. BMI was further included as a covariate and the association remained largely unchanged.

Table 5.5 Associations with 1-year PWL of SNPs at P <1×10^{-4} in combined patients undergoing LSG or LRYGB

Nearest Gene	SNP ID	Chromosome	Base pair position	Minor Allele Frequency	E/O	β	SE	P value	R^2	Rank
KLF3-AS1/KLF3	rs1491199	4	38643014	0.18	G/A	0.28	0.06	6.2×10^{-7}	0.025	1
MAMDC2	rs2975907	9	72688474	0.25	G/A	-0.25	0.05	1.1×10^{-6}	0.024	2
GSAP	rs740158	7	77055836	0.50	T/C	0.21	0.05	3.3×10^{-6}	0.022	3
CASZ1	rs7555879	1	10890026	0.05	G/A	0.49	0.11	3.7×10^{-6}	0.021	4
XYLT1	rs8044934	16	17200759	0.01	G/A	-1.00	0.22	8.5×10^{-6}	0.020	5
STAB2	rs149841328	12	103999874	0.02	G/A	0.71	0.16	1.5×10^{-5}	0.019	6
MICB	rs16899682	6	31443699	0.02	G/C	0.63	0.15	2.3×10^{-5}	0.018	7
DBX2	rs7964973	12	45402893	0.49	T/C	-0.19	0.04	2.3×10^{-5}	0.018	8
LOC101930023	rs790454	12	92615593	0.33	T/C	0.20	0.05	2.4×10^{-5}	0.018	9
LOC105370615	rs77617103	14	89426006	0.10	C/T	0.32	0.07	2.6×10^{-5}	0.018	10
FLT3	rs8001973	13	28650549	0.21	G/T	0.24	0.06	2.6×10^{-5}	0.018	11
INADL	rs58529158	1	62447447	0.03	G/A	-0.59	0.14	2.8×10^{-5}	0.018	12
EPHA5	rs6830981	4	66573287	0.38	T/C	0.19	0.05	3.5×10^{-5}	0.017	13
SCNN1D	rs75809000	1	1223385	0.03	G/C	-0.60	0.14	3.6×10^{-5}	0.017	14
LOC100506422	rs1537292	9	26034314	0.10	C/T	0.31	0.07	3.8×10^{-5}	0.017	15
TTC7A	rs13404033	2	47173359	0.18	C/T	0.24	0.06	3.8×10^{-5}	0.017	16
TYRP1	rs2025556	9	12613216	0.07	A/C	-0.35	0.08	3.9×10^{-5}	0.017	17
MAPT	rs17651507	17	44059010	0.23	A/T	-0.22	0.05	3.9×10^{-5}	0.017	18
LOC101927182	rs114907297	20	40454157	0.04	C/T	0.47	0.11	4.2×10^{-5}	0.017	19
TRA	rs72671955	14	22286970	0.02	T/C	0.66	0.16	4.2×10^{-5}	0.017	20
NDUFA12	rs144250024	12	95399413	0.01	G/A	-0.94	0.23	4.4×10^{-5}	0.017	21
TUSC1	rs4294269	9	25652282	0.21	G/T	-0.22	0.05	5.0×10^{-5}	0.016	22
ADGRL2	rs2209696	1	82987838	0.45	C/T	-0.19	0.05	5.5×10^{-5}	0.016	23
GMPS	rs112029570	3	155646825	0.06	G/C	0.39	0.10	6.0×10^{-5}	0.016	24
GOLGA7	rs16890665	8	41372002	0.16	G/A	-0.24	0.06	6.1×10^{-5}	0.016	25
NCAM2	rs34907658	21	22256852	0.12	G/C	0.27	0.07	6.2×10^{-5}	0.016	26
SLC22A23	rs4959804	6	3317016	0.16	A/G	-0.25	0.06	6.8×10^{-5}	0.016	27
LOC107984303	rs7948696	11	6088112	0.38	T/C	0.18	0.04	7.1×10^{-5}	0.016	28
LOC105371108	rs533465048	16	18169830	0.03	G/C	0.53	0.13	7.1×10^{-5}	0.016	29
GRID2	rs17019814	4	93638479	0.14	A/G	-0.25	0.06	7.2×10^{-5}	0.016	30
CNTNAP2	rs12670868	7	146682211	0.30	A/G	0.19	0.05	7.9×10^{-5}	0.016	31
ITGA4	rs111655017	2	182212439	0.12	G/A	-0.27	0.07	8.2×10^{-5}	0.015	32
SGK223	rs12549872	8	8122710	0.23	T/C	0.20	0.05	8.9×10^{-5}	0.015	33
TENM2	rs2020170	5	165882543	0.42	T/C	0.18	0.05	9.0×10^{-5}	0.015	34
CDH17	rs2446815	8	95137229	0.18	A/C	-0.22	0.06	9.7×10^{-5}	0.015	35

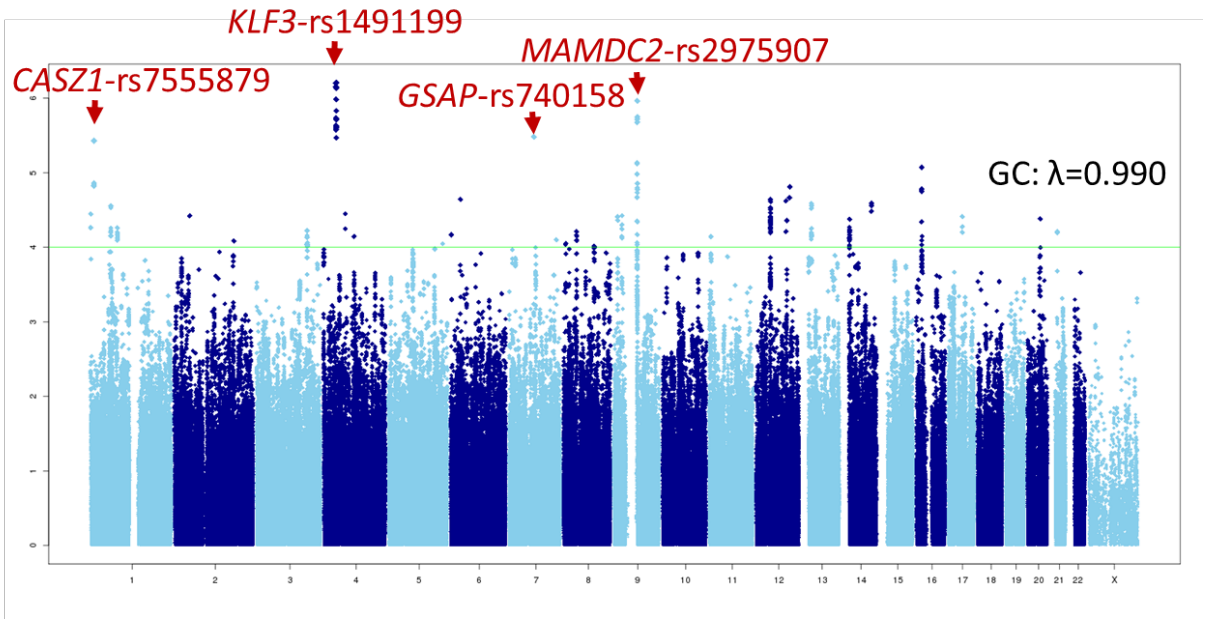


Figure 5.4 Manhattan plot for genome-wide association analysis of 3,327,675 SNPs in 996 overall bariatric surgery patients

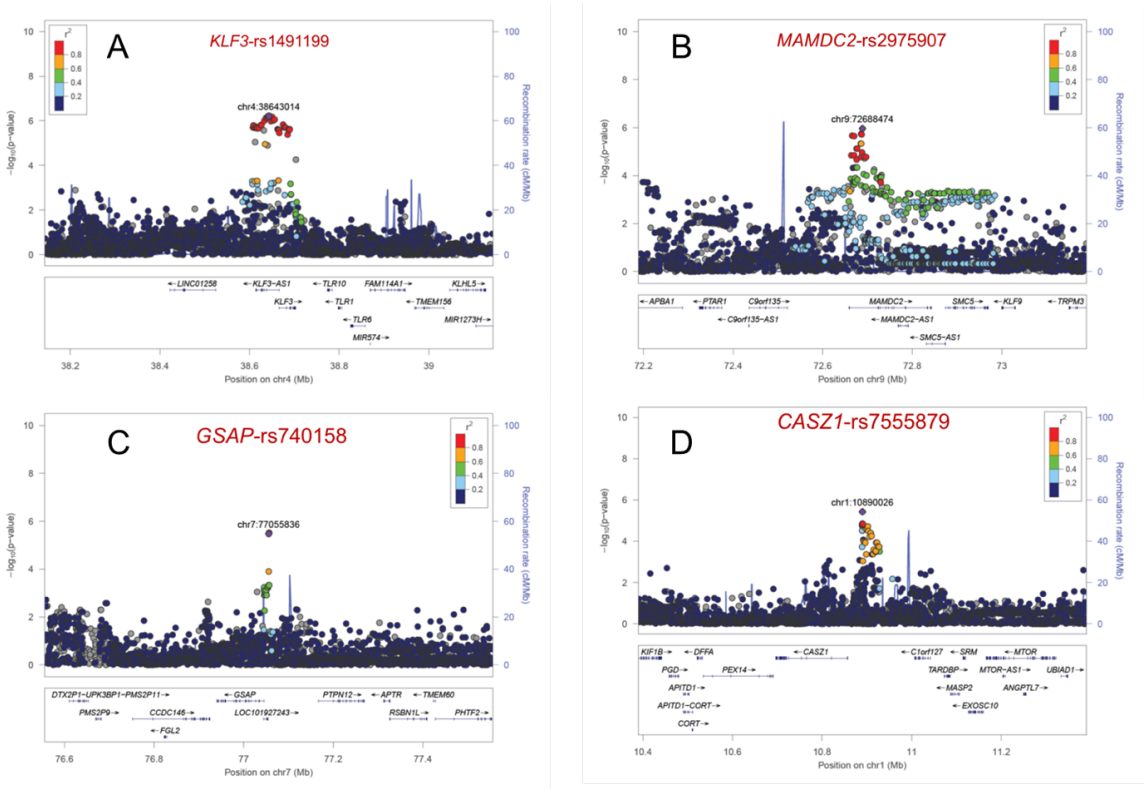


Figure 5.5 Regional plots of 4 loci showed significant association with percentage weight loss at $P < 5\times 10^{-6}$

5.4.3 Genome-wide association study of PWL stratified by type of procedure

Next we performed the association analyses stratified by procedural type (LSG and LRYGB) to identify procedure-specific signals contributing to post-operative weight loss. There were 32 and 50 independent loci significantly associated with PWL at $P<1\times 10^{-4}$ in patients underwent LSG and LRYGB respectively. The most significant signal identified in the overall study samples showed consistent evidence of associations in current subgroup analyses.

5.5 Discussion

In agreement with the previously published findings by Manning et al. (Manning et al., 2015b) and our study 1 in the Chapter 3, the 1-year post-surgery PWL in this genetics cohort also followed a wide and normal distribution, suggesting the important role of biology in determining the weight loss after surgery. Our investigation has highlighted the impact of genetic background on weight loss after bariatric surgery (LRYGB and LSG), using the GRS and GWAS approach. Our results also demonstrated the predictive potential of genetics on weight loss after bariatric surgery.

