Effect of different surgical weight-loss interventions and ethnicity on GLP-1 secretion

Noora AlRasheid

Adipokines and Metabolism Research Group

Division of Medicine

University College London

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Declaration

I Noora Alrasheid confirm 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 this thesis.

Signature:

Date:

Abstract

Background.

Roux–en-Y gastric bypass (RYGB) is the most successful treatment for obesity that results in sustained, long-term weight loss and improved insulin sensitivity. Generally, these benefits are mediated by gut peptides such as glucagon-like peptide-1 (GLP-1). However, their role(s) in some scenarios is yet to be investigated, for example in some patients who, following weight-reducing surgery, develop chronic intolerable nausea and vomiting (N&V) symptoms, in those undergoing a primary obesity surgery endoluminal (POSE) procedure, a new less invasive, incisionless weight-loss technique that seems to be effective for losing weight, or in ethnicities that have a high prevalence of obesity.

Therefore, the aim(s) were to investigate systemic GLP-1 concentrations in three separate groups: 1) patients with prolonged N&V after metabolic surgery (Study I), 2) patients who had POSE for weight loss, compared to RYGB (Study II), and 3) an obesity-prone, Arab population (Study III).

Methods.

Study I: female non-diabetic subjects were studied in five groups. Group 1: patients with N&V after RYGB surgery. Group 2: patients with no symptoms after RYGB surgery. Group 3: morbidly obese patients. Group 4: obese/overweight subjects. Group 5: lean healthy subjects.

Study II: female subjects were studied before surgery (POSE and RYGB), and 1 week, 2 months and 6 months after surgery, in the fasting and postprandial states.

Study III: Arab non-diabetic female subjects were studied before and after a 388.6-kcal liquid mixed-meal challenge.

Blood was collected in the fasting and postprandial states after a defined meal challenge (182.7-kcal meal used in Study I and 388.6-kcal meal used in Studies II and III). Systemic concentrations of glucose, lipids, insulin, GLP-1 and adipokines were determined using a routine chemistry analyser (Roche) or commercial ELISA.

Subcutaneous (SC) adipose tissue organ cultures were incubated with recombinant GLP-1, and leptin concentrations were detected from the conditioned medium.

Results.

Study (I): subjects with N&V post-RYGB had significantly elevated fasting GLP-1 levels compared to those without N&V ($p = 0.035$) and compared to morbidly obese, obese/overweight and lean subjects. Weight loss and glucose, insulin and GLP-1 response to a 180-kcal meal challenge were similar in subjects with and without N&V. Fasting plasma leptin was significantly lower in subjects with N&V compared to those without N&V ($p = 0.04$). *In vitro*, leptin secretion was inhibited by GLP-1.

Study (II): both POSE and RYGB patients lost a significant amount of weight early after surgery, with a concomitant decrease in leptin. However, only RYGB patients continued to lose weight at 6 months. Adiponectin increased at 2 and 6 months, and serum lipid levels were unchanged. First-phase responses (30 minutes) of insulin and GLP-1 were dramatically increased 1 week after RYGB only.

Study (III): in the non-diabetic Arab subjects, the mixed meal provoked a similar GLP-1 response to that reported in Caucasians. However, even in this apparently healthy population, postprandial hyperinsulinemia was evident.

Conclusions.

Subjects with persistent N&V post-RYGB surgery had elevated fasting GLP-1 levels related to their symptoms. Inhibiting GLP-1 with antagonists might help to ameliorate these symptoms. However, potential detrimental effects on weight maintenance and insulin sensitivity need to be considered. GLP-1 directly inhibits leptin secretion, so a decreased leptin level early after RYGB might be explained by an elevated GLP-1 level, and this could at least partially be explained by the alteration in GLP-1.

POSE provides reasonable short-term weight loss without having an effect on GLP-1 in the morbidly obese but it is not as effective as RYGB.

Finally, no significant lesion in systemic GLP-1 levels was seen in a cohort that could explain the obesity phenomenon in this population.

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Abbreviations

ADLQ	Anti-Doping Lab, Qatar
AGRP	Agouti-related peptide
AT	Adipose tissue
AUC	Area under the curve
BBB	Blood–brain barrier
BIB	Bioenteric intragastric balloon
BMI	Body mass index
BSA	Bovine serum albumin
CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CNS	Central nervous system
CPAP	Continuous positive airway pressure
CRC	Clinical research centre
CSF	Cerebrospinal fluid
CT	Computed tomography
Ct	Cycle threshold
CV	Coefficient of variation
DBP	Diastolic blood pressure
DMH	Dorsal medial nucleus of the hypothalamus
DPP-IV	Dipeptidyl peptidase IV
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
EWL	Excess weight loss
FDA	Food and Drug Administration
FFA	Free fatty acids

GB	Gastric band
GEB	Garren-Edwards bubble
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
HDL	High-density lipoprotein
HOMA	Homeostatic model assessment
HRP	Horseradish peroxidate
IL-6	Interleukin 6
Jak	Janus kinase
KIU	Kallikrein inhibitory unit
LCD	Low-calorie diet
LDL	Low-density lipoprotein
LEPR	Leptin receptor
LHA	Lateral hypothalamic area
MCH	Melanin-concentrating hormone
MO	Morbidly obese
MUP	Methyl umbelliferyl phosphate
N&V	Nausea and vomiting
NPY	Neuropeptide Y
NTS	Nucleus of the tractus solitarius
NW	Normal weight
OAT	Omental adipose tissue
OB	Obese
OD	Optical density
OGTT	Oral glucose tolerance test
OM	Omental

OSA	Obstructive sleep apnoea
OW	Overweight
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
POMC	Pro-opiomelanocortin
POSE	Primary obesity surgery endoluminal
PTP	Protein tyrosine phosphatase
PVN	Paraventricular nucleus
PYY	Peptide YY
RNA	Ribonucleic acid
rpm	Revolutions per minute
RYGB	Roux-en-Y gastric bypass
SABS	Spatz adjustable balloon system
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SC	Subcutaneous
SG	Sleeve gastrectomy
SOCS	Suppressor of cytokine signalling
STAT	Signal transducer and activator of transcription
TERIS	Transoral endoscopic restrictive implant system
TNF-α	Tumour necrosis factor-α
TOGA	Transoral gastroplasty
VLCD	Very low-calorie diet
WHO	World Health Organization

Publications arising from this project

Manuscript published

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Conference Proceedings

Al-Rasheid, N., Gray, R., Sufi, P., Atherton, E., Mohamed-Ali, V., Chronically elevated basal GLP-1 mediates persistent nausea symptoms following weight reduction surgery. *Clinical Obesity Treatment Endo 2013* (meeting abstract).

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Chapter 1

Introduction

1.1 Definition of obesity

Obesity is defined as an abnormal and inappropriate accumulation of adipose tissue that has pathological consequences such as insulin resistance and diabetes mellitus, cardiovascular diseases, sleep apnoea, osteoarthritis, cancer and depression [1, 2]. Body mass index (BMI), calculated as [weight (kg)/height $(m)^2$], is used to classify weight as shown in Table 1.1 [3].

1.2 Prevalence of obesity

Obesity is a global health issue with high prevalence that has proven particularly difficult to tackle. Although the effect of obesity on quality of life was observed as early as in the eighteenth century, its definition as a cause of ill health was only recorded in the nineteenth century. Obesity-associated morbidity and mortality rates started to be documented only at the beginning of the twentieth century [4]. In fact, the rapid increase in the incidence of obesity in the last six decades has led the World Health Organization (WHO) to consider obesity as a global health problem of epidemic proportions [5]. In 2010, it was reported that overweight and obesity might result in 3.4 million deaths and 4% of disability-adjusted life-years around the world [6].

Globally, between 1980 and 2013, the number of overweight and obese adults increased from 857 million to 2.1 billion, and the prevalence of overweight and obesity increased by 27.5%. The proportion of overweight men grew from 28.8% to 36.9%, and the proportion of overweight women grew from 29.8% to 38.0% [7].

Classification	BMI (kg/m²)	
	Principal cut-off points	**Additional cut-off points**
Underweight	**< 18.50**	**< 18.50**
Severe thinness	< 16.00	< 16.00
Moderate thinness	16.00–16.99	16.00–16.99
Mild thinness	17.00–18.49	17.00–18.49
Normal range	**18.50–24.99**	**18.50–22.99**
		23.00–24.99
Overweight	**≥ 25.00**	**≥ 25.00**
Pre-obese	25.00–29.99	25.00–27.49
		27.50–29.99
Obese	**≥ 30.00**	**≥ 30.00**
Obese class I	30.00–34.99	30.00–32.49
		32.50–34.99
Obese class II	35.00–39.99	35.00–37.49
		37.50–39.99
Obese class III	≥ 40.00	≥ 40.00

Table 1.1 Classification of BMI according to WHO [2]

Figure 1.1 shows the worldwide prevalence of overweight and obesity, and obesity alone, among adults in developing and developed countries between 1980 and 2013. In developed countries, men were more overweight and obese than women, while in developing countries, women were more overweight and obese [7].

In the United States, the incidence of obesity almost doubled within 10 years, between 1980 and 1990. It was suggested that if this increase in obesity rate was not controlled it would result in a decline in life expectancy [8]. Between 2011 and 2012, over one-third of adults were obese. Overall, the obesity rate was greater in middle-aged (aged 40–59) than younger (aged 20–39) or older adults (aged 60 and above). Moreover, the incidence of obesity was high in non-Hispanic black (47.8%) and Hispanic (42.5%) adults. The incidence of obesity in non-Hispanic Asians (10.8%) was lower than that in non-Hispanic whites (32.6%) [9].

In England, between 1993 and 2012, the percentage of normal-weight adults declined from 41.0% to 32.1% among men, and from 49.5% to 40.6% among women. For both sexes, the overweight percentages were stable (40% for men and 30% for women). The percentages of obesity increased from 13.2% to 24.4% among men, and from 16.4% to 25.1% among women [10].

In Arab countries, over the last three decades, obesity rates have increased in Egypt, Saudi Arabia, Oman and Bahrain among women, and in Bahrain, Kuwait and Saudi Arabia among men [7]. The Arab Gulf countries, in particular Kuwait, Bahrain, Saudi Arabia and United Arab Emirates, have the highest rates of obesity worldwide. The prevalence of obesity in Arab Gulf countries for the last 25 years has been higher among women, whereas the prevalence of overweight for the same period was higher among men [11]. In 2010, the percentages for obesity in females in those countries were as follows: Kuwait 55.2%, Qatar 31.6%, Saudi Arabia 36.4%, Bahrain 37.9% and United Arab Emirates 42% [12].

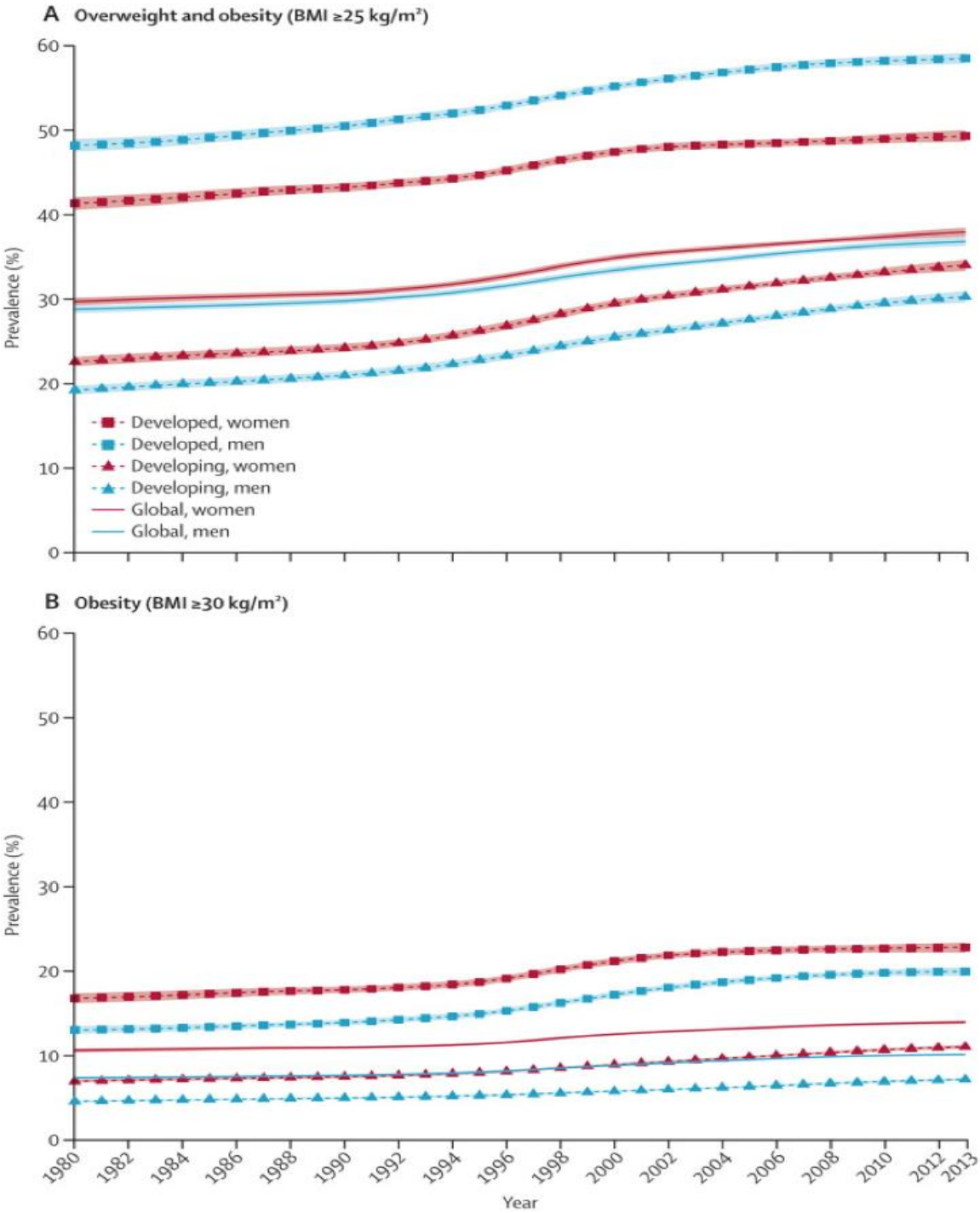

Figure 1.1 Prevalence of overweight and obesity and obesity alone

The prevalence of overweight and obesity (A) and obesity alone (B) among males and females increased between 1980 and 2013 in both developed and developing countries. However, it was lower in developing countries than in developed countries. Also, it was higher among men in developed countries, whereas it was higher among women in developing countries.

1.3 Obesity and insulin resistance

Insulin resistance is a prediabetic condition that is associated with obesity. Some studies have suggested that insulin resistance might result from obesity [13]. Failure of adipocyte differentiation in response to high caloric intake and visceral adipocyte hypertrophy may contribute to insulin resistance. It is possible that secretion of inflammatory cytokines and adipokines from adipocytes causes insulin resistance in liver and muscle tissues. Another possibility is abnormal release of free fatty acids (FFA) from adipocytes and accumulation of ectopic lipids in liver and muscle tissue causing impaired insulin sensitivity in these tissues [14]. However, some obese individuals have no associated metabolic disturbance, have normal insulin sensitivity and are classified as metabolically healthy obese as opposed to pathologically obese [15]. It seems that environmental, behavioural and genetic factors also play a role in mediating healthy or pathological fat accumulation [15].

1.4 Mechanism of satiety

There are two major issues when considering satiety. The first concerns the mechanisms that are attributed to meal termination, whereas the second concerns the mechanisms that govern intervals between meals. These mechanisms involve neural and humoral signals that interact with each other to a degree [16, 17].

Once food is ingested, satiety-inducing signals that reach the brain are initiated by mechanical or chemical stimulation of the gastrointestinal system, neural input related to energy metabolism in the liver, and gut peptide signals such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) [18-21]. CCK is the most studied gastrointestinal hormone that transmits satiety-related information to the brain. Gibbs and colleagues were the first group to report that administering purified or synthetic CCK to animals before a meal decreases nutrient intake in a dose-dependent manner [22]. This hormone is released from endocrine I cells of the duodenum and the jejunum during meal

consumption and acts on its receptors present on sensory fibres of the vagus nerve near the site where food passes from the stomach into the intestine [23]. Once these receptors are stimulated, they send a satiety signal through the vagus nerve afferent fibres to the brain to terminate meal ingestion [24]. Satiety information from the gut converges in the nucleus of the tractus solitarius (NTS) in the brainstem [25].

Furthermore, neuropeptide Y/agouti-related protein (NPY/AGRP) and pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons in the arcuate nucleus are also involved in the satiety process [26, 27]. Leptin and insulin activate the POMC/CART anorexic effects and inhibit (NPY/AGRP) orexigenic effects, which reduces food intake [28-31]. In addition, these neurons project from the arcuate nucleus to other sites in the hypothalamus such as the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA), that are also known to control food intake [31-33].

1.5 Gastric emptying

Gastric emptying depends on complex coordination between the major motor patterns of the stomach. Once food is ingested, an initial gastric relaxation occurs, and the proximal stomach pushes the gastric contents forward by a process of tonic contraction, providing a driving force for gastric emptying. At the same time, peristaltic contractions originating from the mid-corpus progress in the direction of the antrum, cause grinding and sieving of solid food. This repetitive process leads to breakdown of food particles, mixes them with juices and forms a second drive that propels the food content distally. Opening and closing of the pyloric sphincter is the third mechanical factor in the control of gastric emptying. The pyloric sphincter closes the stomach during the terminal phase of a peristaltic contraction wave, preventing any content in the antrum from entering into the duodenum during mixing and grinding [34].

The influence of each of these three mechanical factors depends on the consistency of the food. In the case of liquid, gastric emptying is controlled mainly

by the pressure elicited by the proximal stomach and the opening of the pylorus. Gastric emptying in the case of solid food is controlled mainly by the peristaltic pump of the antrum [35].

The emptying rate of a meal is negatively correlated to its caloric content [36]. Other factors that affect gastric emptying rate are the acidity, osmolarity and viscosity of the meal [37, 38]. Once nutrients enter the duodenum, they activate the vagovagal reflex mechanisms and hormonal signals (e.g. GLP-1, PYY and CCK) which modulate gastric emptying [39-41]. Physiological or artificially induced inhibition of gastric emptying seems to be linked with increased feelings of satiety and fullness and termination of food intake [42-44]. Moreover, it has been found that a subset of patients with anorexia nervosa have markedly delayed gastric emptying, whereas some studies have suggested that obese people have rapid gastric emptying [45-47].

1.6 Hormonal control of food intake

Most human subjects maintain a stable body weight over a number of years. To keep the weight stable, there must be mechanisms that regulate the energy balance, adjusting energy intake and expenditure [48]. These mechanisms involve the gut–brain axis (gut hormones and gut neuronal signals) and adipose–brain axis (adipokines) (Figure 1.2) [49]. Thus, there must be coordination between the brain, gut and adipose tissue. Disturbance in this coordination can impair the regulation of energy homeostasis and result in weight gain and obesity [50].

1.6.1 Adipose–brain axis

Adipose tissue secretes a number of adipokines that provide the brain with information about long-term energy storage. These adipokines may enable the central regulation of energy homeostasis and help in the maintenance of body weight. The best-investigated adipokine that is involved in this action is leptin [51].

Leptin was cloned and sequenced in 1994 by Freidman's group [52]. It is produced in proportion to adipocyte mass, with serum leptin levels increasing as the percentage of body fat rises [53]. Other human organs such as the stomach, mammary tissue, placenta and heart also produce small amounts of leptin [48, 54, 55]. Leptin plays a major role in regulating energy homeostasis and is considered a long-term mediator of energy balance. This hormone is capable of crossing the blood–brain barrier (BBB), and high concentrations have been reported in cerebrospinal fluid (CSF). Leptin suppresses appetite and increases energy expenditure to maintain normal body fat mass [56-60].

It acts on the brain by binding to leptin receptors (LEPR-B) to inform on the status of energy stores. In the brain, it acts through its receptors in the hypothalamus, and in particular in two neuronal systems. The first system is POMC and CART, causing suppression of appetite and increase energy expenditure. The second system is AGRP and NPY, causing an increase in appetite and food intake, and which is suppressed by leptin [61, 62].

The first evidence for leptin as a major mediator of energy balance was provided by Montague et al. They examined severely obese children with a congenital leptin deficiency as a result of a homozygous frameshift mutation in the OB gene. The children were normal weight at birth and rapidly developed severe obesity accompanied by overeating and increased appetite [63]. In another study, by Farooqi et al., subjects with a partial genetic deficiency in leptin had a higher prevalence of obesity, and were found to have a lower serum leptin level than control subjects. In these cohorts, leptin treatment led to an improvement in satiety, reduction in appetite, decreased adiposity, and increased weight loss [60, 64].

Many obese individuals have high circulating leptin levels, and it is not known whether this increase in leptin is a cause or a consequence [65]. In addition, it has een shown that leptin treatment of most obese humans and animals is ineffective in reducing food intake and losing weight, so it has been suggested that an increased level of leptin in obese individuals is associated with leptin resistance [66, 67].

Leptin resistance is most likely preceded by a long period of hyperphagia caused by a disruption in the leptin system [68, 69]. Hyperphagia causes elevation in circulating leptin levels, and as a result, the hypothalamus is exposed to high leptin levels that may attenuate the action of leptin on its receptors in the hypothalamus, leading to insensitivity of the hypothalamus to leptin, and continuous elevation in systemic leptin levels [48]. It has been reported that leptin resistance in the hypothalamus appears after chronic central leptin infusion in rats [70], and it has been shown that overeating causes leptin resistance in human subjects [68].

Leptin binds to its receptor (LEPR-B) in the brain and activates the Janus kinase–signal transducer and activator of transcription (Jak-STAT) pathway, which is the main leptin signal transduction pathway. The binding process of leptin to the extracellular part of its receptor causes structural changes in the receptor that activate its related Jak-2 tyrosine kinase [71], and as a result induces phosphorylation of three intracellular tyrosine residues in LEPR-B. Phosphorylated Tyr985p stimulates SHP2 which involves ERK activation, and SOCS3 suppresses LEPR-B signalling as an inhibitory feedback. Phosphorylated Tyr1077 stimulates STAT5, and phosphorylated Tyr1138 stimulates STAT3 that facilitates the transcription of SOCS3 and causes its accumulation (Figure 1.3) [72-74].

It has been suggested that overproduction of SOCS3 and protein tyrosine phosphatase 1B (PTP1B), which are the negative regulators of leptin signalling, might attenuate LEPR-B activity and therefore contribute to leptin resistance [65]. Destruction of central nervous system (CNS) SOCS3 or LEPR-B Tyr985 reduces food intake and weight in mice [75-77]. Similarly, dysfunction of PTP1B in the brain of mice stimulates leptin signalling and reduces adiposity [78, 79].

In addition, other pathways might also induce the attenuation of leptin signalling. Endoplasmic reticulum (ER) stress and the state of chronic low-grade inflammation that are related to insulin resistance are promoted in peripheral tissues (e.g. adipose tissue, liver and muscle), and may also be involved in the limitation of CNS LEPR-B signalling in obesity (Figure 1.3) [80-82]. It has been

shown in obese mice that increased ER stress in the hypothalamus suppresses leptin signalling. Conversely, reduced ER stress activates leptin signalling [80]. It has also been shown in obese animals and in cultured cell models that stimulation of inflammatory signalling pathways in the hypothalamus can reduce leptin activity, whereas inhibition of inflammatory signalling pathways activates leptin signalling [81].

Therefore, SOCS3, PTP1B, ER stress and inflammation might have direct effects on the attenuation of leptin signal activity in the hypothalamus, and the subsequent development of leptin resistance seen in obese individuals.

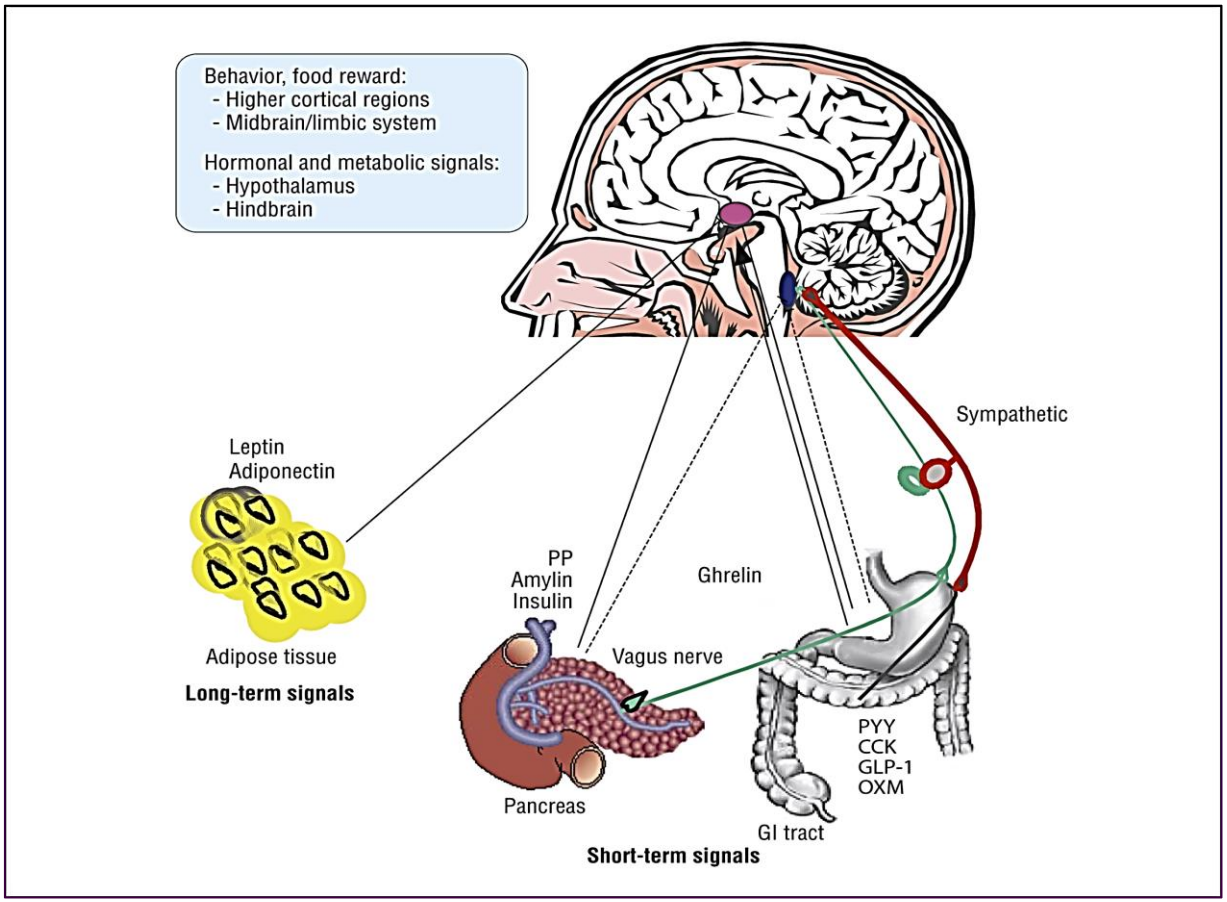

Figure 1.2 Hormonal regulation of energy homeostasis [51]
Gut and adipose tissues play a part in regulating energy homeostasis by producing anorexogenic and orexigenic hormones that act on bran satiety and hunger centres.

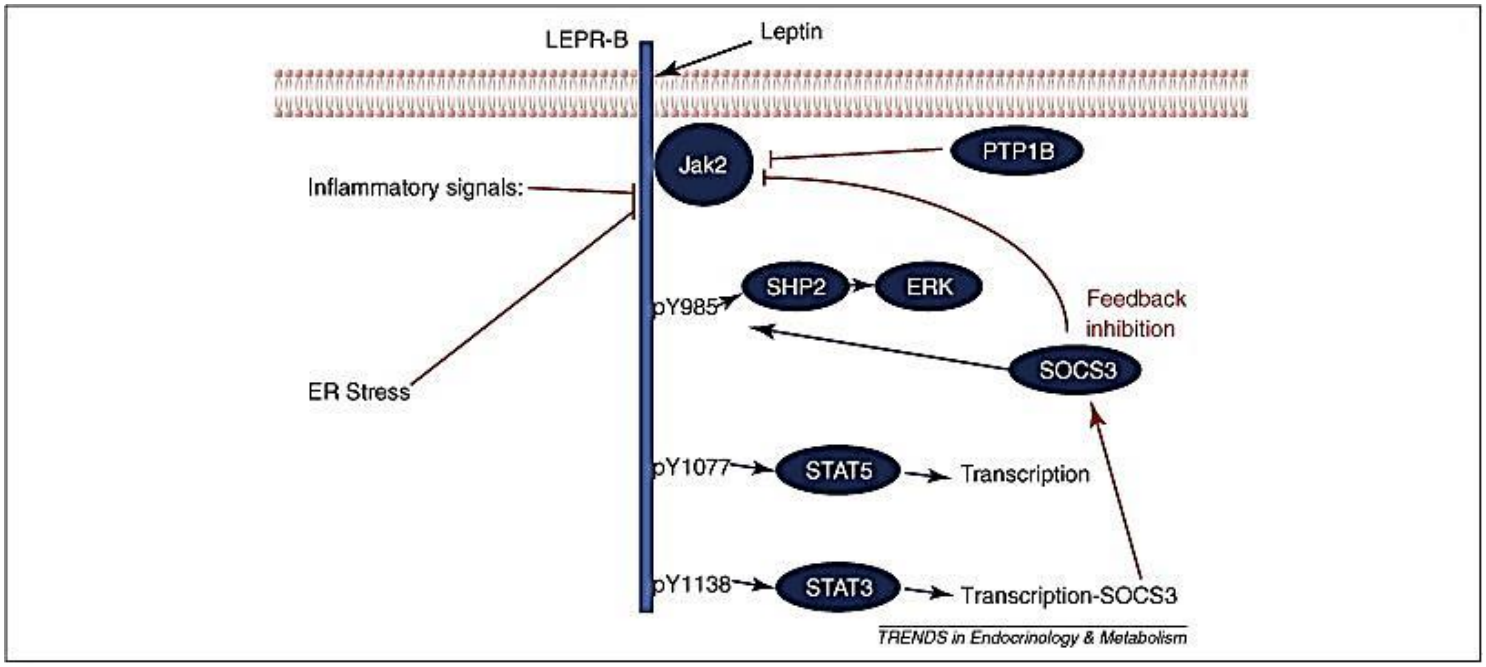

Figure 1.3 Action of leptin on its receptor (LEPR-B), and the development of leptin resistance [33]

Leptin binds to LEPR-B and activates Jak2, which phosphorylates three tyrosine residues on the intracellular tail of LEPR-B. Each one of the phosphorylated residues recruits a unique set of downstream signalling molecules. SHP2 that participates in ERK activation and SOCS3 which is the inhibitor of LEPR-B signalling are recruited by phosphorylated Tyr985 (pY985). The transcription factors STAT5 and STAT3 are recruited by pY1077 and pY1138, respectively. Attenuation of LEPR-B signalling (red lines) is related to a variety of processes, including the feedback inhibition which occurs by STAT3-promoted SOCS3 accumulation. PTP1B, ER stress and inflammatory signals may also induce the inhibition of LEPR-B signalling in obesity.

1.6.2 Gut–brain axis

Besides the important function of the gastrointestinal tract in absorption and digestion of food, it is also considered a major endocrine organ [49]. The production of gut peptides aids digestive function, but lately it has been discovered that it also influences eating behaviour and appetite [83]. Many of the gut hormones participate in controlling appetite through their central action on the brain, mainly on the orexigenic NPY system and the anorectic melanocortin system in the hypothalamus which have a crucial role in energy balance [49]. Recent studies have concluded that manipulation of gut hormones might help in controlling energy balance in obese individuals by reducing appetite and inducing weight loss [83]. The first gut hormone that was shown to affect appetite was CCK; other hormones have subsequently been discovered, such as GLP-1, PYY and ghrelin [84].

1.6.2.1 Ghrelin

Ghrelin is the only known gut hormone that increases food intake [85]. It is secreted by A cells located in the fundus of the stomach [86]. The concentration of ghrelin increases before each meal and decreases postprandially [84], which indicates its short-term effect in appetite regulation [85] through its stimulation of the hypothalamic arcuate nucleus and the production of anabolic NPY and AGRP [86]. The Tschop group showed that chronic administration of ghrelin in rodents results in continuous food intake and weight gain [87], so it seems that ghrelin also plays a role in regulating appetite and body weight in the long term [88].

1.6.2.2 Peptide YY (PYY)

PYY is a 36-amino-acid peptide that is secreted by enteroendocrine L cells of the gut, located in the intestines and mostly concentrated in the ileum and colon. Two forms of PYY are released after meal ingestion, $PYY_{(1-36)}$ and $PYY_{(3-36)}$, which belong to the PP-fold peptide family [89]. $PYY_{(3-36)}$ binds to Y_2 receptors on the

arcuate nucleus, increasing activity of the anorectic melanocortin system and suppressing activity of the orexigenic NPY system [89]. Studies have shown that peripheral administration of $PYY_{(3-36)}$ leads to a decrease in food intake in rodents and humans [90]. However, high levels of $PYY_{(3-36)}$ may cause nausea in dogs [91] and in humans [92]. Witte et al. concluded that the effect of $PYY_{(3-36)}$ in reducing food intake might be due to decreased gastric emptying and nausea [93].

1.6.2.3 Glucagon-like peptide 1 (GLP-1)

GLP-1 is a peptide hormone consisting of 30 amino acids, and is produced in response to nutrient ingestion by enteroendocrine L cells located in the small and large intestines, but mostly distributed in the ileum and colon [89, 94, 95]. GLP-1 is also produced centrally in the brain [96]. L cells are described as open-type epithelial cells that are in direct contact with ingested food in the intestinal lumen [97]. In addition, they are very close to the neurons and microvasculature of the intestine and are affected by neuronal signals and hormones [98, 99].

Proglucagon is a 160-amino-acid prohormone that is produced in enteroendocrine L cells and pancreatic α cells [100, 101]. GLP-1 is synthesized by posttranslational processing of proglucagon by the prohormone convertase 1/3 in L cells. Proglucagon gene expression is activated by β-catenin in intestinal L cells. This activation is mediated by the transcription factor TCF-4 [102]. Variants of TCF-4 are associated with development of type 2 diabetes, indicating that GLP-1 might be involved in the development of this disease [103].

GLP-1 forms

There are multiple forms of GLP-1 present in the circulation, such as GLP-1 (1-37) and GLP-1 (1-36) amide, which are not biologically active. However, the bioactive forms GLP-1 (7-37) and GLP-1 (7-36) amide are the major circulating active forms of GLP-1. It is believed that the addition of the amide group to GLP-1 (7-36) amide is mediated by the enzyme peptidylglycine α-amidating monooxygenase, and this extends the half-life of active GLP-1 in the circulation [104].

GLP-1 has a short half-life of 1–2 minutes in the circulation as it is rapidly broken down by the catalytic enzyme dipeptidyl peptidase IV (DPP-IV), which cleaves off the two NH_2-terminal amino acids, thus forming the inactive metabolites GLP-1 (9-36) amide or GLP-1 (9-37) [105]. DPP-IV is expressed in many cells and tissues including the intestines, kidneys, liver, lungs, CNS and pancreas. It is also expressed in endothelial cells lining the capillaries of the lamina propria, which are located close to the site of GLP-1 secretion. About two-thirds of the total amount of GLP-1 secreted is degraded by DPP-IV before entering the portal circulation. Further degradation is carried out in the liver, and only about 10–15% of intact GLP-1 reaches the systemic circulation [99, 101, 106]. The major route of GLP-1 clearance is through the kidneys [101].

Regulation of GLP-1 secretion

In humans, GLP-1 is released rapidly into the circulation after nutrient ingestion and oral glucose administration, but not intravenous glucose administration [107, 108], and the secretion depends on caloric intake [94]. GLP-1 is secreted in a biphasic pattern postprandially, an early short phase starting within 10–15 minutes, followed by a late longer 30–60-minute phase [107]. Ingested carbohydrate and fat have been shown to be especially strong stimulators of GLP-1 secretion [109]. In addition, direct administration of fat and glucose to the intestinal lumen also stimulates GLP-1 secretion [110, 111].