All SNPs (941 BMI-related SNPs and 49-WHRadjBMI related SNPs) used to construct the GRSs in the present study are the most update variants identified in the GIANT consortium, and the number of SNPs included in our GRSs are highest among the trials examining the association between GRSs of adiposity and weight loss after bariatric surgery (Bandstein et al., 2016, de Toro-Martin et al., 2018, Katsareli et al., 2020). Furthermore, the number of subjects in this trial is so far the largest among its kind of study. We opted to utilise BMI- and WHRadjBMI- associated variants to create GRSs since they are the most meaningful variants related to adiposity, discovered by GWAS (Winkler et al., 2018). The adjusted model (WHRadjBMI), a trait of interest (WHR) adjusted for other genetically correlated traits (BMI), aims at identifying genetic variants related to the trait of interest independently of the correlated traits and increased statistical power (Aschard et al., 2015).

At pre-surgery, the GRS-BMI showed a significant positive association with the baseline BMI; whilst the GRS-WHRadjBMI exhibited a negative association. Previous studies of the WHRadjBMI related SNPs using a genomic-scan approach has revealed that the majority of them had no effect on BMI or had a significant effect on BMI in the opposite direction. Only a few of them are positively associated with the BMI (Aschard et al., 2015, Winkler et al., 2018). They described different biology of these variants as the reason. The WHRadjBMI related SNPs have the opposite direction on BMI perhaps because they affect fat deposition in particular parts of the body; whereas the BMI increasing variants involve generalised fat. This could be the reason why we found the negative association between the GRS-WHRadjBMI and the baseline BMI.

After surgery, the association between GRS-BMI and PWL was not seen at any follow-up visits. Nevertheless, high GRS-WHRadjBMI exhibited a significant association with poor weight loss at 2 and 3 years. Interestingly, the magnitude of association representing by the β-coefficient increased over time, in spite of the decrease in number of subjects. This could be due to: first, the lower baseline BMI in subjects with higher GRS-WHRadjBMI, making the weight loss in the longer term significantly subtle; or second, the GRS-WHRadjBMI play a role in weight regain. Our findings have opened the door to more in-depth research about the impact of genetic predisposition on weight loss dynamics after bariatric surgery.

Similar previous study by Bandstein et al. demonstrated that increasing values of 2 GRSs composed of 7 BMI related SNPs and of 3 WHR related SNPs enhanced the risk of belonging to poor weight loss after bariatric surgery in 238 patients (Bandstein et al., 2016). De Toro-Martin et al. revealed that adding PRS of 186 BMI associated SNPs to a logistic prediction model including pre-op BMI, age, gender and surgical modality improves weight loss prediction in 865 patients before biliopancreatic diversion with duodenal switch (de Toro-Martin et al., 2018). In addition, Katsareli et al. showed a 4.6% decrease of percentage of excess weight loss (%EWL) per a score unit of GRS of 108 SNPs (95 BMI-related SNPs and 13 WHR-related SNPs) in 47 patients with morbid obesity (Katsareli et al., 2020).

However, there were substantial difference in sets of selected genetic variants, statistical approaches, definition of weight loss, and types of bariatric surgery. In this study, the PWL was used since it is less influenced by pre-operative BMI, thus is considered as a better metric for reporting weight loss after bariatric surgery (Corcelles et al., 2016). Furthermore, 2 types of bariatric surgery (LRYGB and LSG) were included in our analysis as they are currently the most common procedures operated worldwide.

In terms of GWAS, the present study revealed novel 35 loci significantly related to 1-year PWL after LSG and LRYGB, and of these, four SNPs including KLF3-rs1491199, MAMDC2-rs2975907, GSAP-rs740158, CASZ1-rs7555879 reached a statistical significance at $P < 5\times10^{-6}$. A previous study by Rinella et al. showed that SNPs in or near several genes including PKHD1, HTR1A, NMBR, and IGF1R were associated with 2-year %EWL after RYGB in 164 patients in the lower quartile of %EWL and 169 patients in the upper quartile of %EWL (Rinella et al., 2013). Another study in 693 individuals undergoing RYGB by Hatoum et al. undiscovered that a 15q26.1 locus near ST8SIA2 and SLCO3A1 was significantly associated with 10-month PWL after RYGB (Hatoum et al., 2013).

The discrepancy in outcomes could be due to the difference in the methodological approach such as procedural type, definition of post-operative weight loss and study population. Both previous studies studied in subjects after gastric bypass surgery whereas more than two-third of subjects in our study underwent LSG. In addition, we excluded subjects whose weight loss was confounded by other factors, enhancing the quality of our clinical data.

We subsequently reviewed literature regarding the function of the significant loci discovered by the GWAS in our study. KLF3-rs1491199 is the first and most significant variant. It is located in the intron region of KLF3-AS1 and upstream of KLF3 gene (Figure 5.5A). KLF3-AS1 encodes long non-coding RNA, KLF3 antisense RNA 1. KLF3 encodes Kruppel like factor 3, which is a member of the Krüppel-like factor (KLF) family of transcription factors and regulates adipogenesis. The KLF3 knockout mice are consistently leaner than their wild-type littermates, mainly due to the reduction in white adipose tissue (Pearson et al., 2011).

The second SNP, rs2975907, is located in the intron of MAMDC2 (Figure 5.5B), which encodes MAM domain containing 2. The defect of this gene causes Kabuki syndrome leading to skeletal abnormalities; short stature; heart defects; and intellectual disability (Kuniba et al., 2009).

The third, GSAP is the nearest gene around SNP rs740158 (Figure 5.5C). GSAP selectively increases amyloid-beta production through a mechanism involving its interaction with both gamma-secretase and its substrate, and it is thought to play a role in Alzheimer's disease (He et al., 2010).

Finally, the fourth SNP, rs7555879, is located in CASZ1 gene, the zinc finger transcription factor, playing a key role in cardiac development and postnatal adaptation. There is evidence demonstrating that the deletion of this gene causes dilated cardiomyopathy in mice and its mutation is also responsible for dilated cardiomyopathy in human (Qiu et al., 2017). It was also reported to be related to T-helper cell differentiation, inflammation and immunity (Bhaskaran et al., 2018).

The replication of the identified SNPs in an independent population is crucially required for the next step of this study. With regards to the diversity of function of the potentially related genes identified through GWAS in our study, studying the expression of these SNPs in gastric and intestinal tissues and their biological function needs to be performed in order to ensure and enlighten the mechanisms behind their association with weight loss after bariatric surgery.

This study has some limitations. First, the number of drop-out subjects was rather high, and this could lead to a selection bias. However, since all bariatric centres participating in this study are tertiary-care hospitals, their patients usually returned to their primary care settings after surgery. Furthermore, Spaniolas et al. has revealed that generally only 28% of patients after surgery completed 12-month assessment (Spaniolas et al., 2016). Hence, this seems to be an inevitable limitation and could be due to the nature of this kind of cohort. Second, the small sample size at post-operative visits, in particular at 2 and 3 years, probably brings about limited statistical power to detect the association between the GRS-BMI and the weight loss. Third, the WHR was not measured in this cohort.

However, this could be a room for further research to test the correlation between GRS-WHRadjBMI and its phenotypic trait as well as the weight loss after surgery. Lastly, although the surgical technique was advised to be standardised among the 5 bariatric centres in 3 countries, it was likely that sometimes this might not have been the case. However, all surgeons tried to minimise this as much as they could.

In conclusion, we have shown that genetic background has an impact on weight-loss success after bariatric surgery. This could lead to developing a novel tool to identify patients who are more likely to benefit from bariatric surgery and patients who need additional support after the surgery in order to maximise the benefit-to-risk ratio. In addition, our finding highlights the importance of the precision medicine.

Chapter 6

Final discussion and conclusion

Chapter 6 Final discussion and conclusion

The main objective of all work in this thesis was to gain insight into the factors associated with weight-loss and metabolic success following LRYGB and LSG, the current two most common bariatric procedures worldwide, using a well-established prospective cohort at the UCLH bariatric centre for weight management and metabolic surgery. Genetic background, body composition, pre- and post-operative clinical characteristics and gut hormones were extensively studied in subjects with a follow-up until 3 years.

Furthermore, we particularly focused on mechanisms mediating T2D remission after LRYGB and LSG by comparing weight-loss-matched patients who did and did not achieve normoglycaemia after 1 year post-surgery in the following factors: gut hormones, insulin sensitivity, β-cell function, genetics, body composition measured by MRI and BIA, and metabolomics. Finally, we performed the GWAS and GRS analysis to identify genetic variants and GRSs that were associated with weight-loss after surgery at the population-based level. All of our findings were thoroughly describes and discussed apropos of current literature on a chapter-to-chapter basis. In this chapter, we summarised the key findings of this work, suggested and discussed potential plans for further research.

6.1 Study 1 detailed in the Chapter 3 (The role of PYY and ghrelin in predicting weight loss after LRGB and LSG)

We reported some predictive factors for 1-year weight loss. Furthermore, the differential changes in PYY and ghrelin between T2D and non-T2D subjects, and the changes between patients with good and poor weight-loss outcomes at 1 year were described. In terms of gut hormones, we specifically pointed PYY and ghrelin out, since they are described as ones of the most influential hormones on weight loss. However, some previous studies showed inconsistent results in their weight-loss and metabolic benefits after bariatric surgery. Moreover, there is a room to develop new therapeutic agents for obesity and T2D based on PYY and ghrelin. The study comparing differential changes of these hormones in patients with T2D vs. patients without T2D and good weight loss vs. poor

weight loss thus gave additional information on to their potential therapeutic effects. Our results showed that:

- T2D related to poorer weight loss after LRYGB and LSG, whereas LRYGB led to greater weight reduction than LSG.
- PWL and WCV at 6 weeks were predictive for 1-year PWL after adjusting for age, gender, T2D, and type of surgery.
- The increase in $\Delta AUC_{0\text{-}180}$ PYY at 6-week post-operation from pre-surgery less than 16,000 pg x min/ mL was significantly associated with 7.7 times of being poor weight loss (<20% of PWL) at 1 year after adjusting for age, gender, T2D, and type of procedures. The $\Delta AUC_{0\text{-}180}$ PYY is an AUC of the increase in postprandial PYY levels from its fasting levels during a MMTT.
- There was no association of circulating AG and DAG levels with 1-year PWL observed in this study. The types of surgery (LRYGB and LSG) had a huge impact on post-operative differential changes of ghrelin.
- At pre-surgery, patients with T2D had significantly greater fasting PYY levels and $AUC_{0\text{-}180}$ PYY than patients without T2D, but $\Delta AUC_{0\text{-}180}$ PYY. At post-surgery, the fasting levels of PYY in T2D slightly dropped, and there was no significant difference between groups. The levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in T2D were significantly higher than non-T2D, suggesting the enhancement of nutrients-stimulated PYY secretion in T2D after bariatric surgery.