The early and rapid release of GLP-1 cannot be explained by direct interaction between the ingested nutrients and L cells, as L cells are dominantly present in the distal gut, and nutrients require more than half an hour to reach this part of the intestine [97, 112, 113]. Therefore, it is suggested that the early postprandial peak of GLP-1 is induced indirectly by the neuroendocrine pathway, unlike the later peak that is attributed to direct contact of luminal nutrients with L cells [111]. Evidence supports the suggestion that the nervous system is involved in the early peak of GLP-1 secretion. Researchers have found that stimulation of rat L cells is prevented by bilateral subdiaphragmatic vagotomy. However, GLP-1

secretion is increased with direct activation of the efferent coeliac branch [110]. *In vitro* studies have shown that acetylcholine has a stimulatory effect on L cells in humans and rodents [98, 114]. In addition, in rodents, duodenal nutrients fail to increase GLP-1 secretion after infusion with atropine and pirenzepine [98]. Somatostatin is an intestinal neuroendocrine hormone that has been found to inhibit GLP-1 secretion. *In vivo* studies have shown that infusion of somatostatin-14 inhibits GLP-1 secretion from L cells in both humans and rats [115, 116].

Leptin is also reported to have stimulatory effects on GLP-1 secretion. Administration of leptin to mice increases GLP-1 secretion. The same effect is observed in human L cells *in vitro* [117, 118]. Both basal and postprandial GLP-1 concentrations are reduced in obese subjects [119, 120]. In addition, increased leptin secretion and leptin resistance are observed in obese patients. Thus, it is hypothesized that as leptin stimulates L cells, leptin resistance may be responsible for the reduction in GLP-1 secretion reported in obese patients [118].

GLP-1 receptors

The GLP-1 receptor (GLP-1R) is a class-2 G protein-coupled receptor [121]. In the early 1990s, Bernard Thorens cloned and sequenced human and rat GLP-1R from their pancreatic islet libraries. Both receptors consist of 463 amino acids [122, 123]. Thorens also discovered two lizard peptides, exendin-4 and exendin 9-39, that can bind to human GLP-1. Exendin-4 shares 53% structural homology with mammalian GLP-1, and acts as a potent agonist; it differs from GLP-1 in that it is more stable, as it is not degraded by DPP-IV. Exendin 9-39 is a human GLP-1 antagonist [123, 124]. Liraglutide is a synthetic GLP-1 derivative of exendin-4, and has closer homology (97%) to human GLP-1 [125]. The GLP-1 receptor is distributed in the pancreas, central and peripheral nervous system, heart, stomach, kidneys, gastrointestinal tract and adipose tissue [101, 126-128].

GLP-1 and appetite

Like other gastrointestinal secreted hormones, GLP-1 is involved in controlling nutrient intake. The presence of GLP-1 receptors in the brain structures which are known to be involved in controlling food intake, including the PVN and dorsal medial nucleus of the hypothalamus (DMH), and area postrema and NTS in the brainstem, indicates the important action of GLP-1 in regulating energy balance [127]. Studies indicate that central administration of exogenous GLP-1 inhibits food intake in rats, which supports the anorexigenic effect of this peptide [129, 130]. GLP-1 infusions increase satiety and decrease energy intake in lean and overweight subjects [131].

Intracerebroventricular injection of GLP-1 has been shown to attenuate the action of the potent orexigenic agents NPY and melanin-concentrating hormone (MCH) in rats [132]. Furthermore, a lesion in the arcuate nucleus that contains the cell bodies of the NPY neurons results in complete inhibition of the anorectic action of GLP-1 in rats [133]. These results suggest that interaction between the anorectic peptide GLP-1 and the orexigenic peptides NPY and MCH may modulate the activity of feeding centres [134]. The anorectic effect of GLP-1 may be mediated either indirectly by acting in a paracrine-like fashion on peripheral GLP-1 receptor expressed in the intestinal vagal afferent neurons, or acting directly on central GLP-1 receptors after crossing the BBB [135, 136]. Intravenous administration of exendin-4 has also been shown to cross the BBB in mice [137]. Moreover, GLP-1 hormone may also indirectly affect food intake by reducing gastric emptying. Subsequently, stimulation of the satiety centre and a reduction in food intake are likely [138]. It is reported that postprandial GLP-1 levels are reduced in obese subjects; however, the mechanism by which obesity decreases GLP-1 levels is not, as yet, clear [139, 140].

GLP-1 and glucose homeostasis

Another important function of GLP-1 is its incretin effect. Gastric inhibitory polypeptide and GLP-1 are considered potent incretin hormones that are capable

of increasing insulin secretion and reducing blood glucose in response to oral nutrient intake. It is estimated that after oral glucose ingestion, about 50–70% of total insulin secretion is induced by this incretin effect. GLP-1 stimulates the biosynthesis and production of insulin from pancreatic beta-cells [106]. The incretin effect has been demonstrated by comparing the insulin response to oral and intravenous administration of glucose, with adjustment of an intravenous infusion of GLP-1 to produce the same peripheral blood glucose concentrations [141]. In a healthy subject, oral administration has been shown to cause a two- to threefold greater insulin response than the intravenous route [101]. Besides the incretin effect, GLP-1 plays a role in maintaining pancreatic beta-cell mass by stimulating beta-cell proliferation and inhibiting apoptosis [142, 143]. In the gastrointestinal tract, GLP-1 inhibits acid secretion. In addition, it reduces the increase in blood glucose levels associated with food ingestion by slowing gastric emptying. Other functions of GLP-1 related to normalizing blood glucose are inhibition of glucose production by the liver, inhibition of glucagon secretion, and an increase in insulin sensitivity [144-146].

The incretin effect seems to be very weak in patients with type 2 diabetes, and GLP-1 has been shown to be significantly reduced in some diabetic patients [147, 148]. Exendin-4, acting as a GLP-1 agonist, is currently used to treat type 2 diabetes. It lowers blood glucose without causing hypoglycaemia, increases insulin secretion by improving beta-cell function and decreases glucagon secretion. However, nausea and vomiting are common side effects of exendin-4; nausea occurs in approximately 50% of patients, and about 4% of them discontinue the treatment because of intolerable nausea [61, 149]. Studies in rats have shown that the effect of peripheral exendin-4 on causing nausea does not require vagal afferent pathways, as subdiaphragmatic vagotomy does not inhibit the nausea effect produced by chronic peripheral exendin-4 administration. Therefore, exendin-4 seems to induce nausea by penetrating the BBB, and subsequent activation of GLP-1R in the brain, mostly in the NTS [150].

GLP-1 and adipose tissue

GLP-1 has an important role in lipolysis and fatty acid synthesis; it has been shown to cause lipolysis in isolated rat adipocytes. It also stimulates fatty acid synthesis in omental adipose tissue cultures at its normal circulating concentrations. In 3T3-L mouse adipocytes, GLP-1 has been shown to induce insulin-dependent glucose uptake and stimulate glucose transporter 1 and 4 protein levels [151].

In rats, GLP-1 decreases macrophage infiltration and secretion of pro-inflammatory cytokines, such as interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α), in adipose tissue. Therefore it may help to inhibit the systemic inflammation associated with obesity and insulin resistance [152]. Some studies suggest that GLP-1 and GLP-1 agonists may regulate adipokine secretion. GLP-1 inhibits visfatin secretion from differentiated 3T3-L1 adipocytes [153]. Also, exendin-4 has been shown to stimulate adiponectin secretion, via a protein kinase A pathway *in vitro*, and to improve cellular insulin sensitivity [154]. In an *in vivo* study, central infusion of exendin-4 in lean and obese male rats resulted in reduced body weight in both lean and obese groups; however, this was accompanied by decreased plasma leptin concentrations only in lean, and not in obese, rats [155].

1.7 Ethnicity and GLP-1 secretion

The rapid increase in global obesity prevalence has increased the need to understand the aetiology of obesity in order to choose the best approaches for managing and preventing this disease. One causative factor of this phenomenon might be related to differences in satiety and incretin hormone secretion among different ethnic groups. As mentioned earlier, low postprandial GLP-1 secretion in obesity and type 2 diabetes might contribute to these disorders [140, 147]. GLP-1 analogue and DPP-IV inhibitor agents have started to be used worldwide as antihyperglycaemic medications and to help in reducing weight [156].

Obesity and type 2 diabetes prevalence is higher in African Americans than in Caucasians, both among adults and adolescents. In a study of 42 severely obese Caucasian and African American adult females, evaluating insulin and GLP-1 secretion in response to an oral glucose tolerance test (OGTT), it was found that fasting and postprandial insulin and GLP-1 secretions were higher in the African Americans than in the Caucasians. Thus the GLP-1 response might be one contributing factor related to the high prevalence of obesity, hyperinsulinemia and type 2 diabetes in African Americans [157].

Another study of 49 obese Caucasian and African American adolescents reported lower postprandial GLP-1 levels in African Americans, supporting the previous finding and a role for lower GLP-1 levels being related to the predisposition of obese African Americans to type 2 diabetes [158].

Similarly, a study in normal-weight, healthy or diabetic young Japanese found that intact postprandial GLP-1 concentrations remained low and did not peak in either the diabetic or non-diabetic groups after either OGTT or a meal challenge [159]. Supporting this result, DPP-IV inhibitors were found to be more effective in lowering HbA1c in Japanese diabetic patients than in non-Japanese diabetic patients [160]. In addition, GLP-1 analogues were also found to be more efficient in lowering HbA1c in Asians than in non-Asians [161].

1.8 Glucose-dependent insulinotropic polypeptide (GIP)

GIP was the first incretin hormone identified and was previously known as gastric inhibitory polypeptide, related to its ability to inhibit gastric acid secretion in dogs [162]. However, subsequent studies revealed its ability to also stimulate insulin secretion in response to nutrient ingestion in humans [163]. GIP is released from enteroendocrine K cells located mostly in the duodenum and proximal jejunum [95]. Glucose and fat are potent stimulators of GIP secretion in human subjects, leading to rapid release of GIP that reaches a peak in 15–30 or 30–45 minutes after oral ingestion of glucose or intraduodenal infusions of fat [164-166]. GIP secretion is proportional to the amount of calories ingested [94]. Furthermore, it

is the rate of intestinal glucose absorption rather than the mere presence of nutrients in the intestine that stimulates GIP production and an increase in GIP levels [167]. Thus, GIP secretion is reduced as a result of intestinal malabsorption, or after intraduodenal administration of pharmacologic agents reducing nutrient absorption [168, 169]. Patients with type 2 diabetes have been found to have a normal GIP plasma level [170]; however, the incretin effect of GIP is diminished and cannot be remedied by administration of exogenous GIP [171, 172].

Like GLP-1, GIP exerts its incretin effects though specific, glycosylated receptors belonging to the secretin B-family of G protein-coupled receptors [173]. Besides the presence of GIP receptors in pancreatic islets, GIP receptors are also found in adipose tissue, gut, several regions of the brain, testis, pituitary, lung, heart, vascular endothelium and bone [174].

1.9 Endogenous DPP-IV activity

DPP-IV, also known as CD26, is a membrane-associated peptidase that consists of 766 amino acids and is expressed in different tissues. In addition to the membrane-bound species, it is also found in a soluble form in the circulation [175-177]. The biological role of DPP-IV relates to both its enzymatic and non-catalytic functions. DPP-IV interacts with various ligands such as ADA, caveolin-1, kidney Na+/H+ ion exchanger 3, thromboxane A2 receptor, CXCR4 and fibronectin, allowing it to mediate different processes including immune regulatory function, such as stimulating T-cell activation [178-182]. The catalytic role of DPP-IV is exhibited both by the membrane-spanning form of the molecule and by the circulating soluble form. DPP-IV breaks down bioactive peptides with an amino-terminal proline or alanine at position 2 [183, 184]. GIP and GLP-1 hormones are peptides with an alanine at position 2 that renders them ideal putative substrates for the aminopeptidase DPP-IV [185]. Thus this enzyme plays an indirect role in regulating glycaemia through inactivation of incretin hormones [105]. DPP-IV inhibitor is used as a therapeutic drug for type 2 diabetes as it prevents the inactivation of GLP-1 by DPP-IV. In October 2006, the Food and Drug

Administration approved sitagliptin as the first selective DPP-IV inhibitor, for use alone or in combination with metformin or thiazolidinedione to treat type 2 diabetes [186].

1.10 Glucagon

Glucagon is a 29-amino-acid peptide hormone that is released from pancreatic α cells [187]. This hormone plays a critical role in the regulation of glycaemia. It opposes the action of insulin during hypoglycaemia by stimulating hepatic glucose synthesis and mobilization, thereby increasing blood glucose concentrations [188-190]. Insulin, GLP-1 and somatostatin inhibit glucagon production. Interestingly, the incretin hormones GIP and GLP-1 have different effects on glucagon secretion, with GIP stimulating but GLP-1 inhibiting glucagon secretion [191-194]. Using isolated islets, it has been demonstrated that the inhibitory effects of GLP-1 are not associated with augmentation of insulin or somatostatin secretion, but involve PKA signalling [193].

1.11 Non-surgical treatment of obesity

1.11.1 Diet

As energy imbalance is one of the major causes of weight gain, the treatment of obesity should be as simple as reducing eating and increasing physical activity! Different dietary approaches are used for weight loss [195]. Low-calorie diet (LCD, 800–1000 kcal/day), very low-calorie diet (VLCD, 450 to < 800 kcal/day) and meal replacement (200–400 kcal/day) therapies are common dietary approaches to reduce excess weight [196]. With VLCD, in the first 6 months, weight loss is about 2 kg per week; this then decreases to 0.8 kg per week over the next 6 months [196]. However, rapid weight regain is reported at 12 months [197]. Thus, diet alone appears to be ineffective in the long-term maintenance of weight loss.

1.11.2 Physical activity

Exercise is commonly considered an important component of a weight-loss programme. However, studies have shown that exercise alone is ineffective as a means of weight loss, and dietary changes seems to be more effective for this [198, 199]. Exercise, however, is the best way of preserving lean body mass, which is often lost when following LCD [200].

1.11.3 Behavioural therapy

Behavioural treatments help obese individuals to change their behavioural pattern by developing adaptive thinking, eating and exercise habits that enable them to reduce their weight and avoid regaining weight. People who add behavioural treatment to caloric restriction and exercise may expect to lose about 5% to 10% of their total weight in about 4 to 6 months [201].

1.11.4 Pharmacotherapy

There are two modes of action of weight-loss drugs, reducing nutrient absorption or reducing food intake. Orlistat is the most popular drug for treating obesity. It is a hydrogenated derivative of a bacterial lipase inhibitor which inhibits pancreatic lipase, thus reducing triglycerides. The digestion of about 30% of orally ingested triglycerides is inhibited by Orlistat. This drug has undesirable effects, such as faecal urgency, incontinence and malabsorption of fat-soluble vitamins. Moreover, this drug has not been effective in long-term (more than 1 year) studies [202].

Sibutramine is a phenethylamine that selectively blocks the reuptake of noradrenaline, serotonin (5-HT) and, to a lesser extent, dopamine, and subsequently reduces food intake. The drug was withdrawn from use in Europe in 2010 due to its related cardiovascular risks [203].

Currently, gut peptides (PYY, GLP-1, ghrelin and oxyntomodulin) are novel targets for developing new obesity treatments. Exendin-4 and liraglutide have been shown to produce ~3 kg reduction of body weight in 26 weeks [204]. However,

nausea is the main side effect of these synthetic GLP-1, which prevents the administration of higher doses that might have greater effects on weight loss [205]. PYY infusion has been found to lower the orexigenic effect of ghrelin by decreasing its level and reducing hunger and food intake in obese and lean individuals [206].

Combination of two or more of the gut peptides may lead to more effective weight loss. PYY and GLP-1 have an additive effect on reducing food intake, both when administrated peripherally in mice, and after intravenous infusion in humans [207].

1.12 Bariatric surgery

Today, bariatric surgery is known as the most effective way of treating obesity and its associated comorbidities. It produces significant weight loss that is maintained long term, improves metabolic abnormalities, and subsequently achieves complete resolution of insulin resistance, diabetes, dyslipidaemia, hypertension and obstructive sleep apnoea in the majority of patients [208]. The exact mechanism(s) by which bariatric surgery produces weight loss is still not fully understood.

Patients that are eligible for bariatric surgery include morbidly obese (BMI greater than 40 kg/m^2) or obese patients with a BMI of more than 35 kg/m^2, with serious comorbidities (NICE guidelines, 2006). Several different types of bariatric surgery are currently available, and they have been classified into different types: restrictive, malabsorptive and a combination of both [209]. The most common types performed are restrictive and a combination of malabsorptive and restrictive (Figure 1.4).

Restrictive procedures involve decreasing the stomach size, thereby reducing the amount of food intake by inducing early satiety. Gastric band (GB), a reversible procedure which involves placing a band around the upper part of the stomach below the gastro-oesophageal junction to reduce stomach capacity [210], and sleeve gastrectomy (SG) which involves excision of 70–80% of the greater

curvature, creating a narrow stomach tube, are examples of the restrictive type of weight-loss procedure [211, 212].

Malabsorptive procedures create a short bowel syndrome by bypassing most of the absorptive part of the small intestine, thereby preventing food from contact with crucial absorptive points of the intestinal tract. Jejunoileal bypass is an example of a malabsorptive procedure that achieves remarkable long-term weight loss. However, because of multiple significant complications in the majority of patients, it has fallen into disrepute and is no longer used [210].

A combination of restrictive and malabsorptive procedures leads to decreased food intake by decreasing stomach size. Roux-en-Y gastric bypass (RYGB) is an example of a restrictive/malabsorptive procedure and is the most common and effective weight-loss operation in current use. In RYGB, a small stomach pouch of approximately 30 ml is created and connected to the small intestine. The rest of the stomach (approx. 400 ml) is bypassed. The small intestine is divided approximately 45 cm below the lower stomach outlet and is re-arranged into a Y-configuration, enabling outflow of food from the small upper stomach pouch via a 'Roux limb'. The Roux limb is constructed using 80–150 cm (31–59 in) of the small intestine, preserving the rest of it for absorbing nutrients. The patient will experience very rapid onset of the stomach feeling full, followed by a growing satiety (or 'indifference' to food) shortly after the start of a meal [210].