  In addition, the post-operative levels of $AUC_{0\text{-}180}$ PYY and $\Delta AUC_{0\text{-}180}$ PYY in T2D remitters were non-significantly higher than non-remitters.

  These findings thus indicate that the impairment of PYY sensitivity and secretion could be mechanisms contributing to T2D. Bariatric surgery perhaps improve these mechanisms, leading to weight loss and diabetes remission.
- In T2D subgroup analysis, we found that:
  - LRYGB would be a preferable option for patients with T2D as it provided more favourable BW outcomes, HbA_{1c} levels, and PYY profiles post-operatively.

- There was no statistically significant difference in weight loss, BW, and BMI between remitters and non-remitters, indicating that other factors than the weight loss play a key role in diabetes remission.
- A trend of higher post-operative levels of meal-stimulated PYY in remitters than non-remitters was observed, suggesting an advantage of PYY on glucose homeostasis.
- A trend of higher post-operative levels of fasting AG and $\Delta AUC_{0\text{-}180}$ AG in remitters than non-remitters was seen. A multivariable logistic regression showed that 6-week $\Delta AUC_{0\text{-}180}$ AG was associated with T2D remission at 1 year after LRYGB and LSG.

  These highlight the role of AG on the remission of T2D.

6.2 Study 2 detailed in Chapter 4 (Factors associated with type 2 diabetes remission after LRYGB and LSG)

In this study, we focused on mechanisms mediating the remission of T2D following bariatric surgery. We compared subjects with and without T2D remission at post-surgery. Since weight loss is generally accepted as a key underlying diabetes remission, the two groups of subjects were matched for PWL (at 20% where it is defined as a successful weight loss after surgery) as well as other demographic factors namely age, gender, type of surgery, BW, BMI, and duration of diabetes. Insulin sensitivity, β-cell function, visceral and ectopic fat measured by MRI and BIA, gut hormones, and metabolomics were compared between groups. The key findings in this study include:

- Subjects who returned to normoglycaemia had significantly greater β-cell function representing by HOMA-β and $AUC_{0\text{-}30}$ insulin than those who did not return to normoglycaemia.
- In terms of gut hormones, ghrelin parameters including fasting AG levels, $AUC_{0\text{-}180}$ AG, fasting AG:DAG and $AUC_{0\text{-}60}$ AG:DAG in remitters were significantly higher than non-remitters. $\Delta AUC_{0\text{-}60}$ FGF-19 in remitters was also significantly greater than in non-remitters.

- In subjects who achieved T2D remission possessed significantly less area of visceral fat and VAT:SAT ratio measured by MRI than subjects without remission.
- $AUC_{0\text{-}30}$ insulin, $AUC_{0\text{-}180}$ AG, fasting AG:DAG showed a significant association with T2D remission examined by a multivariable logistic regression analysis. Furthermore, HOMA-β, fasting FGF-19 levels, $\Delta AUC_{0\text{-}60}$ FGF-19, $AUC_{0\text{-}60}$ AG, and visceral fat area marginally related to the remission.

Our findings suggest that the improvement in β-cell function is the key factor determining diabetes remission. The greater levels of FGF-19 and AG in T2D remitters than non-remitters highlight the advantages of these hormones on the remission, potentially by enhancing the recovery of β-cell function. Our results also support the fundamentals that visceral adipose tissue contributes to cardio-metabolic diseases, in particular T2D.

Of note, in this study, we found that in T2D remitters the levels of AG were significantly greater than non-remitters, whilst in the Study 1, the levels of fasting AG and $AUC_{0\text{-}180}$ AG in remitters were non-significantly greater than non-remitters. This could be due to: first, the small sample size in the Study (only 5 in non-remitters vs. 23 in remitters); second, the different time of measurement (at ≤1-year post-surgery in the Study 1 vs. at 6-year post-surgery in the Study 2). Nevertheless, a trend of higher AG levels in remitters was evident in the Study 1.

- In terms of metabolomics study:
    - The most significant metabolites discriminating remitters vs. non-remitters analysed by sPLS-DA method include size of VLDL and LDL particles, TG content in small HDL, degree of fatty acids unsaturation, the levels of Isoleucine, Leucine, Valine, Alanine, Tyrosine, albumin, glucose, lactate, β-hydroxybutyrate, citrate, and glycoprotein acetyls.
    - The levels of total branched-chain amino acids (BCAAs) and each of them (isoleucine, leucine and valine) in non-remitters were significantly greater than remitters. The cut-off value of $AUC_{0\text{-}180}$ of total BCAAs at ≥91.5 mmol x min/L gives sensitivity of 91%, specificity of 85% for being T2D non-remitters.

- A significant association between visceral fat adipose tissue and total BCAAs was observed.
- The heatmap showed that as the T2D remission status progressed in participants, VLDL and LDL levels decreased, whereas HDL levels increased. These findings highlight diabetic dyslipoproteinaemia and the anti-inflammatory function of HDL.

The metabolomics analysis highlights the role of BCAA metabolism and its link with VAT, mitochondrial function (particularly TCA cycle and fatty-acid oxidation), and inflammation on the diabetes remission.

6.3 Study 3 detailed in Chapter 5 (The role of genetics in predicting weight loss after LRGB and LSG)

Lastly, we tried to identify a correlation between genetics and weight loss after LRYGB and LSG at the population-based level. Hence, the GWAS approach was performed to identify the predictive genetic variants, and GRSs related to anthropometric traits (BMI and WHR adjusted for BMI; WHRadjBMI) that were identified from the GIANT consortium were also tested. Our findings revealed that:

- GRS-BMI showed no association with PWL after surgery, even though it was highly associated with BMI at surgery.
- Increasing GRS-WHRadjBMI was significantly associated with low baseline BMI and poor weight loss after surgery, in particular at 2 and 3 years.
- Subjects in the top decile of GRS-WHRadjBMI experienced significantly lower PWL at 3 years than the bottom decile.
- Stratification of GRS-WHRadjBMI might offer a predictor of post-operative PWL.
- Four genetic loci identified by the GWAS approach (KLF3-rs1491199, MAMDC2-rs2975907, GSAP-rs740158, CASZ1-rs7555879) were significantly associated with the 1-year PWL at $P<5\times 10^{-6}$.

- There is evidence showing that the most significant variant, KLF3-rs1491199, regulates adipogenesis.
- The pathway linking between the other SNPs and the weight loss have not yet been elucidated.

Collectively, our results have highlighted the inter-individual variability in weight loss after bariatric surgery. Thus, identifying patients who would considerably benefit from the surgery and patients who would need post-operative additional support might be of value. This work has suggested that the weight loss and the increase in post-prandial levels of PYY at 6 weeks after LRYGB and LSG may potentially help us to identify patients requiring ongoing support after surgery in order to maximise the benefits from the surgery. Furthermore, our results have highlighted a crucial role of PYY in mediating weight loss and glycaemic improvement after surgery.

The evidence of genetic factors predicting weight loss after surgery reported in the present work would pave the way to select patients who would substantially benefit from the surgery. As a result, these findings could contribute to developing approaches to individualise treatment option based on individual patients' genetics and physiological responses, aiming to maximise beneficial outcomes, reduce surgical risk and minimise the prevalence of complications and suboptimal weight loss. Taken together, these findings may contribute toward improved beneficial outcomes from bariatric surgery and emphasise the importance of precision medicine.

In addition, as the question 'how to put T2D into remission?' is the top priority research question in the field of diabetes (Hopkins et al., 2020), our findings revealed factors apart from weight reduction that could contribute to T2D remission after bariatric surgery. We found that the improvement in β-cell function is the key underlying diabetes remission. The reduction in visceral fat and the increase in AG and FGF-19 circulating levels are also associated with the remission of T2D, potentially via enhancing the β-cell function. The detrimental effects of BCAAs on glucose homeostasis and its association with VAT were observed. In addition, the difference in characteristics of metabolites between groups

suggests that insulin resistance could be another reason of not achieving the diabetes remission.

6.4 Limitations

Overall, the sample size calculation was not performed in any studies in this thesis. The small sample size in the Study 2 and 3 potentially resulted in the under power to detect statistically significant findings. In the Study 1, MMTTs were conducted using a fixed calorie meal not an adjusted energy content for each individual. In addition, the interpretation of PYY and ghrelin levels should be cautious since antidiabetic agents could have an impact on their circulating levels.

In the Study 2, we tried to match T2D remitters and non-remitters with all clinical factors that could contribute to the remission of diabetes. There is still a difference in diabetes duration, glycaemic control and antidiabetic agents between groups, even though it did not reach statistical significance. Of note, the Study 2 is a cross-sectional study. Hence, it cannot prove a causal relationship between factors identified and the T2D remission.

6.5 Future research recommendations

Further research should focus on how the increase in PYY, AG and FGF-19, the reduction in VAT, the mitochondrial function, and circulating levels of BCAAs connect to the improvement in β-cell function, thus diabetes remission after bariatric surgery. Elucidating whether elevated BCAAs are indeed a cause or consequence of T2D is also of value to further investigate. A pharmacological approach targeting PYY, ghrelin and FGF-19 systems, and restoration of BCAA catabolic activity may be of worthy investigation as a potential T2D treatment for non-remitters post-bariatric surgery.

Furthermore, the idea of impaired PYY sensitivity and secretion contributing to T2D is interesting, and studies examining this could bring about additional knowledge on the pathophysiology of T2D. Research exploring the effects of PYY and GRS associated with WHRadjBMI on weight loss after LRYGB and LSG will provide additional insights into the pathophysiology of obesity and how to tackle it.

Study comparing the changes in circulating PYY and ghrelin between bariatric surgery and caloric restriction is needed to confirm the effects of bariatric surgery, in particular LRYGB and LSG, on the evolution of PYY and ghrelin. In addition, studies using a correction of test meal energy content for patient's metabolic rate should be further conducted.

Finally, studies confirming our findings that the GRS associated with WHRadjBMI, 6-week PWL, WCV and AUC PYY are predictors for weight-loss success and for identifying patients who need additional support after bariatric surgery are needed. Larger GWAS studies and studies examining the relationship between body composition using DEXA or MRI scan and weight loss after bariatric surgery are also required. All in all, our results leave a room for future research in order to develop novel agents tackling obesity and T2D, and to identify predictors for selecting patients who will benefit from the surgery and need additional support after the surgery.

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Supplementary appendices

Appendix 1 Participant information sheet for bariatric database and genotyping cohort

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Information Sheet for Research Participants

CONFIDENTIAL

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.

Study title: THE IMPACT OF GENOTYPE ON THE OUTCOME OF OBESITY TREATMENT.

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. 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.

Thank you for reading this.

What is the purpose of the study?

Obesity is one of the major health problems of the 21st century. Currently, worldwide more than 1.7 billion people are overweight and 310 million are obese. Weight gain occurs when energy intake (calories eaten) is greater than energy expended (calories used). Environmental and lifestyle factors are important risk factors for the development of obesity. In addition, the finding that being overweight tends to run in families suggests that changes in DNA make-up (genes) predispose some people to becoming overweight and obese.