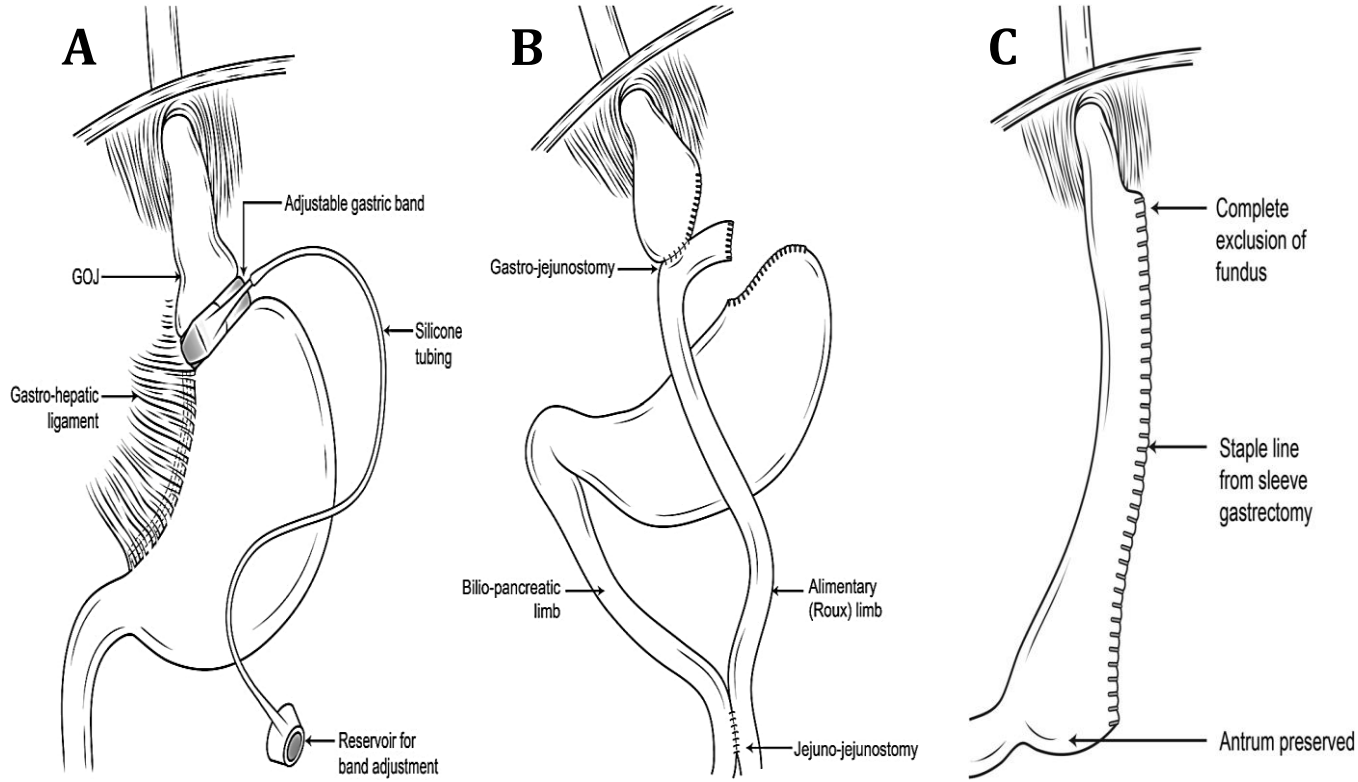

Figure 1.4 Different types of bariatric surgery. A) Adjustable gastric band, B) Roux-en-Y gastric bypass, C) sleeve gastrectomy

1.12.1 Weight loss after bariatric surgery

Non-surgical treatment and bariatric surgery are the usual obesity treatment options. The non-surgical option includes low caloric intake, increasing physical activity, behavioural therapy and different pharmacological treatments. Many studies have demonstrated the efficacy of bariatric surgery in achieving long-term and greater body weight loss, compared with non-surgical treatment [213]. A randomized controlled trial showed that at 2 years, laparoscopic adjustable GB resulted in a greater weight loss, with an average of 21% of initial weight lost, while the non-surgical treatment resulted in only 5.5% of initial weight lost [214]. A meta-analysis reported a mean percentage of excess weight loss (EWL) 2 years after surgery of 61.2% for all bariatric procedures, ranging from 47.5% for GB to 61.6% for RYGB [215]. Greater weight loss was achieved by the RYGB procedure than by GB [216]. A 10-year follow-up of bariatric surgery reported a mean weight loss of 25% for RYGB, and 13.2% for GB. Although greater weight loss is seen with the RYGB procedure, both surgeries maintain long-term weight loss that may last beyond 10 years [217].

Several studies have shown that SG and RYGB achieve a similar amount of weight loss at 6, 12 and 18 months [218, 219]. However, there is still insufficient data with regards to the long-term weight-loss effect of SG [220].

Although both GB and SG are considered constrictive types of bariatric surgery, SG results in greater weight loss than GB. SG involves resection of a great part of the gastric body and antrum where the ghrelin-secreting mucosa is located, and thus attenuates the orexigenic effect of ghrelin [221].

1.12.2 Metabolic advantages of bariatric surgery

Bariatric surgery is currently accepted as a metabolic surgical intervention. Diabetes resolution occurs in 76.8% of patients, and diabetes improves in 86.6% of patients following a bariatric procedure [215, 216]. Moreover, long term, 10 years after bariatric surgery, diabetes resolution has also been reported in 82.9% of patients [210]. However, the type of surgery results in different outcomes:

83.7% diabetes resolution is observed after RYGB, whereas 47.9% is observed after GB. Bypass techniques act more efficiently in terms of improving insulin sensitivity and secretion, especially very early after surgery (first week), even before obtaining a significant weight loss. The SG procedure also induces rapid metabolic changes postoperatively. Fasting serum glucose and insulin are reduced significantly 5 days after SG [222]. However, despite the comparable weight loss achieved by RYGB and SG procedures, better and more durable glycaemic control is seen 2 years after RYGB, but not SG [223]. Thus, the best effect on controlling glycaemia is obtained by the RYGB technique [224].

Unlike RYGB and SG, an improvement in insulin sensitivity and glycaemia is only apparent 6 months after GB surgery, which indicates that the metabolic improvement seen after GB is mostly secondary to weight loss [225].

Improvement in lipid profile had been reported in 20% of patients after bariatric surgery [226]. One year after RYGB, total cholesterol levels are reduced by 16%, triglyceride by 63%, low-density lipoprotein (LDL) cholesterol by 31%, very low-density lipoprotein cholesterol by 74%, and total cholesterol/high-density lipoprotein (HDL) cholesterol risk ratio by 60%; HDL cholesterol concentrations increase by 39%. Moreover, within 1 year postoperatively, 82% of patients discontinue their lipid-lowering medications. This improvement has been observed at 3 months after RYGB and may last up to 1 year [227]. Moreover, a recent study showed that significant improvement in lipid profiles occurs as early as 2 weeks after RYGB and SG [228].

A retrospective analysis by Zhang et al. reported that there is improvement in lipid profiles after an SG procedure, especially in HDL cholesterol and triglycerides, with no change in LDL and total cholesterol [229]. It was also found that the improvement in HDL and triglyceride seen after laparoscopic SG is comparable to that seen after RYGB [230].

GB has also been shown to significantly reduce triglycerides and increase HDL cholesterol at 1 and 2 years postoperatively, and this improvement may persist for up to 5 years [231]. Thus, it seems that the RYGB procedure is better in terms of improving all the variables of lipid profile (triglycerides, HDL, LDL and total

cholesterol) and is more efficient in treating hyperlipidaemia [229].

Hypertension is another component of metabolic syndrome. Improvement in hypertension has been reported in 27% of patients after bariatric surgery [226]. A meta-analysis showed that 78.5% of all patients undergoing bariatric surgery have resolution or improvement in their hypertension over the first and second years postoperatively, but it is not sustained beyond the second year [215]. The reduction in hypertension after bariatric surgery appears to reach a plateau around 2 years postoperatively [232].

Obstructive sleep apnoea (OSA) is a common condition associated with morbid obesity. This condition results from elevation of upper airway collapse that is associated with ineffective respiratory efforts during sleep. Obstruction of the upper airway structures is due to excessive fatty tissue and its laxity, a condition that is associated with morbid obesity [233]. Weight loss is the most effective management of OSA. Bariatric surgery has been found to be very effective in the resolution of OSA [233]. After laparoscopic RYGB, patients with OSA show improvement from the first month postoperatively, and some discontinue the use of continuous positive airway pressure (CPAP) by the sixth month following surgery. Weight loss leads to a reduction in upper airway adipose tissue and improves the pharyngeal neuromuscular control, and subsequently decreases upper airway obstruction [234].

1.12.3 Hormonal changes after bariatric surgery

The advantageous effects of bariatric surgery in terms of weight loss and metabolic improvement are due, at least in part, to alteration in gut hormones and adipokines [235]. Plasma visfatin decreases and adiponectin increases 3 months after an RYGB procedure [236]. RYGB induces an acute decrease in leptin, improvement in insulin sensitivity and increase in postprandial insulin GLP-1 and PYY that are sustained for at least a year, leading to suppression in appetite and long-term weight loss [235, 237, 238]. While immediate improvement in glucose homeostasis following RYGB independent of weight has been explained by the

increased insulin response and enhanced insulin sensitivity caused by exaggerated GLP-1 [235, 239], the exact mechanism by which RYGB alters gut hormone secretion is still uncertain. However, it is hypothesized that bypassing the proximal part of the small intestine causes rerouting of ingested food and digestive fluid, and as a result changes the gastrointestinal relationship with nutrients. Supporting this theory, the beneficial metabolic effects of purely restrictive bariatric procedures, such as GB, usually appear later, following weight loss. These procedures do not produce acute improvement of postprandial glucose metabolism or gut hormones. Rerouting undigested food causes rapid contact between ingested nutrients and L cells located in the small intestine (ileum) at a higher density, and thus directly stimulates these enteroendocrine cells without stimulating the bypassed foregut [240].

Therefore, the RYGB technique induces changes in gastrointestinal endocrinology in a way that reduces overeating and improves glucose homeostasis, by increasing secretion of incretin and satiety hormones including GLP-1, PYY and oxyntomodulin [237, 240].

RYGB may also produce increased GLP-2, a gut repair hormone, and subsequent intestinal mucosal crypt cell proliferation. Furthermore, a study in rats showed an elevation in the number of L cells after gastric bypass and concluded that the elevation in PYY and GLP-1 levels seen after this procedure may be due to the increased L-cell population [241, 242].

The hypothesis that there is metabolic improvement pertaining to increased GLP-1 secretion after RYGB has been argued, as such metabolic advantages can be seen only modestly after subcutaneous administration of GLP-1 receptor agonist, despite resulting in equal levels of circulating GLP-1.

It is thought that the effects of endogenous GLP-1 are mediated by activation of local afferent sensory nerve fibres (vagal nerve) that then activate certain regions in the NTS and hypothalamus which influence insulin and glucose metabolism. This effect is absent with exogenous GLP-1 [240].

Like RYGB, SG may also have an acute effect on appetite and glucose metabolism related to early hormonal changes. Ghrelin secretion decreases, whereas PYY and

GLP-1 increase acutely following SG. Thus SG is no longer considered a purely constrictive type of bariatric surgery [243-245]. The mechanism by which SG results in acute alteration in gut hormones is as yet unclear. However, ghrelin reduction is due to gastric fundus removal [244].

This alteration in gut hormones is not found after a GB procedure. It has been reported that postprandial GLP-1 and PYY levels are increased after RYGB but not GB surgery [246, 247].

1.12.4 Complications of bariatric surgery

High rates of complications, approaching 40%, were reported in 2001 following bariatric surgical treatment of obesity. These complications usually vary according to the procedure performed [248].

1.12.4.1 Roux-en-Y gastric bypass

Stomal stenosis: The anastomotic stenosis rate is 6–20% following RYGB [249]. It usually occurs several weeks after surgery, and patient presents with nausea, vomiting and gastroesophageal reflux; diagnostic and therapeutic endoscopy is used to diagnose the case, and treat it with balloon dilation [250, 251]. Stomal stenosis is more frequent after laparoscopic RYGB than open RYGB [252].

Marginal ulcer: Marginal ulceration has been observed in 1–16% of patients [253]; it occurs close to gastrojejunostomy. Nausea and vomiting, pain, bleeding and perforation are common complaints of this condition [254]. Possible causes of ulceration include: ischaemia at the anastomotic site that results in inadequate tissue perfusion, the presence of a nonabsorbable suture or staple around the area, jejunal injury from excess acid exposure, usage of nonsteroidal anti-inflammatory drugs, and smoking. Upper endoscopy is used to diagnose marginal ulcer [253, 255-257].

Cholelithiasis: Gall stones are a common complication of RYGB; it has been reported that as many as 38% of patients develop cholelithiasis within 6 months of surgery, and symptoms appear in 41% of such patients [258]. Symptomatic patients present with severe right upper quadrant abdominal pain associated with nausea and vomiting [259]. It is believed that developing gallstones is associated with rapid weight loss [260]. Abdominal ultrasound is used to diagnose cholelithiasia [261].

Internal hernias: The Roux-en-Y technique involves the creation of potential internal spaces through which herniation of the small bowel may occur [262]. Internal hernia incidence has been reported in 0–5% of patients undergoing laparoscopic bariatric surgery [252, 263]. Internal hernia can be complicated by small bowel obstruction [262]. Patients present with abdominal pain accompanied by nausea and vomiting. A CT scan of the abdomen and pelvis is used to diagnose internal hernia [264].

Dumping syndrome: Dumping syndrome can be found in up to 50% of patients who have undergone RYGB and is associated with ingesting greater amounts of simple carbohydrates. Dumping may help with losing weight in part by making the patient modify his/her eating habits [265].

Dumping syndrome can be classified into early and late:

Early dumping syndrome — Early dumping syndrome occurs early postprandially, usually within 15 minutes. It is most likely due to rapid food emptying into the small intestine. As a result of hyperosmolality of the food, fluid shifts rapidly from the plasma into the bowel; subsequently, hypotension and a sympathetic nervous system response occur. Patients often experience abdominal pain, nausea and tachycardia [266]. Avoidance of foods containing simple carbohydrates and replacing them with high-fibre, complex carbohydrates and protein-rich foods is recommended. Changing eating habits, such as eating small, frequent meals, and leaving a 30-minute interval between intake of solids and liquid are also advised. Early dumping usually disappears 3 months after

RYGB without requiring medical interference [265].

Late dumping syndrome — Late dumping syndrome occurs 2–3 hours postprandially and is associated with hyperglycaemia followed by insulin response leading to hypoglycaemia. Dizziness, fatigue, diaphoresis and weakness are presentations of late dumping. Similar management to that used for early dumping syndrome is applied to late dumping [265].

Nutritional deficiency: Nutritional depletion is common in severely obese individuals and can be aggravated following RYGB surgery due to reduced food intake and decreased absorption of different micronutrients, in particular iron, vitamin B12, folate, calcium and thiamin, and this deficiency can lead to various conditions, mostly anaemia and neural dysfunction. Therefore, multivitamin and mineral supplementation is crucial after RYGB surgery [267]. However, even with supplementation, patients still experience depletion in some micronutrients [268].

1.12.4.2 Gastric band (GB)

Stomal obstruction: Stomal obstruction is a potential complication that can occur early after GB surgery in about 14% of patients. The causes of obstruction include: incorporation of excess perigastric adipose tissue, using a band with insufficient diameter to include the whole thickness of the tissue, or developing oedema in tissue. The patient usually complains of nausea, vomiting and inability to tolerate food intake. The diagnosis is confirmed with an upper gastrointestinal barium meal and oesophagogastroscopy that illustrate obstruction and no passage of contrast beyond the band [269-271].

Port infection: The incidence of port site infection is between 0.3% and 9% in GB patients. The infection is usually treated with surgical removal of the port; once the infection subsides, the port is reimplanted [272, 273].

Band erosion: Band erosion of the stomach wall is a late complication that has been reported in about 7% of GB patients. Erosion can be caused by stomach wall ischaemia that arises from an excessively tight band, mechanical trauma contributing to the usage of band buckle, or thermal trauma from electrosurgical energy sources when placing the band; infection, stopping losing weight, and nausea and vomiting are clinical signs associated with this condition [274, 275].

Oesophageal dilatation: Oesophageal dilatation has been reported in up to 10% of patients. This condition is known as pseudoachalasia syndrome and may arise when the band is overinflated or on ingesting excessive amounts of food [276].

1.12.4.3 Sleeve gastrectomy (SG)

Stenosis: Stenosis can cause gastric outlet obstruction. Incidence of this complication varies from 0.7% to 4%. Patients usually present with dysphagia, vomiting, dehydration and an inability to tolerate food intake. Endoscopy is used to diagnose this condition [277, 278].

Gastric leaks: Gastric leaks are considered a serious complication of SG that have been reported in up to 5.3% of patients. Fever, tachycardia and tachypnoea are usually experienced by patients. Diagnosis is established by upper gastrointestinal radiograph [279].

Reflux: Gastro-oesophageal reflux has been reported in 47% of SG patients. The classical symptoms of this case are burning pain, heartburn and regurgitation. It is usually treated with a proton pump inhibitor [279, 280].

1.13 Endoscopic weight-loss surgery

An endoscopic approach for obesity management has developed as a result of an attempt to produce some of the anatomical manipulations and physiological effects of traditional weight-loss surgery with a less invasive and incisionless

procedure as well as fewer perioperative complications and a shorter recovery time [281].

Other indications for performing an endoscopic procedure are considering such intervention in individuals with early-stage obesity who do not require invasive bariatric surgery, in morbidly obese individuals before proceeding with invasive bariatric surgery to lower peri- and postoperative risks associated with obesity, and in patients with metabolic disorders, in particular type 2 diabetes, as a primary surgical intervention [281].

As with bariatric surgery, the endoscopic technique can be constrictive, reducing gastric capacity by using a space-occupying object or gastric suturing or stapling, malabsorptive, creating malabsorption by preventing passage of ingested nutrients through the duodenum and the proximal part of the jejunum, or a combination of both [281].

1.13.1 Constrictive endoscopic methods

1.13.1.1 Intragastric balloon

This technique was one of the first endoscopic gastric restrictive procedures available. The first intragastric balloon was the Garren-Edwards bubble (GEB) that was mostly used in the United States during the 1980s. The Food and Drug Administration (FDA) approved GEB in 1985 [282].

The bioenteric intragastric balloon (BIB) was first used in 1999. This technique of intragastric balloon has been widely studied worldwide. In spite of that, BIB has not been approved by the FDA and is not used in United States; it is approved and used in many other regions such as Europe, Australia, Canada, Mexico, India and several South American countries [281]. It is performed through placement of an intragastric adjustable silicone elastomer balloon with spherical shape, therapy which reduces gastric volume and enhances satiety. The balloon is then removed after about 3–6 months, after achieving the desirable weight, by using endoscopy with or without sedation.

It has been shown that balloon treatment lasting for 6 months results in an

average weight loss of 15 kg [283]; however, the main concern related to such a technique is weight regain after balloon removal [284]. Moreover, although it carries less risk than invasive bariatric surgery, some complications are recorded following this procedure, such as gastric ulceration and erosion, oesophageal laceration and small bowel obstruction [282]. A retrospective study showed a low complication rate of about 2.8%, with EWL of 33.9% after BIB [285].

It has been reported that leptin decreases significantly at 6 months (at the time of balloon removal), whereas no significant change is seen in adiponectin, PYY or ghrelin after 6 months of having the intragastric balloon [286-288].

The Spatz adjustable balloon system (SABS) was introduced to overcome the limitations associated with BIB. The balloon volume can be adjusted while in the stomach and can be left in the stomach for 1 year; 36% and 48.8% EWL has been reported at 6 and 12 months, respectively [289]. A study was conducted to compare the 1-year effect on weight loss between BIB and SABS. In the BIB procedure, two balloons were used; the first balloon was placed for 6 months, after which it was extracted, and patients were given a 1-month holiday before placing the second balloon for another 6 months. It was concluded that both procedures yield similar weight loss [290].

A new technique used for intragastric balloon, a thin-walled, gas-filled, swallowable intragastric balloon (Obalon) has been designed recently. This technique does not require the use of upper gastrointestinal endoscopy to remove the balloon, and it allows gastric volume titration by swallowing additional balloons (up to three balloons). It has been reported that this procedure induces significant weight loss at 4, 8 and 12 weeks without serious complications; however, nausea and gastric pain are the most common side effects [291].

1.13.1.2 Endoscopic gastric stapling/suturing

The endoscopic stapling procedure was designed to create a small and restrictive gastric cavity, therapy which induces early satiety and reduces food intake. Endoluminal gastric stapling was first done in 2007, and since then, different

locations, techniques and devices have been used. In the beginning, it was tested in rats, and gastric plication of the greater curvature was found to induce greater weight loss than plication of the anterior wall [292]. Human trials showed a similar result, with 23% EWL after plication of the gastric anterior wall, and 53% EWL after plication of the greater curvature [293, 294].

Endoluminal vertical gastroplasty is one of the suturing techniques; it uses an EndoCinch device to create a continuous suture pattern from the proximal fundus to the terminal body, forming a narrow tube-like shape of the stomach [282].

Transoral gastroplasty (TOGA) is another endoluminal procedure that restricts stomach volume by creating endoscopic stapling of the lesser curvature; 24% EWL at 6 months is reported for this technique [294].

Transoral endoscopic restrictive implant system (TERIS) is a recent endoscopic technique that uses a permanent implant as a restrictor, with a 10-mm orifice for food passage, and which is usually placed at the gastric cardia; the restrictor can be removed at any time. However, TERIS may result in serious complications such as gastric perforation or pneumoperitoneum [281].

Nausea, vomiting and pain are common side effects seen after gastric stapling [294]. It has been reported that reoperation is required for complications such as intractable nausea, total dysphagia or total gastric obstruction [293, 295, 296].
A new novel procedure has been used recently for weight reduction and is known as primary obesity surgery endoluminal (POSE); details of this procedure are mentioned in Chapter 4.

1.13.2 Malabsorptive endoscopic methods

Beside the weight-loss effect of endoluminal malabsorptive procedures, they have a positive effect on obesity-related metabolic disorders [281].

1.13.2.1 Duodenojejunal bypass sleeve

A duodenojejunal bypass sleeve is performed using endoluminal implants; a 60-cm sleeve is placed in the duodenum to bypass the duodenum and proximal jejunum, thus allowing food passage without contact with the duodenum or biliary and pancreatic secretions. This therapy delays digestion and alters production of some gut peptides such as GLP-1 [282].

A trial on obese diabetic patients undergoing this technique showed a good result after 52 weeks, with improved glycaemia and 39% EWL [297].

1.13.2.2 Gastroduodenojejunal bypass sleeve

This novel endoluminal approach is used in order to get physiological effects similar to those seen after gastric bypass bariatric surgery. The device is 120 cm long; it is placed in the stomach and extended through duodenum and jejunum endoscopy, and is then anchored at the gastroesophageal junction with endoscopic and laparoscopic techniques. This sleeve prevents nutrient absorption throughout the stomach, duodenum and jejunum [281].

A trial was conducted on 24 obese patients, of whom 12 were obese and underwent gastroduodenojejunal sleeve to assess the efficacy of this technique on weight loss as well as glycaemic control. Seventeen patients completed the trial, and evaluation at 12 weeks revealed 39.7% EWL, with complete resolution of diabetes in these patients and discontinuation of antihyperglycaemic medication [298].

1.14 Aims of this thesis

The aims of this thesis were to:

1- Investigate whether persistent nausea and vomiting symptoms after RYGB could be due to exaggerated systemic GLP-1 levels.

2- Investigate whether GLP-1 may directly inhibit adipocyte leptin secretion.

3- Compare a novel technique (POSE) with RYGB with regards to effective weight loss and induction of favourable metabolic and hormonal changes.

4- Investigate whether obesity and insulin resistance are associated with decreased postprandial levels of GLP-1 in subjects of Arab origin.

Chapter 2

Materials and Methods

2.1 Study population

2.1.1 Patient recruitment

<u>Study 1:</u> Prolonged nausea and vomiting after metabolic surgery

Subjects were recruited from a bariatric clinic (North London Obesity Surgery Service, Whittington Hospital, London, UK) and by local advertising at University College London. Group 1 consisted of ten female patients with nausea and vomiting symptoms. These patients had undergone laparoscopic RYGB and were characterized by persistent nausea and vomiting that had started at least 1 year after having had the surgery and lasted for over 6 months. Endoscopies were done to exclude a mechanical cause for their symptoms, such as anastomotic ulcer or stomal stenosis (section **1.12.4.1**). Group 2: ten patients without nausea and vomiting. These patients had undergone laparoscopic RYGB but reported no abnormally prolonged symptoms of nausea and vomiting. Age, gender, ethnicity, BMI and time of surgery were all matched with Group 1. Group 3: seven morbidly obese subjects from those awaiting bariatric surgery. Group 4: six overweight/obese subjects not scheduled for any surgery. Group 5: nine normal-weight healthy subjects. The last couple of groups were recruited by local advertising. The treating surgeon referred the symptomatic patients for the study. The asymptomatic patients and those on a waiting list were contacted by phone and they agreed to participate in the study.

<u>Adipose tissue study</u>

Six non-diabetic morbidly obese patients undergoing laparoscopic bariatric surgery (gastric bypass, gastric sleeve or GB) were recruited from a bariatric clinic (North London Obesity Surgery Service, Whittington Hospital, London, UK). Morbid obesity was classified as BMI ≥ 40 kg/m^2.

<u>Study 2:</u> Comparison of primary obesity surgery endoluminal (POSE) and RYGB

The surgeon explained both POSE and RYGB to patients awaiting weight reduction surgery, along with the risks and benefits of each of the procedures.

Patients were then allowed a period of time with the information on both procedures, to consider having either one of the surgeries. Following this, six of the patients agreed to undergo POSE, while the remaining majority preferred to go for RYGB.

<u>Study 3:</u> Post-absorptive and postprandial GLP-1 concentrations in an obesity-prone population (Arabs)

Twenty-seven healthy female subjects were recruited from the staff of the Anti-Doping Lab, Doha, Qatar (ADLQ), of whom seven were obese, eight overweight and 12 normal weight.

Exclusion criteria were patients on current treatment of warfarin, malignancy or terminal illness.

The studies were approved by the national ethics committees. The studies that were carried out in London were approved by Whittington Health; the REC reference number is 12/LO/0625. The study in Doha was approved by the Institutional Research Board of the ADLQ (SCH-ADL-070). Written informed consent was obtained from all subjects.

2.1.2 Demographic and anthropometric data

Weight (kg) and height (m) were measured, and BMI was calculated. Blood pressure and pulse were measured using a digital monitor (Datex-Ohmeda Patient Monitor, GE Healthcare, UK). Patient information including demographic data (date of birth, gender, ethnicity), surgery type (gastric bypass, POSE), medical history, current medication, weight-loss history, history of any physical activity, smoking habits and alcohol consumption were obtained and recorded.

2.1.3 Meal challenge test

Patients were asked to attend the bariatric clinic after an overnight fast. Blood samples were drawn from an antecubital vein into one tube with no anticoagulant added (serum), one with EDTA as the anticoagulant, one with NaF as the

anticoagulant (for measurement of plasma glucose concentrations), and another with EDTA along with a DPP-IV inhibitor to prevent breakdown of the active form of GLP-1. Patients were asked to ingest a mixed meal (different number of calories according to each study), and blood was collected at 0 minutes (prior to meal) and at 30, 120 and 180 minutes after the meal.

Blood samples were placed in a centrifuge (3000 rpm, 15 minutes, 23 °C), and the plasma or serum was collected and stored at −80 °C until analysis.

2.1.4 Intervention treatment

Symptomatic patients who suffered from chronic nausea and vomiting following RYGB were treated with subcutaneous injection of octreotide (50 mg) three times a day for 3 months.

2.2 Blood measurement

2.2.1 Assays

Plasma glucose levels were determined with glucose hexokinase reagent (Roche, CA, USA). Serum-specific insulin levels were determined by ELISA (Mercodia, Sweden). Serum triglycerides, total, LDL and HDL cholesterol were assayed with commercial reagents (total cholesterol: Boehringer Mannheim, Sussex, UK; triglycerides: Roche Diagnostics, Herts, UK). All lipid assays were done by Dr David Wickens (Chemical Pathology, Whittington Hospital, London, UK). Insulin resistance was calculated using homeostatic model assessment (HOMA) = (glucose in mmol/L × insulin in mIU/L)/22.5 [299].

2.2.2 Adipokine ELISA

Adipokine concentrations in serum including total adiponectin and leptin were determined by commercially available two-site ELISA (R&D Systems, UK). Standards and samples were pipetted into the 96 wells of a microplate pre-coated with a monoclonal antibody specific for the target adipokine. After incubation, any

adipokine present was bound to the antibody pre-coated onto the bottom of the well. The unbound substances were then washed away, followed by the addition of a 'detecting' enzyme-linked monoclonal antibody specific for the target antigen, adipokine. After washing, to remove any unbound antibody-enzyme reagent, the substrate solution was added to the wells, and colour developed in proportion to the amount of adipokine bound in the initial step. The colour development was stopped at specified times as per the manufacturer's instructions, and the intensity of the colour was measured using a microplate reader (Opsys MR, Dynex, UK) as absorbance at 450 nm, with correction at 540 nm.

2.2.3 DPP-IV inhibitors

Two different DPP-IV inhibitors were used.

Aprotinin (non-specific protease inhibitor): aprotinin (500 kallikrein inhibitory units (KIU)/ml blood) from Sigma-Aldrich was used as DPP-IV inhibitor.

P32/98 (50 mg) powder form (MW 260.4 g): a potent and selective inhibitor of DPP-IV (Enzo Life Sciences) was prepared by adding 5.84 ml of distilled water to the 50 g of inhibitor in order to obtain a final concentration of 330 μM of stock solution; 50 μL of the stock solution was added to 4 ml of blood.

2.2.4 GLP-1 ELISA

Two different commercially available ELISA kits were used to measure the bioactive form of GLP-1.

GLP-1 ELISA kit (ALPCO immunoassays, ALPCO Diagnostic, USA): this ELISA was used to measure the active form of GLP-1 (7-36) in human EDTA-plasma by a sandwich technique with two selected GLP-1 (7-36)-specific antibodies. Standards, controls and samples were pipetted into the 96 wells of a microplate that was coated with streptavidin. Then, a mixture of biotinylated GLP-1 (7-36)-specific antibody and a horseradish peroxidate (HRP)-conjugated GLP-1 (7-36)-specific antibody was added to each well and left for overnight incubation, during

which a sandwich immunocomplex was formed and adhered to the walls of the plate; unbound substances were then washed out. The substrate solution was then added to each of the wells for detection of immunocomplex, incubated in a timed reaction and then measured using a spectrophotometric microplate reader (Opsys MR, Dynex, UK) as absorbance at 450 nm, with correction at 620 nm.

GLP-1 ELISA kit (Millipore, Billerica, MA, USA): this kit was used to measure both the active forms of GLP-1 (7-36) and GLP-1 (7-37) in plasma. Standards, controls and plasma samples were added to a GLP-1 monoclonal antibody-coated microwell plate that binds specifically to the N-terminal region of active GLP-1 protein in the wells. The plate was incubated for at least 20 hours at 4 °C. The plate was then washed to remove unbound materials; an anti-GLP-1-alkaline phosphatase detection conjugate was added to the immobilized GLP-1 in the wells, and the plate was incubated for 2 hours at room temperature. After washing off unbound conjugate, methyl umbelliferyl phosphate (MUP) solution was added and left for at least 20 minutes to form a fluorescent product, umbelliferone, when in the presence of alkaline phosphatase-conjugated GLP-1. The MUP reaction was stopped by adding dipotassium phosphate reagent. The plate was read using a GENios Microplate Reader (TECAN Group Ltd, Switzerland) with an excitation/emission wavelength of 350/460 nm.

2.3 Adipose tissue

2.3.1 Adipose tissue sample collection

Abdominal subcutaneous and intra-abdominal greater omentum adipose tissue (AT) (about 5 g each) were obtained during the intraoperative period, placed in serum-free medium (Cellgro, Mediatech, Manassas, USA) and then transferred quickly to the laboratory.

2.3.2 GLP-1 recombinant preparation

Recombinant human GLP-1 (9-36) amide was obtained from Polypeptide Laboratories and diluted in phosphate-buffered saline (PBS) with 0.1% bovine

serum albumin (BSA) to provide a 1 nM stock concentration. The peptide was then further diluted in PBS to obtain three different concentrations: 1, 0.5 and 0.1 nM.

2.3.3 Adipose tissue organ culture with recombinant GLP-1

Subcutaneous and omental AT (0.05 g) were dissected, and each was incubated in 500 µl Cellgro serum-free media with 5% penicillin-streptomycin or three different concentrations of GLP-1 solution (0.1, 0.5 and 1 nM) for 4 or 16 hours (37 °C, 95% O_2/5% CO_2). Following incubation, the conditioned medium was collected and stored at −80 °C until assessment of leptin secretion.

2.3.4 Collagenase digestion of adipose tissue

Collagenase digestion of AT was used to obtain the adipocytes. AT was weighed, and 5 g each of subcutaneous (SC) and omental (OM) AT (SAT and OAT, respectively) were used for collagenase digestion. Both AT were minced in a boat using a scalpel and forceps. The minced tissue was placed in a 50-ml universal tube. To each tube, 1 ml of collagenase, 1.5 ml of BSA and 7.5 ml of Cellgro were added. The tubes were covered with parafilm and then incubated in a shaking water bath (~160/minute) at 37 °C for 45 minutes. The cell suspension was then filtered through a tissue sieve containing 8-mesh, transferred into a 15-ml Falcon tube and spun for 5 minutes (3000 rpm at 4 °C). The adipocytes were decanted and collected into an Eppendorf tube and taken immediately for RNA extraction.

2.4 Adipocyte gene expression

2.4.1 Cell RNA extraction

Adipocytes were collected and homogenized using TRIzol reagent (Invitrogen, Life Technologies, UK). Then, chloroform was added and mixed thoroughly (0.2 ml chloroform per 1 ml TRIzol). The mixture was centrifuged at 3200 rpm for 15 minutes at 4 °C and was separated to form an upper colourless aqueous phase,

interphase, and lower red phenol-chloroform phase. The aqueous phase that contained the total RNA was then transferred to a new Eppendorf tube and mixed gently with an equal volume of 100% isopropanol. The mixture was incubated at −20 °C for 1.5 hours, then centrifuged (3200 rpm, 15 minutes, 4 °C). Subsequently, the RNA pellet that appeared on the bottom of the Eppendorf tube was washed twice in 75% ethanol (Sigma-Aldrich, UK) and finally re-suspended in nuclease-free water (Invitrogen, Life Technologies, UK).

The quality and concentration of the RNA extracted were assessed spectrophotometrically using a NanoDrop-1000 spectrophotometer (Thermo Scientific, USA). The concentration of RNA was recorded as the optical density (OD) at 260 nm, and the quality reported as the ratio of OD at 260 nm to that at 280 nm. A 260/280 ratio >1.7 was acceptable quality for RNA.

2.4.2 cDNA synthesis

For synthesis of the first strand of cDNA, a reverse transcription reaction mixture was made with MultiScribe Reverse Transcriptase (1.25 units/µl), dNTP (ATP, CTP, GTP, UTP; 500 µL each), Oligo $d(T)_{16}$ (2.5 µM), RNase inhibitor (0.4 units/µl), $MgCl_2$ (5.5 mM) and reaction buffer (TaqMan Reverse Transcription Reagents, Roche, New Jersey, USA); 500 ng of total RNA was added to the mixture, and the mixture was then incubated in a thermal cycler (Genius, Techne, UK) at 42 °C for 1 hour, at 72 ˚C for 15 minutes, and held at 4 °C. After that, the cDNA was stored at −20 °C.