Being overweight or obese is associated with several diseases such as type 2 diabetes, raised blood pressure and heart disease. Weight loss improves these diseases and results in people living for longer. Current methods of getting people to lose weight include diets, tablets and weight-loss (bariatric) surgery. However, patients respond differently to these treatments with some patients losing a large amount of weight and others losing less. This variability in weight loss may also be due to differences in genes. An understanding of how changes in genes affect weight loss will help us gain better knowledge of how body weight is regulated and help us develop new treatments for obesity.

We plan to look at the genetic make-up of patients attending the obesity and bariatric clinics at UCLH and to examine how variations in DNA affect weight loss achieved with diet, tablets or different types of bariatric operations.

Why have I been chosen?
All patients attending the bariatric/obesity services at the University College London Hospital will be invited to participate in the study. You should not take part in this study if you have been previously diagnosed with HIV, hepatitis B or hepatitis C.

Do I have to take part?
No, taking part is voluntary. 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 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 would happen if I decide to take part?
If you are interested in taking part in the study we will arrange to see you in person to ensure that you understand the purpose of the study and to answer any questions you may have. If you are happy to proceed we will ask you to sign a consent form. Any visits related to the study will be arranged to coincide with your normal outpatient appointments. We will collect information that is taken as part of your routine clinical care. We will need to take one additional blood sample or alternatively request you to provide a sample of your saliva so that we can analyse your DNA. We will ask you to fill in four questionnaires in order to assess your eating habits, mood, and quality of life. You will have the opportunity to ask any questions you may have with regard to these. We will also ask your permission to use your samples in future ethically approved research. You will be free to decide whether you wish your samples to be involved with these potential future studies or not.

We might also invite you to take part in additional studies that would involve you eating nothing from 8pm on the night before coming to the hospital. Some of these studies will involve you eating specific meals or drinking glucose, and blood taking. Before taking part in any of these future studies you will be sent detailed information as to the purpose of each of these studies and details of what these involve. You will be free to decide whether you want to be involved with these future studies or not.

What are the possible disadvantages and risks of taking part?
Blood taking can cause slight discomfort and very occasionally may cause localised bruising.
What are the possible benefits of taking part?
Whilst there are no immediate benefits for those participating in the study, it is hoped that a clearer understanding how changes in DNA affect weight loss will help us gain better knowledge of how body weight is regulated with implications on drug development and future anti-obesity treatments.

What if something goes wrong?
In the event of any adverse events occurring as a consequence of your participation in this study, you will be compensated through the University College London Hospitals NHS Trust insurance scheme.

Will my taking part in the study be kept confidential?

All the information you give us will be confidential and used for the purposes of this study only. The data will be collected and stored in accordance with the Data Protection Act 1998 and will be disposed of in a secure manner. The data will be used in a way that will not allow you to be identified individually.

What happens when the research study stops?
Once the study has finished, the results of the study can be made available to you.

What will happen to the results of the research study?
The results are likely to be published in the twelve 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 of the study can be made available to you should you wish.

Who has reviewed this study?
This study has been reviewed by the one of UCLH NHS Foundation Trusts Research Ethics Committees.

What do I do now?
Think about the information on this sheet and ask me if you are not sure about anything. If you agree to take part sign the consent form. The consent form will not be used to identify you. It will be filed separately from all other information.

Contact for further information
If you have any further questions about the study contact:
Dr Andrea Pucci, andrea.pucci@ucl.ac.uk
Dr Kusuma Chaiyasoot, kusuma.chaiyasoot.15@ucl.ac.uk

THANK YOU VERY MUCH FOR YOUR HELP!

Appendix 2 Consent form for bariatric database and genotyping cohort

Centre for Diabetes & Endocrinology
University College London
Rayne Institute
5, University Street
London WC1E 6JJ
☎ 0207 6790991
Fax 0207 6796583
Email:
a.pucci@ucl.ac.uk
kusuma.chaiyasoot.15@ucl.ac.uk

Centre Number:
Patient Identification Number for this study:

UCLH Project ID number: **09/H0715/65**
Form version: Version (6.2)

➢

➢ CONSENT FORM

Title of project: ***Evaluation the impact of carrier status of obesity-linked genetic variants on the outcome of medical weight loss and bariatric surgery.***

Name of Principal Investigator: Professor Rachel Batterham

Please initial box

1. I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I confirm that I have had sufficient time to consider whether or not want to be included in the study. ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
4. I understand that sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from the UCL research team or from regulatory authorities or from the NHS Trust -where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
5. I agree to take part in the above study. ☐

➢

6. I understand that my blood or saliva sample taken for DNA analysis is viewed as a gift and I give permission for the sample to be stored. ☐
7. Do you give permission for the sample to be used in future research linked to this project? Please circle yes or no as appropriate. [Yes] [No]

Continued on next page/

Centre Number: UCLH Project ID number: **09/H0715/65**
Patient Identification Number for this study: Form version: Version (6.2)

CONSENT FORM

Title of project: ***Evaluation the impact of carrier status of obesity-linked genetic variants on the outcome of medical weight loss and bariatric surgery***

Name of Principal investigator: **Professor Rachel Batterham**

Name of patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher (to be contacted if there are any problems)	Date	Signature

- **Comments or concerns during the study**

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.

1 form for Patient;
1 to be kept as part of the study documentation,
1 to be kept with hospital notes

Appendix 3 Participant information sheet for a mixed meal tolerance test

University College London Hospitals NHS
NHS Foundation Trust

Centre for Obesity Research
University College London
Rayne Institute
London WC1E 6JJ
☎ 02076790991
Fax 02076796583
Email:
r.batterham@ucl.ac.uk
kusuma.chaiyasoot@nhs.net
j.makaronidis@nhs.net

Information Sheet for Research Participants

CONFIDENTIAL

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.

Study title: THE IMPACT OF GENOTYPE ON THE OUTCOME OF OBESITY TREATMENT.

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like any more information.

What is the purpose of this study?

Obesity is one of the major health problems of the 21st century. In the UK currently ~ 66% of the adult population are overweight, defined as a body mass index (BMI: weight in kilograms divided by the square of height in meters) >24.9 kg/m^2. Overweight and obesity (BMI > 40 kg/m^2) results from chronic disruption of energy balance such that energy intake exceeds energy expended. Recent progress in our understanding of body weight regulation has identified a complex system whereby signals of nutrient status inform brain circuits which control subsequent feeding behaviour. However, we still have much to learn about body weight control mechanisms and we plan to undertake a series of studies in healthy normal weight volunteers to increase our understanding of body weight control.

Environmental and lifestyle factors such as diet and exercise affect body weight. However, twin, family and migration studies have demonstrated that there is also a

large genetic component. Recent studies looking at minor variations (polymorphisms) in individuals' DNA have identified new genes which may potentially regulate body weight. A clearer understanding of how these polymorphisms effect energy balance may help us to treat obesity in the future.

The reasons why weight-loss surgery is so effective are unclear. Research has shown that gut hormones regulate body weight by altering hunger, taste and how much energy the body uses. In normal-weight people eating causes a change in blood and saliva levels of these gut hormones which signal to the brain that food has been eaten. In overweight people gut hormone levels are changed and as a result the brain does not receive the appropriate signals after a meal. Our previous research has shown that weight-loss surgery alters appetite, taste and gut hormone levels and that these changes cause the subsequent weight loss. However, these changes in appetite, taste and gut hormones are highly variable and we plan to investigate whether this variability is due to genetic make-up. New research suggests that the amount of gut hormones present in saliva plays a key role in controlling the taste of food. However, we do not know whether weight loss alters the amount of gut hormones in saliva or whether these are altered by a person's genetic make-up. We plan to study the effects of genetic variation on appetite, taste, energy usage by the body and gut hormone levels in blood and saliva before and after weight-loss.

Why have I been chosen?

We are recruiting patients, aged 18-65 years, BMI>35 kg/m^2 who are attending our weight management clinics to lose weight either through dieting or who will undergo gastric bypass surgery or sleeve gastrectomy. You should not take part in this study if you have been previously diagnosed with HIV, hepatitis B or hepatitis C.

Do I have to take part?

No, taking part is entirely voluntary. It is up to you to decide if you would like to take part. If you did decide to take part, you will be given a copy of this information sheet and a copy of the signed consent form to keep. A decision to take part is not binding, and you are free to withdraw from the study at any time without the need to give a reason for withdrawing your consent.

What would happen if I decide to take part?

a) ***Obtaining your consent and arranging your study visits***

If you are interested in participating we will arrange to see you in person to ensure that you understand the purpose of the study and to answer any questions that you have. If you are happy to proceed we will ask you to sign a consent form. We will then agree on dates convenient for you to undertake the meal studies. The first of these

dates should be within the two weeks prior to starting your weight loss program or your planned operation, the second date after 6 weeks.

b) Meal study days

The blood levels of gut hormones are affected by diet, so we will ask you to eat nothing after 8 pm the night before each visit and to drink only water. For 24 hours before each study day you will be asked to refrain from taking strenuous exercise and drinking alcohol. Your meal study out-patient appointments will be booked for 9 am and each visit including your follow-up assessment will take approximately 3 hours.

When you arrive a plastic cannula will be placed into a vein on one arm and this will be used to collect blood samples throughout the study. You may feel a little discomfort when the cannula is inserted. You will then be asked to relax for approximately 30 minutes. A baseline blood sample will then be taken from the cannula. Blood will be taken from your cannula throughout the study. The amount of blood taken for each study blood sample is approximately equivalent to two teaspoonfuls. The total amount of blood taken during all both visits is less than the amount taken when giving blood. At each occasion when blood is taken we will ask you to complete a questionnaire asking you how hungry you feel, we will explain how to complete this form prior to the study.

You will then be asked to consume a liquid replacement meal within a 15-minute period. You will need to complete the whole drink so that we can ensure that the calories you consume on each day are the same. Following this drink you will need to stay for 3 hours so that we can continue to take blood samples. During the study we will ask you to spit (a quarter of a teaspoon each time) into a collection tube so that we can collect and then measure gut hormone levels in your saliva. We will also ask your permission to use your samples in future ethically approved research. You will be free to decide whether you wish your samples to be involved with these potential future studies or not.

We will ask if you would be willing to have additional meal studies undertaken in the future to allow us to look at the longer-term effects of dieting or surgery on your appetite and gut hormones. These studies would be undertaken on a maximum of three occasions over a 2-year period. These studies would coincide with your normal follow up appointments.

What are the risks and disadvantages in taking part?

The placement of the cannula in your vein can be uncomfortable for a few seconds and very occasionally may cause a slight bruise.

What are the benefits of taking part?

There is no direct benefit to you from the experiment. However, it is hoped that information gained from these studies will increase our understanding of body weight regulation and help with the development of new treatments for obesity.

What if something goes wrong?

In the event of any adverse events occurring as a consequence of your participation in this study, you will be compensated through the University College London Hospitals NHS Trust insurance scheme.

Will my information be kept confidential?

All the information you provide us with will be kept strictly confidential and used only for the purposes of this study. The data will be collected and stored in accordance with the data protection act 1998. The data will be disposed of in a secure manner. The results from the study will be anonymised prior to publication.

What happens when the study finishes?

We would be happy to make the results of any studies available to you, at your request. The results from this study are likely to be published in a peer reviewed scientific journal.

Who has reviewed this study?