2.4.3 Real-time polymerase chain reaction (real-time PCR)

Target gene expression was measured using TaqMan real-time PCR. The primers of β-actin (a housekeeping gene) and GLP-1 receptor used were obtained from Qiagen Company, UK. SYBR Green solution (12.5 µl), primer (2.5 µl), cDNA (10 µl) and nuclease-free water were mixed to form 25 µl of mixture. The reaction was carried out in triplicate in a 384-well plate using 10 µl total volume per well of the

mixture. The PCR reaction conditions were 50 °C for 2 minutes, 95 °C for 15 minutes, followed by 40 cycles of 94 °C for 15 seconds, 56 °C for 30 seconds and 76 °C for 30 seconds in an ABI Prism 7900HT sequence detection system (Applied Biosystems, UK).

The cycle threshold (Ct) value was defined as the number of cycles required for the fluorescent signal to cross the threshold, and this was used to measure gene expression. Data were expressed as the Ct ratio of β-actin to the target gene.

2.5 Statistical analysis

Data were analysed using SPSS version 20 for Apple (Statistical Package for the Social Sciences, SPSS UK Ltd, Chertsey, UK). Normally distributed data are expressed as the mean (standard deviation), and non-normally distributed data are expressed as the median (interquartile range) in text and in tables. Non-parametric data were tested using a Wilcoxon–Mann–Whitney U test. Significance was defined as $p \leq 0.05$.

Chapter 3

Elevated GLP-1 mediates chronic nausea and vomiting

3.1 Introduction

3.1.1 Nausea and vomiting (N&V) after bariatric surgery

Bariatric surgery is, to date, the only intervention that results in significant and sustained weight loss for morbid obesity. Procedures such as RYGB, which combines gastric restriction with bypass of the stomach and proximal intestine, are more effective than other forms of metabolic surgery (GB and SG) [300]. However, a proportion of patients develop severe, sustained and debilitating N&V symptoms of unknown cause after RYGB, as well as with other procedures such as SG, which starts late after the surgery (at least 1 year), persists for more than 3 months and is not related to mechanical obstruction [301, 302]. The mechanism underlying this phenomenon is unknown, but the symptoms are similar to those experienced by patients with gastroparesis characterized by delayed gastric emptying of solids without evidence of mechanical obstruction. Symptomatic improvement occurs in these patients with gastric electrical stimulation which has been shown to enhance vagal autonomic function, decrease gastric sensitivity to volume distension and activate central control mechanisms for N&V through thalamic pathways [303].

3.1.2 GLP-1 is associated with nausea

A recognized side effect of GLP-1 analogues used to treat diabetes is nausea which occurs in up to 50% of patients [304]. Studies in rats suggest that the nausea-inducing effects of peripheral exendin-4, a GLP-1 receptor agonist, are mediated by a vagal-independent pathway that appears to involve BBB penetration and subsequent GLP-1R activation in the CNS, most likely in the NTS [150].

Postprandial GLP-1 plasma levels are significantly increased following RYGB, and remain elevated for at least a year [305]. Whether the increase in GLP-1 levels after surgery could also be a contributing factor to the persistent N&V experienced by some patients has not been reported.

3.1.3 Leptin and nausea

There is also data suggesting a relationship between leptin and symptoms of severe, but not mild to moderate, nausea in women during pregnancy [306, 307]. Furthermore, the most common side effect of treatment with metreleptin is nausea [308]. Thus, whether leptin may provoke N&V in a subset of women following RYGB has not been investigated.

Thus, the hypothesis of this study was that elevated GLP-1 levels might be associated with persistent N&V experienced by a subset of patients following RYGB, and that administration of GLP-1 inhibitor may ameliorate these symptoms. Secondly, GLP-1 directly alters leptin secretion by adipocytes, and, together, GLP-1 and leptin augment the symptoms of N&V.

The aims of this study were to investigate whether increased GLP-1 and leptin levels after surgery could be a contributing factor to persistent N&V, by comparing post-absorptive and postprandial levels of GLP-1 and leptin between asymptomatic subjects and patients with persistent N&V following RYGB, and between normal-weight, overweight, obese and morbidly obese subjects not scheduled for surgery. The second aim was to determine the possibility of crosstalk between gut peptides and adipokines, by investigating the effect of GLP-1 on leptin secretion *in vitro*.

3.2 Methods

3.2.1 *In vivo* study

Study population

Forty-two non-diabetic female subjects stratified into five groups participated in this study: obese subjects with persistent and prolonged N&V after RYGB (Group 1: n = 10), asymptomatic postoperative obese subjects (Group 2: n = 10), morbidly obese subjects (Group 3: n = 7), overweight/obese subjects (Group 4: n = 6) and normal-weight healthy subjects (Group 5: n = 9). Subjects in Group 1 had upper gastrointestinal tract endoscopy and imaging to exclude an obstructive cause for

their symptoms. Weight, height, BMI, BP and pulse were recorded. Exclusion criteria were as mentioned in section **2.1.1**.

Meal challenge test

Day of visit: subjects attended the research clinic at the Whittington Hospital between 8:30 and 10 AM after an overnight fast. The postoperative patients were studied 18.8 ± 3.0 months after surgery. A cannula was inserted into a forearm vein, and fasting blood samples were collected for measurement of plasma glucose, lipids, insulin, leptin, adiponectin and GLP-1. Subjects consumed a 182.7-kcal solid meal (10.3 g of fat, 18.9 g of carbohydrate and 3.6 g of protein), and additional blood samples for GLP-1, glucose and insulin were collected 45, 120 and 180 minutes after the meal; 250 μl of aprotinin was added immediately to each blood sample taken for GLP-1 to prevent degradation by DPP-IV enzyme. Blood samples were centrifuged at 3000 rpm for 15 minutes at 23 °C and stored at −80 °C until analysis. This meal size was used as subjects with postoperative N&V were unable to tolerate a larger meal.

Treatment intervention

One symptomatic patient received subcutaneous injections of octreotide three times a day for 3 months, after which the meal challenge test was repeated (as above), and blood was taken in the fasted state for measurement of GLP-1 level.

Assay

Glucose, total cholesterol, LDL, HDL and triglycerides were assayed at the Whittington Hospital as mentioned in section **2.2.1**. Insulin, leptin and adiponectin were determined by ELISA (sections **2.2.1** and **2.2.2**). GLP-1 was measured using ELISA (ALPCO immunoassays, ALPCO Diagnostic, USA), as detailed in section **2.2.4**.

HOMA was calculated as mentioned earlier in section **2.2.1**.

3.2.2 *In vitro* study

Subjects

Abdominal SAT and OAT samples were obtained from morbidly obese non-diabetic patients undergoing bariatric surgery (n = 6), for use in organ cultures.

Tissue culture

SAT and OAT (0.05 g) were incubated in 500 µl of three different concentrations of human GLP-1 (0.1, 0.5 and 1.0 nM) as well as Cellgro (control 0) for 4 or 16 hours. Culture media were collected, and leptin levels were determined by ELISA (section **2.2.2**).

Gene expression in cells

Collagenase digest for SAT and OAT was done to obtain the adipocyte fraction; the method is described in section **2.3.4**.

RNA extraction from SC and OM adipocytes was carried out as described in section **2.4.1** and followed by cDNA synthesis (section **2.4.2**).

GLP-1 receptor expression was quantified using real-time PCR; the method is described in section **2.4.3**.

3.3 Results

3.3.1 Patients' characteristics

Patients' characteristics are shown in Table 3.1. All subjects were female, non-diabetic, normotensive and normolipidaemic. Appendix 1 shows the medications that were used by participants in the different groups. The groups were matched for age, and the two postoperative groups were matched for BMI and postoperative duration (18.8 ± 3.0 months). BMI on the day of study varied according to the group. The mean BMI of morbidly obese patients and normal-weight subjects were 46.3 and 21.3 kg/m^2, respectively. The BMI of the postoperative and the obese/overweight patients was not significantly different.

	Group 1 N&V (n = 10)	Group 2 No N&V (n = 10)	Group 3 MO (n = 7)	Group 4 OW (n = 6)	Group 5 NW (n = 9)
Age (years)	41.4 ± 3.4	38 ± 3.1	39.3 ± 4.3	45.5 ± 3.9	34.7 ± 4.6
BMI (kg/m²)	30.6 ± 2.3	31.2 ± 2.0	46.3 ± 1.7	31.8 ± 1.9	21.3 ± 0.7
SBP (mm Hg)	119 ± 3	109 ± 3	127 ± 7	121 ± 12	121.3 ± 1
DBP (mm Hg)	73 ± 5	72 ± 3	81 ± 4	79 ± 2	76.6 ± 12
Basal glucose (mmol/l)	5.0 ± 2.4	4.5 ± 0.5	5.07 ± 0.9	5.2 ± 0.7	4.7 ± 0.5
Basal insulin (mIU/l)	5.0 ± 2.4	4.5 ± 1.5	10.2 ± 7.5	9.4 ± 4.1	4.1 ± 2
HOMA-IR	1.2 ± 0.6	0.9 ± 0.3	2.4 ± 2.2†	2.1 ± 0.8†	0.8 ± 0.4
Total cholesterol (mmol/l)	3.5 ± 0.35	4.1 ± 0.12	4.0 ± 0.43	4.4 ± 0.26	4.9 ± 0.38
HDL cholesterol (mmol/l)	1.2 ± 0.12	1.4 ± 0.12	0.99 ± 0.07	1.3 ± 0.14	1.9 ± 0.29*
LDL cholesterol (mmol/l)	1.8 ± 0.29	2.4 ± 0.25	2.5 ± 0.41	2.7 ± 0.23	2.6 ± 0.29
Triglycerides (mmol/l)	1.1 ± 0.23	0.85 ± 0.05	1.1 ± 0.18	0.81 ± 0.24	0.81 ± 0.13

Table 3.1 Patients' characteristics

SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment – insulin resistance, HDL: high-density lipoprotein, LDL: low-density lipoprotein, N&V: nausea and vomiting, MO: morbidly obese, OW: obese and overweight, NW: normal weight. † denotes $p < 0.05$ compared to the post-surgery and normal-weight groups; * denotes $p < 0.05$ comparing the normal-weight group to all other groups.

3.3.2 BMI in postoperative groups

The patients with N&V and those without symptoms were followed up after surgery over a period of 14–18 months to find out if those with symptoms lost more weight. The figure below shows that weight loss following RYGB surgery was not significantly different in subjects with and without N&V for up to 18 months (Figure 3.1).

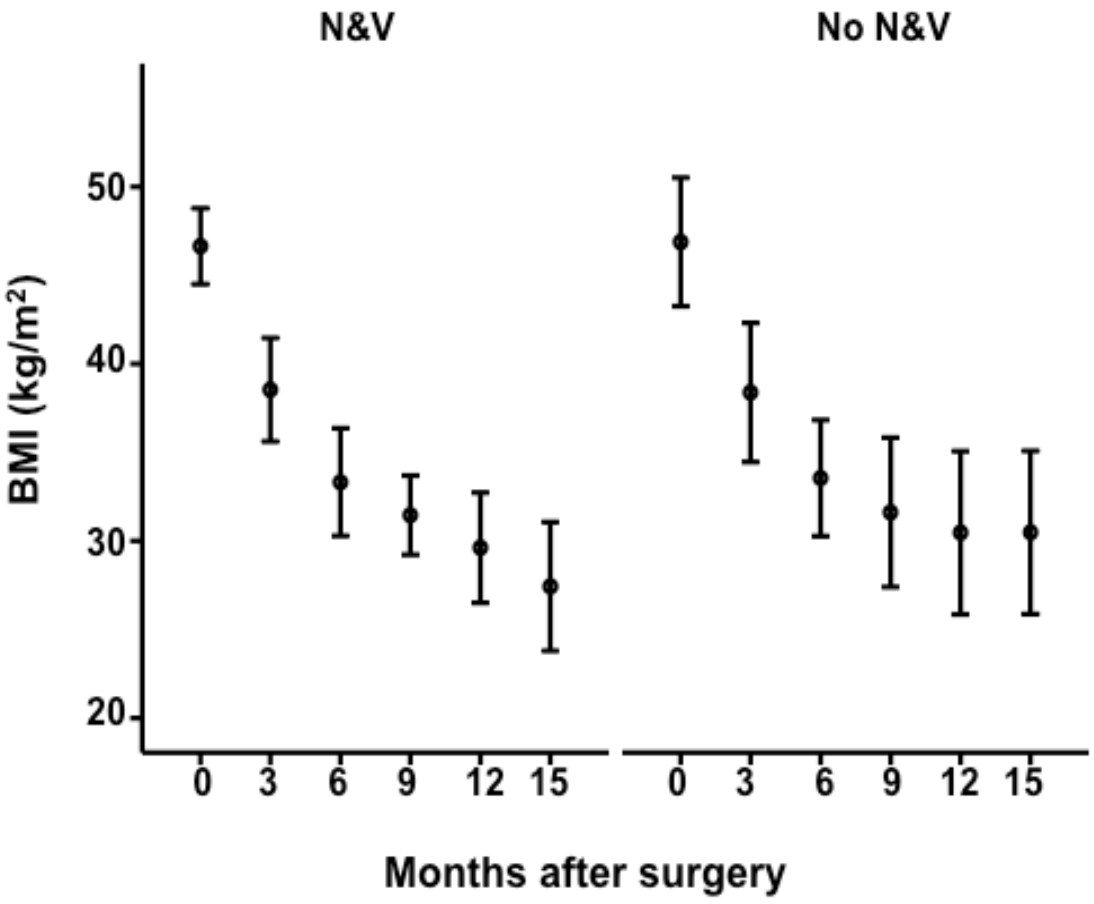

Figure 3.1 Weight loss following Roux-en-Y gastric bypass in subjects with and without persistent nausea and vomiting symptoms

BMI in patients with and without persistent N&V symptoms was recorded for 15 months following RYGB (n = 10 per group). There were no significant differences in the change in BMI over time between the two groups. Data are shown as mean and standard deviation and comparisons carried out by t-test. N&V: nausea and vomiting, BMI: body mass index, RYGB: Roux-en-Y gastric bypass.

3.3.3 Post-absorptive and postprandial circulating GLP-1 levels

Fasting plasma GLP-1 levels were significantly higher in the subjects with persistent N&V post-RYGB surgery compared to all other groups ($p = 0.007$), as shown in Figure 3.2. This difference was significant ($p = 0.035$) despite similar BMI ($p = 0.86$) in the two postoperative groups. Postprandial GLP-1 levels did not increase significantly at 45, 120 and 180 minutes following the meal challenge compared to fasting levels in either operative or non-operative groups (Table 3.2).

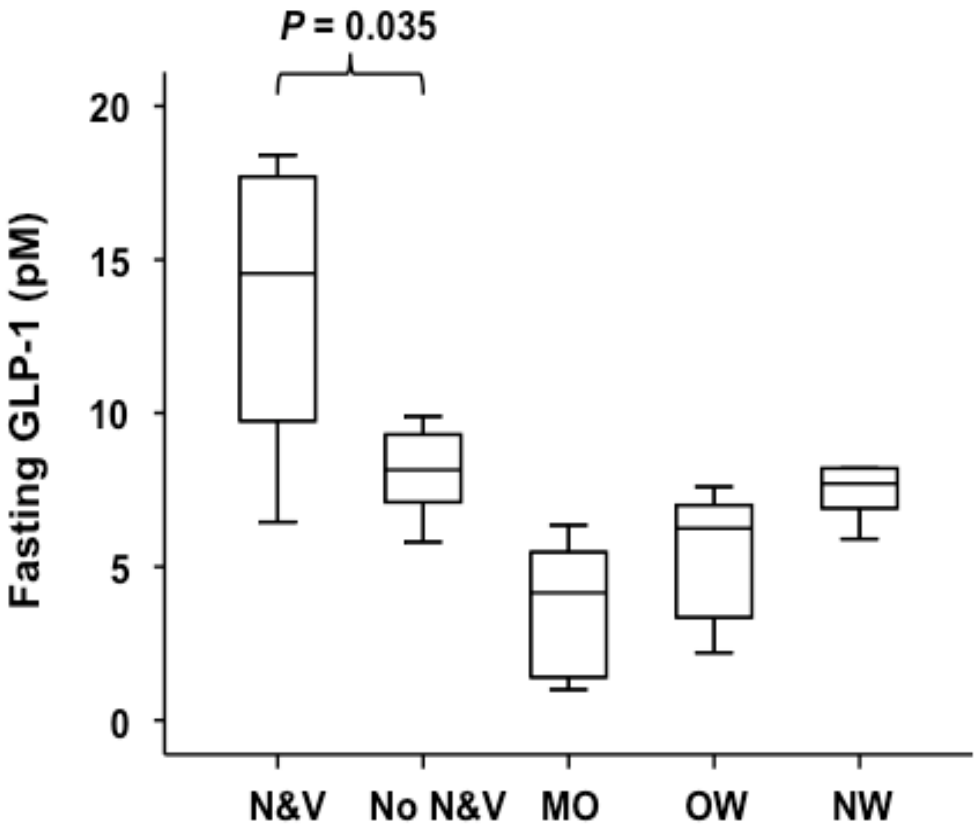

Figure 3.2 Changes in fasting levels of GLP-1

Fasting plasma GLP-1 levels. In subjects with persistent N&V, fasting GLP-1 levels were elevated (p = 0.035) compared to subjects without N&V, morbidly obese subjects, obese and overweight subjects and lean subjects. Data are shown as median and interquartile ranges and comparisons made by Mann–Whitney U test. Groups: postoperative nausea and vomiting (N&V) n = 10; postoperative non-N&V n = 10; morbidly obese (MO) n = 7; obese and overweight (OW) n = 6; normal weight (NW) n = 9.

	Group 1 N&V	Group 2 No N&V	Group 3 MO	Group 4 OW	Group 5 NW
Fasting GLP-1 (PM)	14.5 (10–17.5)	8.3 (7.0–9.5)	4.0 (1.5–5.2)	6.0 (3.0–7.1)	8.0 (7.0–8.5)
45 min PP GLP-1 (PM)	16.5 (11.9–94.8)	7.6 (4.9–8.4)	4.9 (3.7–10.0)	7.3 (4.4–10.5)	7.4 (4.5–10.9)
120 min PP GLP-1 (PM)	13.1 (10.1–90.2)	8.1 (4.7–8.0)	4.4 (3.5–11.0)	8.1 (4.1–10.2)	6.2 (4.3–8.3)
180 min PP GLP-1 (PM)	14.4 (9.5–89.6)	5.6 (4.2–7.8)	4.0 (3.1–10.0)	7.6 (3.2–10.6)	6.0 (4.6–7.7)

Table 3.2 Fasting and postprandial GLP-1 levels in the five different groups

Data are expressed as median and interquartile range. N&V = postoperative nausea and vomiting, No N&V = postoperative without nausea and vomiting, MO = morbidly obese, OW = obese and overweight, NW = normal weight.

3.3.4 Fasting systemic adipokine levels and BMI

Fasting systemic leptin and BMI are shown in the graph below (Figure 3.3). As expected, morbidly obese subjects had high systemic leptin levels. Subjects with postoperative N&V had significantly lower plasma leptin ($p = 0.04$) compared to those without N&V, similar to the levels in normal-weight subjects. As expected, adiponectin levels were lower in the morbidly obese and obese/overweight groups and highest in the normal-weight group. However, levels were lower in subjects with postoperative N&V compared to asymptomatic subjects, but this did not reach significance ($p = 0.30$) (Figure 3.4).

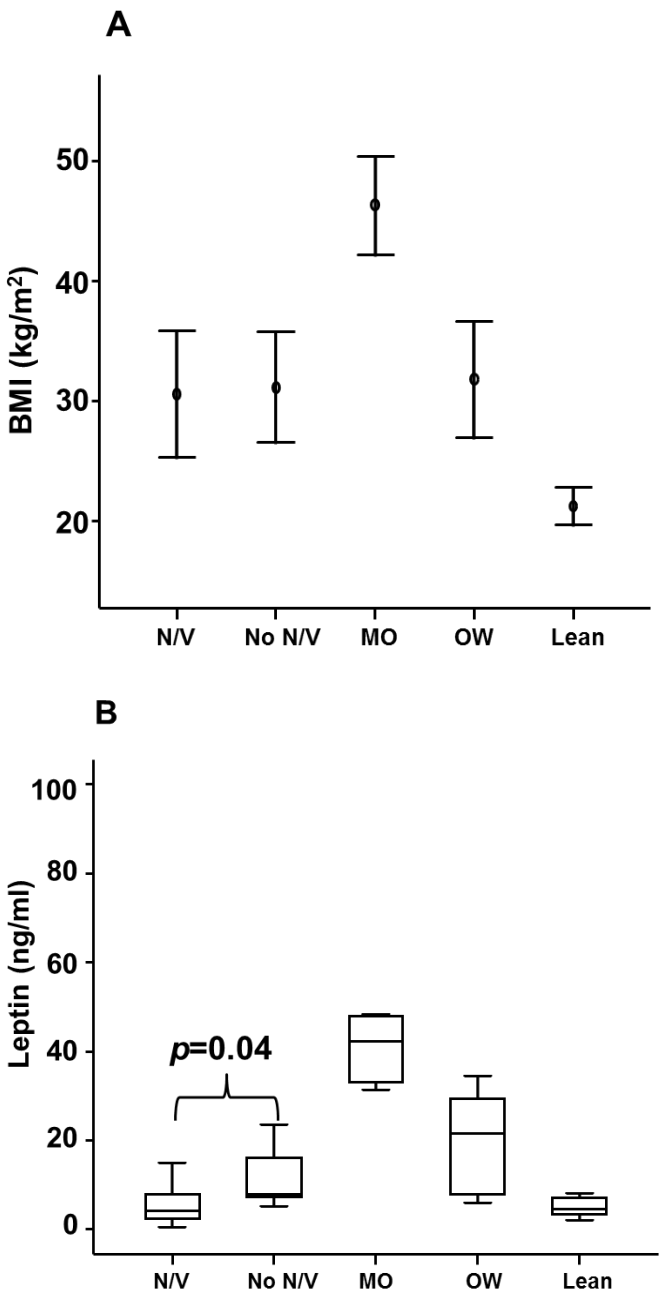

Figure 3.3 BMI and fasting systemic leptin

Although BMI (A) were similar between N&V and non-N&V groups, plasma leptin levels (B) were significantly lower in N&V groups compared to non-N&V groups ($p = 0.04$) and to morbidly obese and obese/overweight groups ($p < 0.05$). Levels were comparable between the N&V group and the lean group ($p = 0.9$). Data in (A) are shown as mean (SD) and in (B) as median (interquartile range). Groups: postoperative nausea and vomiting (N&V) n = 10; postoperative non-N&V n = 10; morbidly obese (MO) n = 7; obese and overweight (OW) n = 6; normal weight (NW) n = 9.

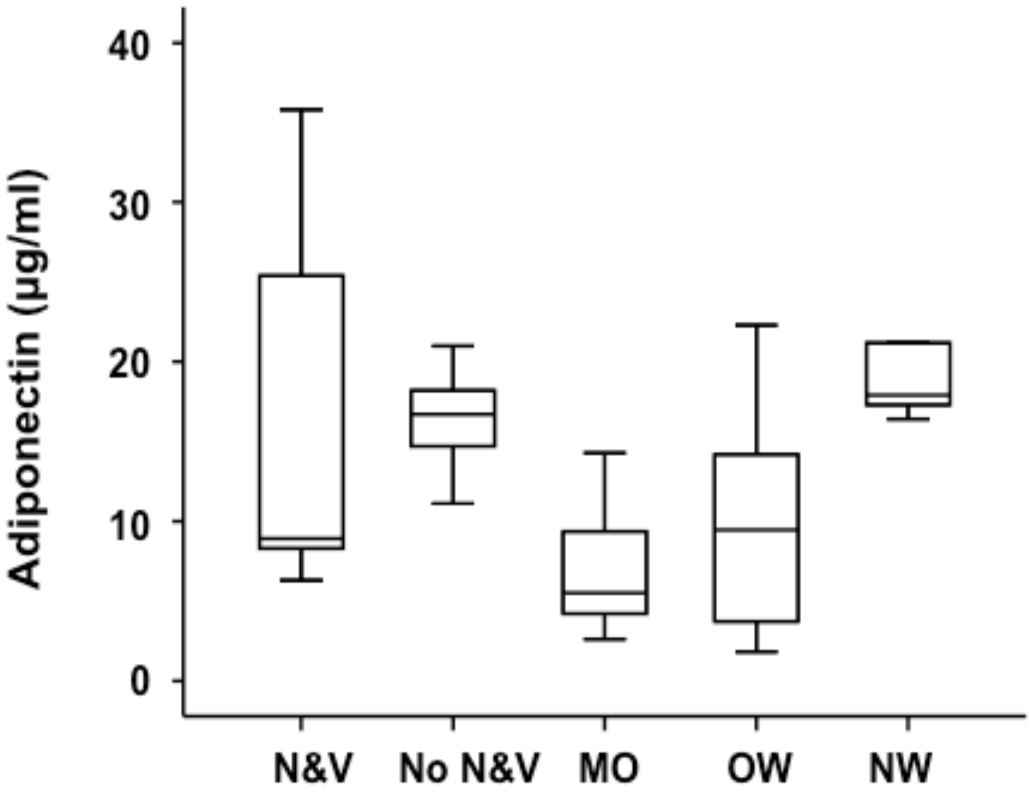

Figure 3.4 Fasting plasma adiponectin levels

Plasma adiponectin was not significantly different between N&V and non-N&V groups ($p = 0.2$). Circulating adiponectin levels were significantly lower in the morbidly obese group compared to no N&V and normal-weight groups ($p < 0.05$), whereas the levels were comparable between the normal-weight and postoperative N&V and no N&V groups ($p > 0.05$). Data are shown as median (interquartile range); comparisons were made by Mann–Whitney U test. Groups: postoperative nausea and vomiting (N&V) n = 10; postoperative non-N&V n = 10; morbidly obese (MO) n = 7; obese and overweight (OW) n = 6; normal weight (NW) n = 9.

3.3.5 Glucose and insulin response

There was no significant difference in the glucose and insulin response to the meal challenge in subjects with and without postoperative N&V (Table 3.3, Figures 3.5 and 3.6).

	Group 1 N & V	Group 2 No N & V	P value
Fasting glucose (mM)	4.4 (4.2- 4.9)	4.3 (4.0-5.0)	0.5
45 min PP glucose (mM)	4.9 (4.8-6.2)	5.4 (4.4-7.0)	0.9
2 h PP glucose (mM)	4.3 (3.9-4.8)	4.2 (3.5-4.8)	0.8
3 h PP glucose (mM)	4.3 (4.1-4.8)	4.3 (4.0-4.7)	0.7
Fasting insulin (mIU/ml)	5.5 (2.9-6.4)	4.7 (3.5-5.5)	0.6
45 min PP insulin (mIU/ml)	13.1 (5.1-32.8)	19.2 (11.3-32.2)	0.4
2 h PP insulin (mIU/ml)	4.2 (2.8-4.8)	5.1 (4-10.4)	0.2
3 h PP insulin (mIU/ml)	3.3 (2.8-5.4)	3.8 (2.8-4.7)	0.9

Table 3.3 Fasting and postprandial (45, 120 and 180 minutes) glucose and insulin plasma levels

Data are expressed as median and interquartile ranges. N&V: nausea and vomiting, PP: postprandial, min: minute, h: hour.

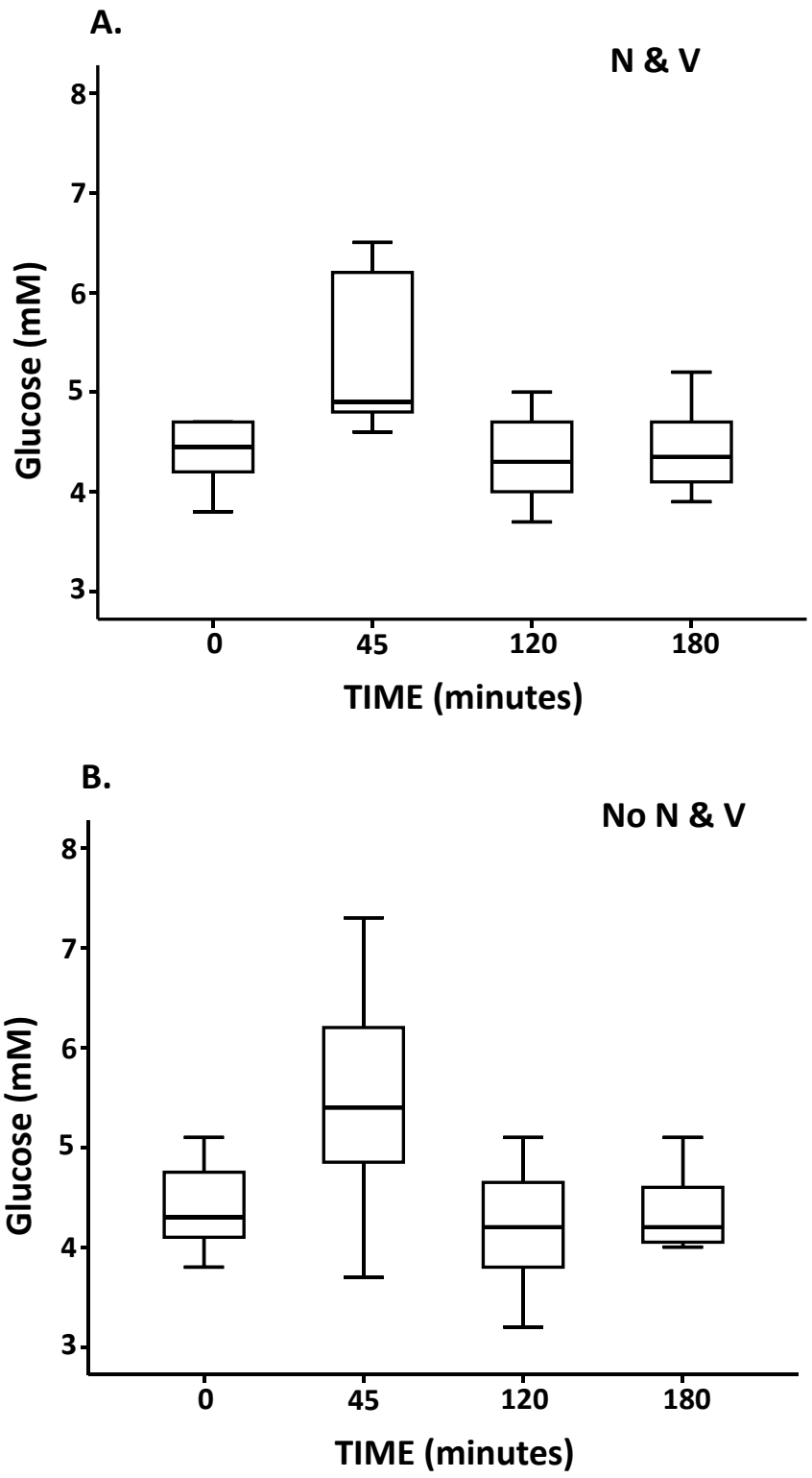

Figure 3.5 Glucose response to a meal challenge in subjects with (A) and without (B) nausea and vomiting after Roux-en-Y gastric bypass surgery

Following a 182.7-kcal meal challenge, the glucose responses were similar in subjects with and without N&V symptoms (A and B) after Roux-en-Y gastric bypass surgery. Data are shown as median and interquartile ranges and comparisons made with a Mann–Whitney U test.

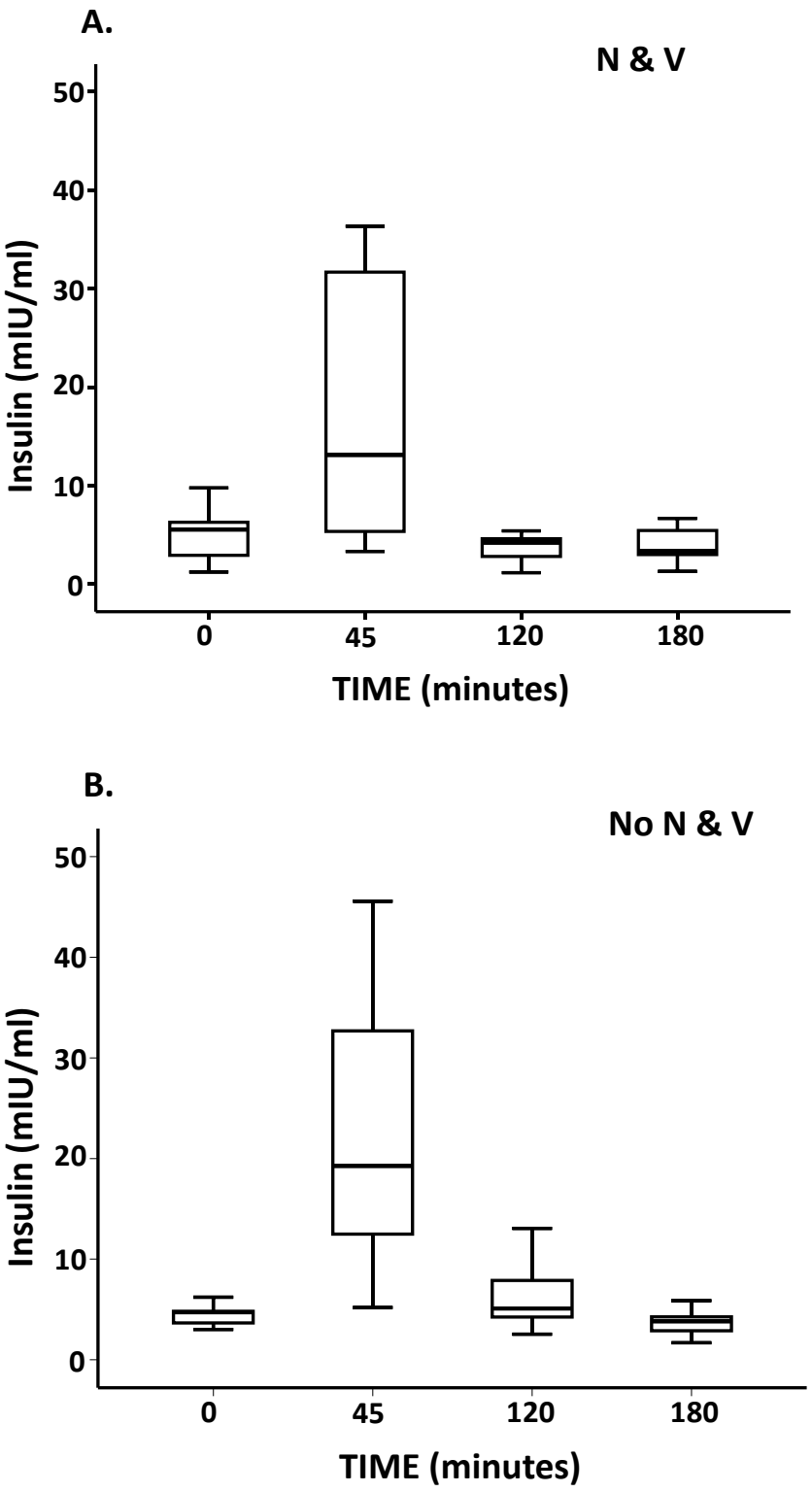

Figure 3.6 Insulin response to a meal challenge in subjects with (A) and without (B) nausea and vomiting after Roux-en-Y gastric bypass surgery

Following a 182.7-kcal meal challenge, the insulin responses were similar in subjects with (A) and without N&V symptoms (B) after Roux-en-Y gastric bypass surgery. Data are shown as median and interquartile ranges and comparisons made with a Mann–Whitney U test.

3.3.6 Treatment intervention

Octreotide injection (50 mg three times a day) was given to one patient with persistent N&V. The patient reported amelioration in N&V symptoms with the onset of the treatment, and her basal plasma GLP-1 levels decreased (basal GLP-1 pre-treatment versus post-treatment: 6.35 versus 5.24 pM).

3.3.7 Leptin secretion in response to GLP-1 (*in vitro*)

Minimal leptin secretion was detected from either SAT or OAT after 4 hours' incubation (Figure 3.7 A). However, there was significant suppression of leptin secretion in SAT at all concentrations of GLP-1 used (0.1, 0.5 and 1 nM) after 16 hours' incubation (Figure 3.7 B). Leptin secretion from OAT was very low, and significantly lower than that from SAT, so that the effect of GLP-1 on leptin secretion could not be recognized.