This study was originally reviewed by the one of UCLH NHS Foundation Trusts Research Ethics Committees in 2009. The Harrow Research Ethics Committee reviewed amendments made to this study in 2015.

What do I do now?

Think about the information on this sheet and ask one of us if there is anything you are unsure about. If you agree to take part, sign the consent form. The consent form will not be used to identify you. It will be filed separately from all other information.

Contact for further information

If you have any further questions about the study, please contact:

Professor Rachel Batterham, r.batterham@ucl.ac.uk
Dr Kusuma Chaiyasoot, kusuma.chaiyasoot@nhs.net
Dr Janine Makaronidis, j.makaronidis@nhs.net

THANK YOU VERY MUCH FOR YOUR HELP!

Appendix 4 Participant information sheet for MRI study

University College London Hospitals NHS
NHS Foundation Trust

Centre for Medical Imaging,
2nd Floor Charles Bell House,
43-45 Foley Street,
London W1W 7TS
☎ 02076798156

Email: n.sakai@ucl.ac.uk
margaret.hall-craggs@nhs.net
stuart.taylor1@nhs.net

Patient information sheet (Cohort 2)

Title of project: **Magnetic resonance imaging in obesity**

Name of Chief Investigator: **Professor Stuart Taylor**

R&D reference number: **17/0703**

Introduction

- We are asking if you will take part in this research study which is contributing to a PhD.
- We want you to understand what the research involves and why it is being done.
- Please read through this information sheet carefully and discuss it with others if you wish.
- Take as much time as you need to consider taking part.

What is the purpose of this research study?

- Obesity is associated with an increased risk of other health problems including type 2 diabetes, liver disease and osteoarthritis. After weight loss (through diet, medications or surgery), patients can see improvements in or recover from these health problems.

- We want to learn more about how obesity causes these health problems and how and why they improve after weight loss.

- Magnetic resonance (MRI) scans can be used to measure fat in the body. These are safe scans which do not use any harmful radiation.

- We want to measure the fat content of individual organs such as bone and the liver, using MRI. This will help us understand more about how fat affects their function and how things improve with treatment. In the future, we hope this will improve treatment of obesity.

- We would also like to see if there is a link between the fat content of muscles and their function and strength by performing muscle tests.

Why have I been chosen?

- You have been chosen for this study because you have been referred to the weight management services at UCLH.

Do I have to take part?

- No - it is up to you whether you take part or not.
- A member of your clinical care team will give you this information sheet and a researcher will then speak to you about the study.
- You can change your mind at any time during the research without giving a reason.

What will happen if I take part?

- A researcher will go through the consent form with you. You will have the opportunity to ask questions. You will be asked to read and sign the consent form.
- The research doctor will ask you questions and record this information for the study.
- Your name, date of birth and contact details will be recorded on a list by the research doctor.
- You will be asked to up to 4 MRI scans, lasting about 40 minutes each. Some people may have had an MRI scan before. The scan we are doing will seem like a normal scan, but we can make some special measurements from it.

- You will complete an MRI safety checklist and have the opportunity to ask questions about the scan.

- You will have up to 4 MRI scans over a period of up to 5 years.

- Alongside the MRI scans, we would like to test your muscle strength and function by carrying out tests on your muscles. ***This is optional*** in addition to the MRI scans.

- If we discover a result that the research doctor thinks may be important for your health, we will tell you about it. With your permission, we will tell your GP and the other doctors looking after you.

- Unless there is a problem, we will not inform your GP about the study.

- With your permission, we will compare the MRI result with clinical information your doctors already routinely collect from you. This may include height and weight measurements, the results of blood tests, medications you take, results of biopsies and other scans, other similar clinical tests and details of any treatment you are given. You do not have to undergo additional tests other than the MRI scan (and optional muscle tests) if you agree to take part in our study. We can obtain all the data we require from your hospital notes and computerised patient records. We would like to collect the information for a maximum of 10 years after you join the study.

- If you are also taking part in other research studies, with your permission we will ask the organisers of these studies if they will share any information or results. We are particularly interested in any biopsies or tissue samples you have had taken and the results of any genetic testing you consent to as part of other studies. We will tell you if we use any data collected as part of other studies. By sharing information across different studies, we hope to improve the usefulness of our research.

- With your permission, we would like to share anonymized data from your MRI scans with UCL scientists working in computer engineering to help us develop new ways to interpret the scans. Nobody will be able to identify you and we will not share your personal details.

- We cannot provide information on your individual results, but will provide details of the results of this research if you ask for it.

- With your permission, we will securely store your contact details indefinitely following the completion of this study so that you can be contacted to be given feedback from research studies that may be useful for your clinical care.

- With your permission, we may approach you about participating in future, related studies.

Will any samples or tissue be used in this research?

- No – we are only asking you to undergo MRI scans and optional muscle testing. However, as noted above, we may compare the MRI/muscle test results with blood tests and biopsies (or other samples) which you have had taken as part of your routine clinical care or other research studies you have agreed to take part in.

What will I be asked to do?

The study will involve:

- Your permission for the MRI scans – we will discuss this with you and ask you to sign a form.

- The scans themselves.

- The first scan will be performed around the time you agree to take part in the study. We will then ask you to undergo up to 3 further scans about 6 - 12 months apart. In total, we are asking you to have up to 4 scans. We will try to time the scans so that they take place on the same day as other hospital appointments that you may be attending.

- ***Optional*** *muscle strength testing. This would involve a series of manual tests on your muscles carried out by research physiotherapists within 2 weeks of each of the MRI scans.*

Are there any disadvantages in taking part?

- The MRI scan can sometimes be uncomfortable and quite noisy, and you will need to stay still in the scanner. You will be able to talk to the radiographers running the scan at all times via a microphone and you can press a button to stop the scan at any time. We do not give you any injections as part of the scan.

- The magnets used in the MRI scanners are very strong and some people cannot be scanned safely, for example people with heart pacemakers. Our radiographers are highly skilled and will check that it is safe for you to go into the scanner.

- The MRI scan will mainly look at the liver, pancreas, bone and muscles. However, we will also obtain imaging information about the other organs in your abdomen such as bowel, kidneys etc. We may therefore detect an incidental abnormality in one of these organs which may need further investigation to clarify its nature. The vast majority of such findings are incidental and of little importance. If we do identify an incidental abnormality, we will inform your doctor of the finding via the clinical report we provide for the MRI scan. Scan reports will be provided to your team and will be available to your GP - you will not need to contact your GP separately for any scan results. If you have private medical insurance you are advised to check with the company before agreeing to take part in the study.

Are there any advantages in taking part?

- This research may help you and others in the future but will not benefit you in the short term.

- We hope to understand your disease better and this will help us to improve treatment of obesity.

- Taking part in this study will not affect any treatment you are receiving at this or a different hospital.

What will happen to the results of the study?

- Everything we discover will be published in medical journals for everyone to see and for other researchers to learn from. Results may not be available for several years.

- Nobody will be able to identify you from anything we publish. No personal information will be given to anyone who is not directly working on this research.

What if relevant new information becomes available?

- If this happens then one of the doctors running the study will talk to you about it.

What will happen if I don't want to carry on with the study?

- You can withdraw from the study at any time without giving a reason.
- We will need to use the data collected up to your withdrawal.

Who is organising and funding the research?

- This research is being organised by Professor Stuart Taylor, Professor Margaret Hall-Craggs, Professor Rachel Batterham and Dr Naomi Sakai.
- It is run within the Department of Medical Imaging, University College London Hospital NHS Trust, together with the Centre for Obesity Research at University College London.
- This research is funded by the Biomedical Research Council and sponsored by University College London.
- No one, including your doctor, receives any payment for being involved in this study.

Who has reviewed the study?

- The study has been reviewed by the South Central – Hampshire A Research Ethics Committee (18/SC/0177).

What will happen to the information collected about me?

- All information collected from you including the medical images will be strictly confidential and pseudoanonymised.
- Forms will be kept in the Centre for Medical Imaging office. All forms will be stored in a locked filing cabinet and the office will be locked when not attended.
- All information relating to the study kept on computers will be in encrypted files. This information will be kept confidential and only available to researchers directly involved in this study.
- Any information which leaves the hospital in either electronic form or hard copy will have all identifiable information removed such as name and address so that you cannot be recognised from it.

What if I have a problem or would like further information about the study?

- If you have any concern about any aspect of the study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact us by email.

- It is very unlikely that participants in the study will be harmed by having an MRI scan. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. There are no special compensation arrangements for this study. You will be given a copy of this information sheet to keep.

- If you wish to complain or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. You can contact the Patient Advice and Liaison Service at UCLH on 020 3447 3042. Please ask your research doctor if you require further information.

- In the unlikely event that you are harmed by taking part in this study, compensation may be available. If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your research doctor, please make the claim in writing to Professor Stuart Taylor, who is the Chief Investigator for the research and is based at the Centre for Medical Imaging. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Thank you for reading this information sheet. If there is anything you are unsure about, please ask.

Dr Naomi Sakai/Professor Stuart Taylor/Professor Margaret Hall-Craggs

Appendix 5 MRI safety questionnaire for patients

University College London Hospitals NHS
NHS Foundation Trust

MRI Safety Questionnaire for Patients (*Complete for every visit*)

Please ensure you remove all the following items before entering the scan room: transdermal patches, external prostheses, belt, keys, coins, bank cards, watch, earrings and other jewellery, rail tickets, hair clips and any loose metallic objects.

Name		**Date of Birth**	
Hospital Number		**Weight (kg)**	
First line of address			

	Circle
1. Have you ever had a cardiac **pacemaker**, implantable cardioverter defibrillator (ICD) or REVEAL devices?	Yes* / No
2. Have you ever had any cardiac surgery or operations to your heart (including **cardiac stents**)?	Yes* / No
3. Have you ever had surgery to your head, neck, spine, eyes or ears?	Yes* / No
4. Have you ever had any metal fragments in your eyes?	Yes* / No
5. Have you ever had any metal fragments in any other part of your body (*e.g. shrapnel, bullets, debris etc)*?	Yes* / No
6. Have you ever had any operations involving the use of metal implants (not dental) or devices, plates, clips, IVC filters, stents or coils?	Yes* / No
7. Have you ever had any type of electronic, mechanical or magnetic implant (*e.g. neurostimulator, shunt, cochlear implant, drug infusion pumps etc)*?	Yes* / No
8. Have you had any surgery to any part of your body in the past 3 months?	Yes* / No
9. Have you ever had renal (*kidney*) disease, renal failure or a renal transplant?	Yes* / No
(For women of child bearing age) 10. Is there any possibility that you are pregnant?	Yes* / No
11. Are you breastfeeding?	Yes* / No

***Important Note:** If you have answered **YES** to any of the above questions please contact the Imaging Department to avoid a wasted journey. If you are not fluent in English please bring an interpreter or contact the Imaging Department to have one arranged.

12. Do you wear dentures, a dental plate or a brace?	Yes / No
13. Do you have epilepsy or have you had a seizure in the past?	Yes / No
14. Do you wear coloured contact lens or hearing aid?	Yes / No

I have read understood and answered all the relevant questions above and am happy to undergo an MRI scan. I understand I may also receive a contrast injection as a routine part of this examination.

Signature (*Patient, Parent or Guardian*)..... **Date** .....

MRI Radiographer/authorised member of staff: I confirm that I have supervised the completion of this safety checklist and based on the information provided I am satisfied that it is safe to proceed with the MRI examination. I have also confirmed the patients ID in line with departmental policy.

Patient Allergies: Y / N .....

☐Tick if safe for Gadolinium GFR (if known):..... Date:.....

Glaucoma? Y / N Myasthenia Gravis? Y / N Heart Problem? Y / N .....

☐ Tick if safe for Buscopan

Radiographer Signature ..... **Date** .....
Radiographer name (PRINT).....

Appendix 6 Weight loss outcomes, glycaemic indices, PYY, AG and DAG profiles after bariatric surgery, divided patients by type of surgery

	LRYGB (n = 27)					LSG (n = 58)					P-value
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	between groups
BW, kg	123.1 ± 23.1	109.1 ± 20	92.4 (81, 111.2)	85.8 (74, 103)	<0.001	126.7 ± 22.3	112.6 ± 20.5	92 (85.3, 108.1)	89.5 (80.8, 105.8)	<0.001	0.17
n	27	26	27	27		57	57	53	56		
BMI, kg/m²	45.5 (42.3, 50.7)	41.2 ± 5.5	35.2 (32.5, 39.5)	32.6 (29.8, 35.3)	<0.001	44.4 (40.6, 50.4)	40.5 ± 6.3	34.3 (30, 36.9)	32.3 (30, 38.5)	<0.001	0.05
n	27	26	27	27		57	57	53	56		
PWL, %	0	8.6 (7.1, 9.7)	21.2 ± 5.9	27.4 ± 7.1	<0.001	0	9 (7.2, 11.6)	21.5 ± 5.4	24.5 ± 7.5	<0.001	0.04
n	27	26	27	27		58	57	53	56		
WCV, kg/week	0	-1.83 (-2.35, -1.53)	-0.63 (-0.92, -0.49)	-0.27 (-0.37, -0.15)	<0.001	0	-2.2 (-2.85, -1.82)	-0.71 (-0.81, -0.5)	-0.11 (-0.28, -0.02)	<0.001	0.02
n	27	26	26	27		58	57	53	53		
HbA_{1c}, mmol/mol	38.3 (35.5, 44.8)	36 ± 5.7	35.1 ± 4.5	34.5 (33, 36.8)	<0.001	39.9 (35.5, 46.7)	36.7 ± 5.6	36.3 ± 4.9	35 (33, 39)	<0.001	0.97
n	24	17	22	24		58	45	51	55		
Fasting PYY level, pg/mL	63 (36, 89)	74 (52, 113)	81 (59, 143)	86 (52, 112)	0.07	59.5 (43.5, 83.5)	59 (41, 77)	64 (51, 81)	47 (35, 74)	0.12	0.04
n	27	25	24	21		56	50	48	48		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	24,653 (19,993, 39,117)	52,035 (36,049, 68,974)	56,511 (31,913, 64,825)	42,742 (24,777, 0,695)	<0.001	24,209 (19,154, 29,048)	31,954 (25,047, 40,701)	28,633 (20,332, 39,530)	22,297 (18,133, 29,915)	<0.001	<0.001
n	27	25	24	21		55	49	48	47		
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	16,923 ± 11,138	31,788 (24,445, 53,241)	33,873 (18,116, 47,850)	26,625 (16,061, 41,206)	<0.001	13,753 ± 7,421	20,842 (13,445, 29,183)	14,886 (8,209, 28,127)	14,408 (5,635, 21,511)	<0.001	<0.001
n	27	25	24	21		55	49	48	47		

Fasting AG,	8	5.3	6.8	9.6	0.02	7.6	3.1	3.2	3.5	<0.001	<0.001
fmol/mL	(5.2, 12.1)	(3.4, 9.1)	(5.3, 10)	(7, 13.8)		(4.8, 10.4)	(1.6, 4.9)	(1.9, 5.7)	(2.3, 5.9)		
n	26	25	23	22		55	50	44	49		
$AUC_{0\text{-}180}$ AG,	1,142	839	1,045	1,255	0.01	945	544	608	707	<0.001	<0.001
fmol x min/mL	(636, 1,542)	(603, 1,546)	(809, 1,515)	(928, 1,890)		(689, 1,498)	(373, 940)	(410, ,027)	(587, 989)		
n	26	25	23	22		53	49	44	49		
$\Delta AUC_{0\text{-}180}$ AG,	-328	-173	-320	-451	0.72	-386	32	-22	56	<0.001	0.05
fmol x min/mL	(-907, -63)	(-419, 67)	(-451, -44)	(-591, -156)		(-722, -93)	(-229, 211)	(-411, 265)	(-207, 223)		
n	26	25	23	22		53	49	44	49		
Fasting DAG,	108 ± 48	93 ± 41	96	94	0.04	98 ± 38	46 ± 8	48	38	<0.001	<0.001
fmol/mL			(72, 145)	(68, 144)				(34, 64)	(28, 48)		
n	26	25	23	22		56	50	47	49		
$AUC_{0\text{-}180}$ DAG,	12,312 ± 5,206	10,888	12,094	14,215	0.02	13,159 ± 5,718	6,318	7,769	6,139	<0.001	<0.001
fmol x min/mL		(7,567, 17,162)	(9,753, 16,737)	(10,386, 19,028)			(4,364, 8,487)	(4,570, 9,401)	(4,925, 7,831)		
n	26	25	23	22		54	49	47	48		
$\Delta AUC_{0\text{-}180}$ DAG,	-6,312	-4,342	-4,532	-4,218 ± 3,597	<0.01	-4,490	-1,542	-1,958	-550 ± 1,479	<0.001	0.02
fmol x min/mL	(-9,596, -2,740)	(-5,895, -2,112)	(-8,912, -3,107)			(-6,530, -2,457)	(-3,203, -329)	(-2,897, -22)			
n	26	25	23	22		54	49	47	48		
Fasting AG:DAG	0.09	0.075	0.079	0.105	0.49	0.076	0.07	0.068	0.091	0.48	0.86
	(0.05, 0.117)	(0.053, 0.088)	(0.052, 0.098)	(0.085, 0.128)		(0.057, 0.115)	(0.033, 0.133)	(0.039, 0.117)	(0.056, 0.138)		
n	26	25	23	22		55	50	44	49		
$AUC_{0\text{-}180}$ AG:DAG	0.092	0.085	0.084	0.09	0.43	0.086	0.091	0.089	0.112	0.002	0.03
	(0.064, 0.123)	(0.062, 0.117)	(0.068, 0.109)	(0.079, 0.113)		(0.06, 0.108)	(0.053, 0.146)	(0.053, 0.146)	(0.094, 0.144)		
n	26	25	23	22		53	49	44	48		
$\Delta AUC_{0\text{-}180}$	0.05	0.039	0.063	0.125	0.36	0.063	-0.029	0.009	0.064	0.47	0.76
AG:DAG	(0.025, 0.136)	(-0.055, 0.098)	(0.01, 0.107)	(-0.022, 0.209)		(0.02, 0.149)	(-0.185, 0.078)	(-0.122, 0.156)	(-0.196, 0.209)		
n	26	25	23	22		53	49	44	48		

Appendix 7 Weight loss outcomes, glycaemic indices, PYY, AG and DAG profiles after bariatric surgery, divided patients by T2D status

	Patients without T2D (n = 57)					Patients with T2D (n = 28)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
BW, kg	122.4 (110.1, 142)	109.3 (98.5, 124.8)	96.1 ± 17.9	90.7 ± 16.8	<0.001	123.9 (106.5, 140.8)	109.2 (93.6, 122.6)	97.1 ± 21.3	93.9 ± 21.4	<0.001	0.001
n	56	56	53	55		28	27	27	28		
BMI, kg/m²	43.7 (41.1, 50.1)	39.3 (35.9, 46.2)	33.9 (30, 38.4)	31.7 (29.4, 35.5)	<0.001	45.8 (41, 51)	40.6 (36.7, 45)	35.7 (32.5, 38.6)	33.7 (30.5, 38.9)	<0.001	0.02
n	56	56	53	55		28	27	27	28		
PWL, %	0	8.9 (7.1, 11.3)	22.3 ± 5.6	26.9 ± 7.6	<0.001	0	9 (7.1, 10.2)	19.6 ± 5	22.6 ± 6.6	<0.001	0.002
n	57	56	53	55		28	27	27	28		
WCV, kg/week	0	-2.17 (-2.63, -1.73)	-0.73 (-0.92, -0.6)	-0.2 (-0.35, -0.03)	<0.001	0	-2.2 (-2.7, -1.68)	-0.5 (-0.69, -0.44)	-0.17 (-0.23, -0.08)	<0.001	0.53
n	57	56	53	53		28	27	26	27		
HbA_{1c}, mmol/mol	36.6 (35.2, 40.2)	34.1 ± 4.1	33.9 ± 3.7	34 (32, 35)	<0.001	49.2 (43.2, 54.1)	40.4 ± 5.6	39.2 ± 4.6	39 (36, 42)	<0.001	<0.001
n	54	38	46	52		28	24	27	27		
Fasting PYY level, pg/mL	53 (36, 64)	61 (41, 77)	59 (45, 79)	48 (35, 69)	0.15	84 (61, 110)	72 (53, 110)	83 (66, 164)	85 (50, 108)	0.19	0.41
n	55	50	47	47		28	25	25	22		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	22,578 (18,923, 27,640)	33,450 (26,418, 50,432)	28,523 (20,581, 45,084)	22,471 (18,092, 35,217)	<0.001	28,508 (22,808, 37,674)	44,970 (30,597, 61,682)	47,589 (31,486, 63,535)	32,704 (24,135, 59,084)	<0.001	0.003
n	55	49	47	46		27	25	25	22		
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	13,200 (9,320, 19,493)	23,055 (15,415, 31,667)	15,518 (10,709, 31,720)	14,509 (5,560, 22,510)	<0.001	12,878 (6,818, 19,688)	28,793 (18,130, 41,847)	24,803 (16,719, 38,076)	20,172 (13,859, 41,481)	<0.001	0.003
n	55	49	47	46		27	25	25	22		

Fasting AG,	8	3.2	4	5.2	<0.001	7.6	4.2	5.7	5.5	0.07	0.28
fmol/mL	(4.5, 11)	(1.4, 5.4)	(1.9, 6.9)	(3.1, 7.6)		(5.2, 12.2)	(2.9, 5.9)	(3.9, 8.9)	(2.8, 9.1)		
n	54	50	44	48		27	25	23	23		
$AUC_{0\text{-}180}$ AG,	1,062	652	799	870	<0.001	1,022	613	786	769	0.09	0.74
fmol x min/mL	(689, 1,489)	(476, 1,038)	(486, 1,145)	(653, 1,239)		(606, 1,564)	(424, 1,084)	(491, 1,222)	(603, 1,269)		
n	53	49	44	48		26	25	23	23		
$\Delta AUC_{0\text{-}180}$ AG,	-392	41	-32	-8	<0.001	-350	-203	-288	-205	0.2	0.17
fmol x min/mL	(-840, -78)	(-240, 231)	(-401, 265)	(-277, 195)		(-710, -134)	(-326, 44)	(-717, -11)	(-395, 115)		
n	53	49	44	48		26	25	23	23		
Fasting DAG,	97	52	60	48	<0.001	106	60	68	44	<0.001	0.06
fmol/mL	(67, 131)	(37, 79)	(40, 78)	(31, 77)		(75, 118)	(35, 82)	(44, 114)	(36, 82)		
n	55	50	46	48		27	25	24	23		
$AUC_{0\text{-}180}$ DAG,	11,650	7,215	8,779	7,841	<0.001	12,812	6,989	8,701	7,516	<0.001	0.35
fmol x min/mL	(8,494, 17,304)	(5,617, 11,465)	(6,344, 11,436)	(5,686, 12,795)		(8,235, 15,645)	(4,685, 10,975)	(6,777, 11,776)	(5,429, 10,909)		
n	54	49	46	47		26	25	24	23		
$\Delta AUC_{0\text{-}180}$ DAG,	-4,441	-2,244	-2,039	-982	<0.001	-5,325	-2,588	-3,388	-1,024	0.001	0.09
fmol x min/mL	(-7,485, -2,546)	(-3,989, -822)	(-3,554, -498)	(-2,634, -2)		(-8,787, -3,625)	(-4,945, -883)	(-5,898, -2,168)	(-3,295, -191)		
n	54	49	46	47		26	25	24	23		
Fasting AG:DAG	0.083	0.073	0.067	0.089	0.06	0.076	0.075	0.079	0.115	0.67	0.19
	(0.057, 0.116)	(0.023, 0.107)	(0.034, 0.114)	(0.055, 0.128)		(0.05, 0.102)	(0.054, 0.125)	(0.045, 0.106)	(0.088, 0.145)		
n	54	50	44	48		27	25	23	23		
$AUC_{0\text{-}180}$ AG:DAG	0.089	0.086	0.09	0.106	0.47	0.078	0.1	0.081	0.106	0.11	0.78
	(0.062, 0.117)	(0.056, 0.142)	(0.057, 0.138)	(0.081, 0.133)		(0.062, 0.111)	(0.06, 0.155)	(0.056, 0.109)	(0.089, 0.12)		
n	53	49	44	47		26	25	23	23		
$\Delta AUC_{0\text{-}180}$	0.066	-0.028	0.009	0.067	0.32	0.054	0.063	0.068	0.097	0.19	0.09
AG:DAG	(0.01, 0.149)	(-0.169, 0.073)	(-0.106, 0.112)	(-0.125, 0.204)		(0.026, 0.13)	(-0.076, 0.118)	(0.01, 0.202)	(-0.206, 0.232)		
n	53	49	44	47		26	25	23	23		

Appendix 8 Weight loss outcomes, glycaemic indices, PYY, AG and DAG profiles after bariatric surgery, divided patients by good and poor weight loss at 1 year

	Poor weight loss (n = 15)					Good weight loss (n = 68)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
BW, kg	137	118	107.9	107.5	<0.001	121.3	107.8	90.1	86.7	<0.001	<0.001
	(116, 142.5)	(105.2, 131)	(92.1, 21.4)	(92, 121.8)		(108.6, 137.9)	(95.6, 122.2)	(81, 105.1)	(77, 95)		
n	15	15	14	15		68	67	66	68		
BMI, kg/m²	45.8 (41.7, 51.6)	39.9 (37.6, 47.4)	36.9 (33.8, 43.3)	38.8 (33.2, 43.5)	<0.001	43.9 (41, 50.3)	39.4 (35.9, 43.2)	34 (30.1, 37.8)	31.8 (28.5, 34.9)	<0.001	<0.001
n	15	15	14	15		68	67	66	68		
PWL, %	0	6.9 (6.3, 8.9)	15.4 (12.2, 18.6)	15.5 (11, 19)	<0.001	0	9.4 (7.8, 11.3)	21.6 (19.3,26.1)	26 (23.5, 31.1)	<0.001	<0.001
n	15	15	14	15		68	67	66	68		
WCV, kg/week	0	-1.87	-0.49	0.02	<0.001	0	-2.2	-0.72	-0.21	<0.001	0.41
		(-2.2, -1.68)	(-0.61, -0.31)	(-0.15, 0.19)			(-2.78, -1.73)	(-0.9, -0.58)	(-0.35, -0.08)		
n	15	15	14	14		68	67	65	66		
HbA_{1c}, mmol/mol	42.1	35.4 ± 6.8	35	39	<0.01	39.9	36.7 ± 5.4	36	35	<0.001	0.45
n	(33.3, 50.8)		(31.5, 40)	(31.5, 40.5)		(35.5, 45.4)		(33, 38)	(33, 38)		
	14	10	13	13		66	52	60	66		
Fasting PYY level,	50	60	64	40	0.4	60	65	72	60	0.04	0.57
pg/mL	(39, 102)	(36, 84)	(48, 78)	(33, 67)		(40, 85)	(44, 89)	(54, 95)	(41, 92)		
n	15	13	13	11		68	62	59	58		
$AUC_{0\text{-}180}$ PYY, pg x	24,209	31,651	37,275	22,297	0.04	24,226	37,718	34,103	25,210	<0.001	0.34
min/mL	(19,154, 32,743)	(24,390, 45,361)	(19,356, 48,105)	(11,552, 43,440)		(19,713, 29,933)	(29,641, 57,964)	(21,349, 52,895)	(20,411, 42,676)		
n	15	13	13	11		67	61	59	57		
$\Delta AUC_{0\text{-}180}$ PYY, pg	12,878	20,842	23,428	15,345	<0.05	13,200	26,439	19,263	15,908	<0.001	0.11
x min/mL	(6,994, 20,453)	(12,620, 28,653)	(9,987, 32,543)	(3,539, 33,900)		(9,086, 19,275)	(16,782, 42,318)	(11,446, 36,801)	(8,830, 26,366)		
n	15	13	13	11		67	61	59	57		

Fasting AG,	7 (4.8, 11.9)	4.1	5.3	5	<0.01	7.9	3.5	4.6	5.7	<0.001	0.89
fmol/mL		(2.4, 7)	(3.7, 8.2)	(3.5, 6.1)		(4.8, 11.4)	(1.8, 5.7)	(2.2, 7.2)	(2.8, 7.9)		
n	14	13	12	12		67	62	55	59		
AUC_{0-180} AG,	1,035	544	623	808	<0.01	1,035	660	842	894	<0.001	0.94
fmol x min/mL	(756, 1,418)	(420, 798)	(483, 1,051)	(630, 1,091)		(669, 1,520)	(466, 1,057)	(507, 1,222)	(621, 1,292)		
n	13	13	12	12		66	61	55	59		
ΔAUC_{0-180} AG,	-290 (-932, -193)	-206	-331	82	0.02	-374	-2	-44	-97	<0.01	0.86
fmol x min/mL		(-345, 37)	(-555, -81)	(-269, 149)		(-773, -77)	(-266, 05)	(-409, 230)	(-291, 210)		
n	13	13	12	12		66	61	55	59		
Fasting DAG,	105 (64, 124)	56	65	46	<0.001	98	53	61	46	<0.001	0.57
fmol/mL		(31, 77)	(44, 75)	(39, 66)		(74, 131)	(37, 81)	(42, 90)	(35, 82)		
n	14	13	13	12		67	62	57	59		
AUC_{0-180} DAG,	12,501 ± 4,877	6,740	8,729	7,463	<0.01	13,025 ± 5,706	7,215	8,739	7,786	<0.001	0.86
fmol x min/mL		(4,819, 9,450)	(7,442, 10,490)	(5,533, 9,128)			(5,186, 12,705)	(5,584, 12,320)	(5,411, 11,654)		
n	13	13	13	12		66	61	57	58		
ΔAUC_{0-180} DAG,	-4,917	-2,297	-3,026	-997	<0.01	-5,321	-2,402	-2,771	-872	<0.001	0.58
fmol x min/mL	(-6,192, -3,034)	(-3,385, -1,477)	(-3,892, -827)	(-2,209, -245)		(-8,221, -2,546)	(-4,401, -679)	(-4,050, -895)	(-2,921, -50)		
n	13	13	13	12		66	61	57	58		
Fasting AG:DAG	0.076	0.075	0.078	0.105	0.74	0.077	0.075	0.069	0.096	0.24	0.79
	(0.05, 0.117)	(0.053, 0.131)	(0.064, 0.122)	(0.074, 0.117)		(0.055, 0.115)	(0.037, 0.115)	(0.038, 0.107)	(0.058, 0.137)		
n	14	13	12	12		67	62	55	59		
AUC_{0-180} AG:DAG	0.089	0.086	0.072	0.109	0.64	0.087	0.09	0.089	0.106	0.17	0.99
	(0.059, 0.117)	(0.056, 0.129)	(0.05, 0.118)	(0.092, 0.126)		(0.062, 0.111)	(0.059, 0.142)	(0.063, 0.132)	(0.083, 0.131)		
n	13	13	12	12		66	61	55	58		
ΔAUC_{0-180}	0.099	0.063	0.102	0.008	0.95	0.052	-0.015	0.019	0.082	0.44	0.9
AG:DAG	(0.042, 0.174)	(-0.076, 0.113)	(-0.023, 0.163)	(-0.182, 0.189)		(0.012, 0.14)	(-0.142, 0.08)	(-0.056, 0.128)	(-0.136, 0.211)		
n	13	13	12	12		66	61	55	58		

Appendix 9 Anthropometric parameters, glycaemic indices, PYY, AG and DAG profiles after bariatric surgery in patients with T2D, divided by type of surgery

	LRYGB (n = 10)					LSG (n = 18)					P-value
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	between groups
BW, kg	116.2 ± 20.8	99.9 ± 14.4	91.7 ± 19.4	85 ± 17.6	<0.001	128.5 ± 24.9	113.3 ± 23.1	100.3 ± 22.3	98.9 ± 22.1	<0.001	0.36
n	10	9	10	10		18	18	17	18		
BMI, kg/m²	44.7 ± 5.2	39.5 ± 4.6	35.5 (30.2, 38.8)	32.6 ± 4.1	<0.001	46.3 ± 7.4	40.7 ± 6.6	35.7 (33.1, 40.5)	35.6 ± 6.8	<0.001	0.25
n	10	9	10	10		18	18	17	18		
PWL, %	0	8.7 (7, 9.9)	20 ± 6.2	25.6 ± 7.1	<0.001	0	9.3 (7.1, 11.7)	19.4 ± 4.3	21 ± 5.9	<0.001	0.06
n	10	9	10	10		18	18	17	18		
WCV, kg/week	0	-1.9 ± 0.43	-0.59 ± 0.38	-0.25 ± 0.15	<0.001	0	-2.54 ± 0.87	-0.6 ± 0.3	-0.11 ± 0.18	<0.001	<0.01
n	10	9	9	10		18	18	17	17		
HbA_{1c}, mmol/mol	45.9 (41, 53)	38.9 ± 5.8	38.1 ± 4.4	37.5 (35.5, 41.3)	<0.001	51.4 (44.8, 60.7)	41.3 ± 5.5	39.9 ± 4.7	40 (35.5, 42)	<0.001	0.94
n	10	9	10	10		18	15	17	17		
Fasting PYY level, pg/mL	100 ± 66	77 (55, 197)	122 (86, 246)	104 ± 31	0.26	83 ± 27	63 (44, 98)	72 (61, 83)	73 ± 34	0.73	0.21
n	10	10	10	7		18	15	15	15		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	34,280 ± 16,776	61,093 (49,308, 83,318)	63,535 (52,652, 69,776)	63,917 (56,324, 72,023)	<0.001	27,556 ± 7,953	34,598 (27,705, 45,353)	37,356 (22,673, 43,200)	29,088 (22,297, 42,610)	<0.01	<0.01
n	10	10	10	7		17	15	15	15		
Δ$AUC_{0\text{-}180}$ PYY, pg x min/mL	16,278 ± 12,236	44,226 ± 30,303	42,561 ± 34,899	44,701 ± 20,732	<0.01	12,221 ± 7,886	24,775 ± 14,384	22,736 ± 12,401	19,288 ± 12,052	<0.01	0.05
n	10	10	10	7		17	15	15	15		

Fasting AG,	9.1	5.9	8.9	13.8 ± 8.1	0.31	6.4	3.5	4.6	4 ± 2.2	<0.001	0.01
fmol/mL	(5.3, 12.6)	(3.6, 9.9)	(5.2, 20.6)			(5.2, 11.6)	(2.4, 5)	(2.6, 8.1)			
n	10	10	9	8		17	15	14	15		
AUC_{0-180} AG,	1,246	834	1,222	1,445	0.12	965 (581, 1,565)	476	640	621	<0.001	<0.01
fmol x min/mL	(790, 1,555)	(613, 1,648)	(852, 1,778)	(980, 3,232)		16	(330, 674)	(387, 1,040)	(393, 935)		
n	10	10	9	8			15	14	15		
ΔAUC_{0-180} AG,	-328	-188	-288	-447 ± 1,095	0.84	-354 (-674, -144)	-206	-291	-28 ± 257	0.02	0.63
fmol x min/mL	(-856, -11)	(-657, 42)	(-756, -130)			16	(-290, 62)	(-727, 67)			
n	10	10	9	8			15	14	15		
Fasting DAG,	118 ± 53	88 ± 48	126	94	0.06	99 ± 36	49 ± 22	53	40	<0.001	0.001
fmol/mL			(77, 180)	(62, 114)				(41, 69)	(35, 45)		
n	10	10	9	8		17	15	15	15		
AUC_{0-180} DAG,	13,115 ± 5,133	11,133 ± 6,601	11,916	12,030	0.09	13,301 ± 6,720	6,550 ± 3,211	7,535	5,972	<0.001	<0.001
fmol x min/mL			(10,052, 18,064)	(9,834, 16,494)				(5,036, 8,729)	(4,907, 7,516)		
n	10	10	9	8		16	15	15	15		
ΔAUC_{0-180} DAG,	-8,113 ± 8,428	-4,716 ± 4,259	-7,267	-4,022 ± 3,407	0.06	-4,927 ± 3,548	-2,169 ± 1,779	-2,852	-750 ± 1,012	<0.001	<0.05
fmol x min/mL			(-18,386, -3,754)					(-3,643, -1,958)			
n	10	10	9	8		16	15	15	15		
Fasting AG:DAG	0.074	0.076	0.064	0.131 ± 0.027	0.32	0.076	0.068	0.084	0.098 ± 0.044	0.85	0.54
	(0.051, 0.105)	(0.065, 0.097)	(0.047, 0.093)			(0.046, 0.141)	(0.053, 0.15)	(0.044, 0.119)			
n	10	10	9	8		17	15	14	15		
AUC_{0-180} AG:DAG	0.093	0.104	0.082	0.109	0.33	0.07	0.084	0.075	0.101	0.36	0.65
	(0.064, 0.125)	(0.063, 0.178)	(0.071, 0.118)	(0.091, 0.117)		(0.06, 0.106)	(0.049, 0.135)	(0.051, 0.112)	(0.083, 0.124)		
n	10	10	9	8		16	15	14	15		
ΔAUC_{0-180} AG:DAG	0.05	0.034 ± 0.166	0.04	0.149	0.58	0.059	0.032 ± 0.184	0.118	0.069	0.2	0.4
	(0.028, 0.13)		(0.014, 0.145)	(-0.188, 0.23)		(0.018, 0.166)		(-0.007, 0.226)	(-0.206, 0.248)		
n	10	10	9	8		16	15	14	15		

Appendix 10 Anthropometric parameters, glycaemic indices, PYY, AG and DAG profiles after bariatric surgery in patients with T2D, divided by diabetes remission status at 1 year

	Non-remitters (n = 5)					Remitters (n = 23)					P-value between groups
	Baseline	6 weeks	6 months	1 year	P-value	Baseline	6 weeks	6 months	1 year	P-value	
BW, kg	115.2 ± 24.6	102.4 ± 19	84 ± 12.9	88 ± 22.2	<0.001	126 ± 23.8	110.3 ± 21.9	99.4 ± 21.9	95.2 ± 21.5	<0.001	0.52
n	5	5	4	5		23	22	23	23		
BMI, kg/m²	44.9 ± 10.8	39.9 ± 8.5	34.8 (26.5, 35.8)	34.2 ± 9.3	<0.001	45.9 ± 5.7	40.4 ± 5.5	36.7 (32.5, 39.5)	34.6 ± 5.4	<0.001	0.84
n	5	5	4	5		23	22	23	23		
PWL, %	0	8 ± 1.5	19.9 ± 3.3	21.5 ± 7.3	<0.001	0	9.6 ± 2.5	19.5 ± 5.3	22.9 ± 6.6	<0.001	0.91
n	5	5	4	5		23	22	23	23		
WCV, kg/week	0	-1.6	-0.63	-0.18 ± 0.12	0.009	0	-2.25	-0.49	-0.16 ± 0.19	<0.001	0.85
n	5	(-3.13, -1.43) 5	(-0.68, -0.46) 4	4		23	(-2.72, -1.83) 22	(-0.74, -0.44) 22	23		
HbA_{1c}, mmol/mol	53	47.5 ± 5.2	43.8 ± 6.2	47.8 ± 12.5	0.2	47.5	39 ± .6	38.4 ± 3.9	38.1 ± 3.6	<0.001	0.79
n	(48.1, 67.8) 5	4	4	5		(41, 54.1) 23	20	23	22		
Fasting PYY level, pg/mL	104 (71, 140)	91 (59, 198)	73 (71, 217)	106 ± 44	0.94	84 (51, 106)	69 (51, 109)	84 (63, 164)	77 ± 33	0.22	0.97
n	5	4	4	4		23	21	21	18		
$AUC_{0\text{-}180}$ PYY, pg x min/mL	31,541 ± 16,337	28,624 (27,222, 103,922)	25,588 (19,429, 132,634)	37,748 ± 21,440	0.44	29,707 ± 11,440	45,353 (35,918, 61,682)	49,821 (38,384, 63,535)	43,225 ± 21,392	<0.001	0.61
n	5	4	4	4		22	21	21	18		
$\Delta AUC_{0\text{-}180}$ PYY, pg x min/mL	12,657 ± 11,857	16,412 (10,930, 69,907)	12,649 (6,353, 93,844)	10,908 (5,151, 40,146)	0.33	13,966 ± 9,454	29,513 (21,158, 41,847)	28,382 (19,985, 38,076)	22,713 (15,111, 41,481)	<0.001	0.35
n	5	4	4	4		22	21	21	18		

Fasting AG,	5.3	3.1	3.5	5.2	0.12	7.6	4.9	5.8	5.5	0.13	0.94
fmol/mL	(5.2, 8.8)	(2.5, 5.4)	(2.3, 7.8)	(0.8, 8.7)		(5.1 12.2)	(3.2, 7)	(4, 11.9)	(2.8, 12.1)		
n	4	4	4	4		23	21	19	19		
$AUC_{0\text{-}180}$ AG,	1,057	765	734	697	0.02	1,022	613	786	769	0.08	0.47
fmol x min/mL	(583, 1,549)	(345, 1,494)	(386, 1,186)	(350, 1,041)		(683, 1,569)	(465, 934)	(589, 1,310)	(613, 1,509)		
n	4	4	4	4		22	21	19	19		
$\Delta AUC_{0\text{-}180}$ AG,	-319	216	-37	-259	0.51	-384	-207	-331	-205	0.18	0.62
fmol x min/mL	(-358, 398)	(-100, 529)	(-299, 213)	(-527, 241)		(-790, -134)	(-400, -9)	(-755, -129)	(-395, 115)		
n	4	4	4	4		22	21	19	19		
Fasting DAG,	110	69	90	42	0.01	106	60	67	44	<0.001	0.9
fmol/mL	(69, 154)	(35, 90)	(39, 114)	(25, 82)		(75, 116)	(35, 79)	(44, 117)	(38, 82)		
n	4	4	4	4		23	21	20	19		
$AUC_{0\text{-}180}$ DAG,	14,082 ± 8,385	8,920	9,934	7,226	0.05	13,074 ± 5,774	6,117	8,696	7,516	0.002	0.66
fmol x min/mL		(5,312, 12,484)	(8,176, 18,352)	(4,183, 10,132)			(4,685, 10,975)	(6,203, 11,776)	(5,462, 11,278)		
n	4	4	4	4		22	21	20	19		
$\Delta AUC_{0\text{-}180}$ DAG,	-5,359	-1,161	-1,651	-463	0.02	-5,325	-2,726	-3,551	-1,118	0.003	0.64
fmol x min/mL	(-8,191, -4,044)	(-5,877, -853)	(-7,513, 2,030)	(-5,440, 799)		(-8,787, -2,676)	(-4,945, -928)	(-5,898, -2,387)	(-3,295, -197)		
n	4	4	4	4		22	21	20	19		
Fasting AG:DAG	0.067	0.058	0.056	0.085 ± 0.059	0.72	0.076	0.077	0.089	0.115 ± 0.037	0.64	0.87
	(0.035, 0.097)	(0.043, 0.081)	(0.038, 0.078)			(0.051, 0.12)	(0.06, 0.139)	(0.052, 0.117)			
n	4	4	4	4		23	21	19	19		
$AUC_{0\text{-}180}$ AG:DAG	0.074	0.096 ± 0.056	0.05	0.098	0.19	0.078	0.11 ± 0.065	0.082	0.107	0.16	0.73
	(0.046, 0.143)		(0.046, 0.095)	(0.075, 0.109)		(0.063, 0.111)		(0.068, 0.127)	(0.089, 0.124)		
n	4	4	4	4		22	21	19	19		
$\Delta AUC_{0\text{-}180}$	0.044	-0.057 ± 0.233	0.031	-0.107	0.39	0.057	0.05 ± 0.161	0.091	0.113	0.35	1.0
AG:DAG	(-0.098, 0.083)		(0.002, 13.5)	(-1.57, 0.207)		(0.026, 0.137)		(0.01, 0.202)	(-0.205, 0.232)		
n	4	4	4	4		22	21	19	19		