3.3.8 GLP-1 receptor expression in adipocytes

Both SC and OM adipocytes showed the presence of GLP-1 receptors. There was no difference in mRNA expression of GLP-1 receptors between SC and OM adipocytes (Figure 3.8).

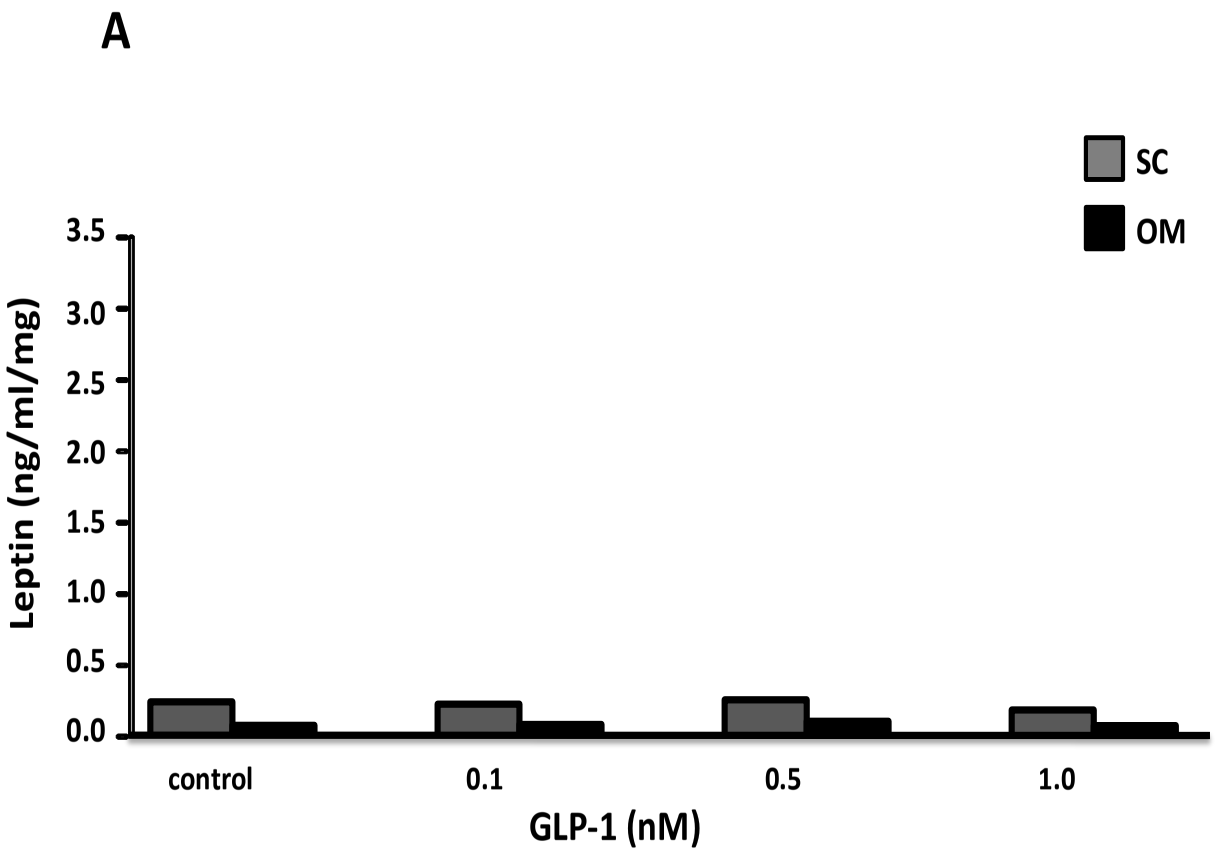

Figure 3.7 (A) Effect of GLP-1 on leptin secretion from subcutaneous (SC) and omental (OM) adipose tissue *in vitro* after 4 hours' incubation

Very little leptin secretion was detected from SC and OM adipose tissue in response to GLP-1 treatments and control (0, 0.1, 0.5 and 1.0 nM) after 4 hours' incubation.

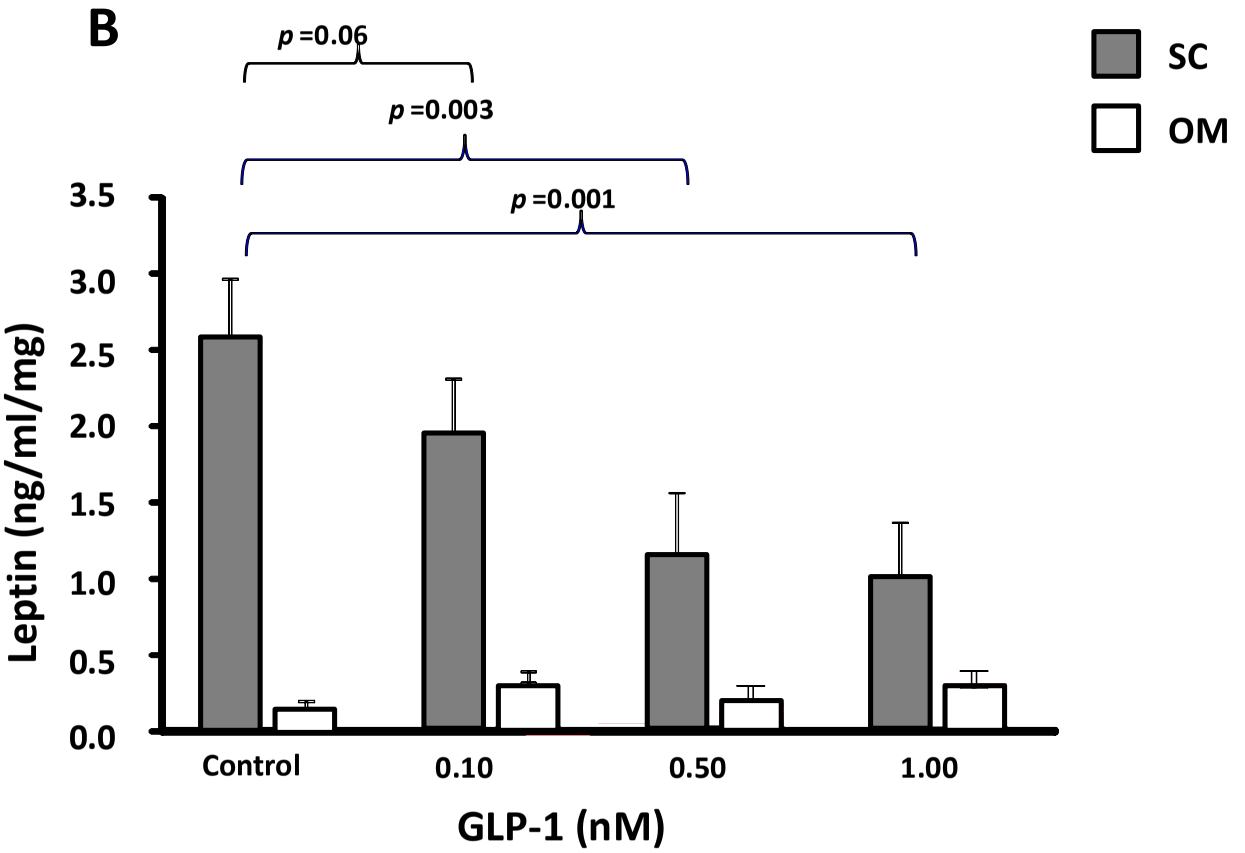

Figure 3.7 (B) Effect of GLP-1 on leptin secretion from subcutaneous (SC) and omental (OM) adipose tissue *in vitro* after 16 hours' incubation

Leptin secretions were significantly reduced in response to GLP (0.5 and 1.0 nM concentrations) compared to the control in SC adipose tissue after 16 hours' incubation, whereas very little secretion of leptin was detected from OM adipose tissue in response to control and GLP-1 (0.1, 0.5 and 1.0) after 16 hours.

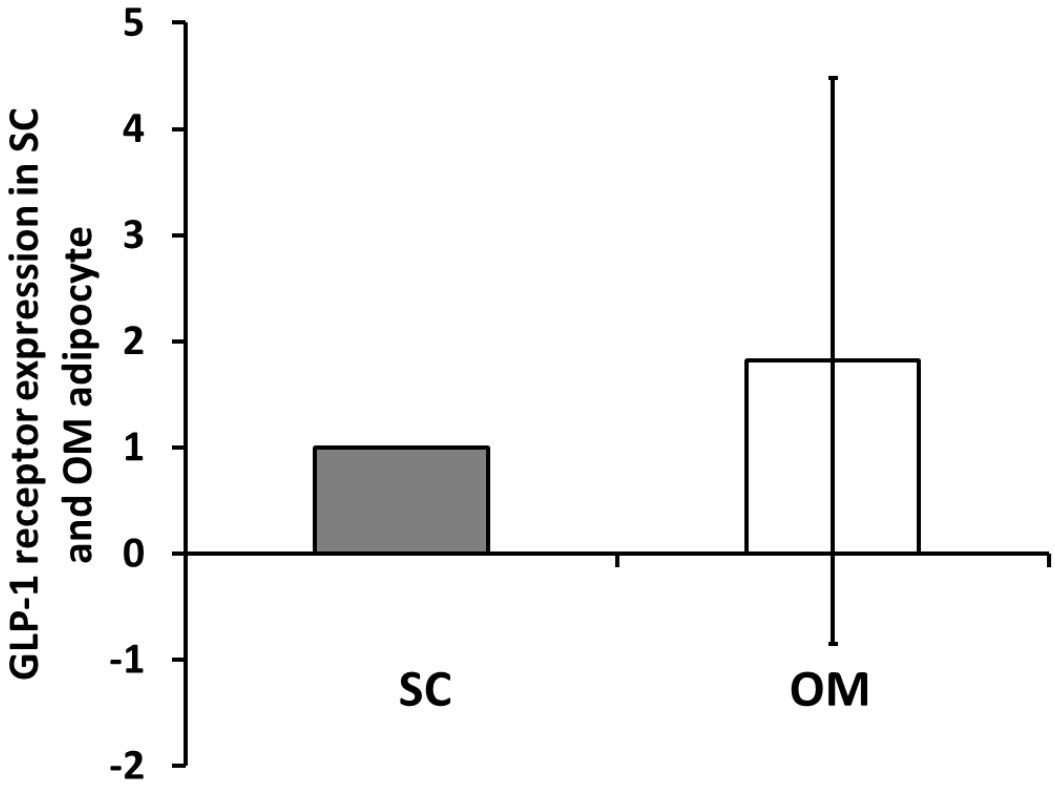

Figure 3.8 GLP-1 receptor mRNA expression in subcutaneous (SC) and omental (OM) adipocytes

Expression of GLP-1 receptor in SC (control) and OM adipose tissues was calculated by the $2^{-\Delta\Delta CT}$ method (n = 3). A paired-sample t-test indicated no significant difference in the level of expression between the two depots. Data are presented as mean and standard deviation. SC = subcutaneous, OM = omental.

3.4 Discussion

3.4.1 Elevated basal GLP-1 is associated with chronic symptoms of nausea and vomiting

N&V is a common side effect experienced by the majority of patients undergoing RYGB, but symptoms usually disappear shortly after the operation [309]. However, approximately 1–5% of patients present with difficult-to-control persistent N&V despite the absence of mechanical abnormalities [301]. In this study, it was found that symptomatic patients have significantly higher basal, but not postprandial, GLP-1 levels, suggesting that non-mechanical chronic N&V symptoms after RYGB surgery may be due, at least in part, to chronically elevated GLP-1 levels. Increased GLP-1 concentrations may therefore also explain similar symptoms seen after other bariatric procedures such as SG [310]. Postprandial GLP-1 responses were not apparent in all groups. This might be due to the small amount of caloric intake that was inadequate to stimulate postprandial GLP-1 secretion. However, this still does not detract from the result that GLP-1 was elevated, although only evidence for elevated fasting levels mediating chronic nausea/vomiting is provided by this study.

A significant number of diabetic patients treated with exendin-4 also experience N&V [150], providing further support for the role of elevated GLP-1 levels in the generation of symptoms.

Exendin-4 induces nausea by penetrating the BBB and subsequently activating GLP-1R in the medial NTS [150, 304]. Whether GLP-1 induces N&V by direct actions on the NTS or indirectly through the vagal afferent pathway is not known. However, endogenous GLP-1 has a very short half-life and is rapidly degraded by DPP-IV enzyme, which makes it unlikely to cross the BBB.

Despite higher basal GLP-1 levels in symptomatic patients compared to those without symptoms postoperatively, weight loss, insulin sensitivity and adiponectin levels were not significantly different in the two groups. Thus, the beneficial effects of RYGB on improving insulin sensitivity and weight loss were not affected by elevated basal GLP-1 levels and the symptoms of N&V.

3.4.2 Leptin secretion *in vivo* and *in vitro*

Contrary to data for the persistent N&V that accompanies pregnancy [306, 307], systemic leptin levels were lower in the symptomatic compared to asymptomatic subjects, despite similar postoperative BMI. The *in vitro* study showed that chronic (16 h) but not acute (4 h) exposure to GLP-1 inhibited leptin secretion from human SAT but not OAT. Leptin secretion from OAT was significantly lower, and the effect of GLP-1 on leptin secretion was unclear. GLP-1 has been shown to inhibit visfatin, and exendin-4 to stimulate adiponectin secretion from 3T3-L1 adipocytes [153, 154]. Acute administration of synthetic human GLP-1 to obese patients with and without type 2 diabetes mellitus reduces circulating IL-6 only in those with type 2 diabetes mellitus, without affecting levels of leptin, adiponectin or obestatin [311]. Chronic central infusion of exendin-4 reduces circulating leptin levels in lean but not obese rats [155]. Therefore, it appears that only chronic, but not acute, exposure to elevated levels of GLP-1, either *in vivo* or *in vitro*, leads to inhibition of leptin.

It has been suggested that leptin modulates peripheral GLP-1 secretion from L cells [118], which confirms that it plays a part in regulating peripheral GLP-1 production. As leptin stimulates GLP-1 secretion in a negative feedback mechanism, GLP-1 may directly inhibit leptin secretion.

Inhibition of leptin secretion by GLP-1 was observed in SAT, the major depot contributing to its systemic levels. That this is a direct effect on secretion rather than a reflection of differences in fat mass in patients with and without N&V is substantiated by the fact that the groups with and without N&V had similar BMI, insulin sensitivity and adiponectin levels, as well as similar lipid profiles.

3.4.3 Treatment intervention

The non-mechanical N&V symptoms experienced by some patients were associated with high baseline levels of GLP-1. We hypothesized that the symptoms may be ameliorated by treatment with GLP-1 inhibitors, but potential detrimental

effects on weight maintenance and insulin sensitivity need to be considered. One of the patients in this study was treated with octreotide, a somatostatin analogue that inhibits GLP-1 secretion [312], and reported an improvement in N&V symptoms, with a concomitant reduction in basal and postprandial GLP-1 levels. However, octreotide also suppresses other gut hormones, such as PYY, which also decrease appetite and increase weight loss and are increased after RYGB surgery [312]. Therefore, specific GLP-1 antagonists, such as exendin 9-39, might be more beneficial for improving N&V symptoms, without interfering with secretion of other gut peptides that may potentially affect weight loss and insulin sensitivity adversely.

3.4.4 GLP-1 receptor gene expression in adipocytes

It was observed in the *in vitro* study that, unlike SAT, leptin secretion from OAT in response to control and GLP-1 conditions was significantly lower. This could not be explained by low expression of GLP-1 receptors in the OM depot, as it was found that GLP-1 R mRNA was expressed equally in both SC and OM adipocytes (the secretory site of leptin). Vendrell et al. previously reported that lean and obese human adipocytes of the SC, compared to OM, express more GLP-1R [126]. Perhaps in morbid obesity this depot-specific difference in GLP-1R is lost.

This study showed a direct effect of GLP-1 on the secretion of an adipokine, leptin, by human AT. Chronic elevation of the gut peptide has an inhibitory effect on both systemic leptin levels and its secretion from AT. Previous reports of decreased leptin levels early after RYGB, prior to significant reduction in BMI [19], might be explained by GLP-1-mediated inhibition of leptin. The findings in this study suggest that persistent N&V after RYGB surgery may be mediated by elevated fasting GLP-1 levels. Further studies are required to determine if treatment with specific GLP-1 antagonists ameliorates N&V symptoms without detrimental effects.

3.4.5 Study limitations

The GLP-1 ELISA kit used in this study did not measure all active forms of GLP-1. Other kits that detect all active forms of GLP-1 might have given more comprehensive results and also shown conclusively if postprandial GLP-1 levels in N&V patients are higher than those in non-N&V patients.

Only one symptomatic patient was treated with a GLP-1 antagonist; therefore, increasing the number of patients treated with specific GLP-1 antagonists is required to be able to reach any conclusions on the effect of reducing GLP-1 in order to manage patients with chronic N&V following several forms of bariatric surgery.

Chapter 4

Differential effect of primary obesity surgery endoluminal (POSE) compared to Roux-en-Y gastric bypass (RYGB)

4.1 Introduction

4.1.1 Primary obesity surgery endoluminal (POSE)

The long-term challenges facing metabolic surgery are to help identify which procedure is most suited to individual patients and to develop more efficient and cost-effective, and less invasive procedures, while investigating other therapeutic strategies that provide similar weight loss and metabolic benefits. Bariatric surgery, in keeping with other invasive procedures, has a morbidity rate of 2–17% and a mortality rate of 0.1–0.3%, creating a need to identify less invasive and safer alternatives [313].

POSE is a new form of weight-loss surgery which is performed endoscopically inside the stomach (Figure 4.1). The procedure has been successfully performed in a small number of pioneering centres around the world, mainly in the United States and recently in Europe [314]. This procedure is generally offered to patients seeking weight-loss surgery who have a BMI between 30 and 40. POSE may also be a treatment option for morbidly obese people who are not suitable for more invasive weight-loss procedures [314].

The POSE procedure is performed under general anaesthesia and takes 60–90 minutes [315]. The technique consists of using a multifunctional endoscopic platform known as an incisionless operating platform (IOP) that is introduced into the mouth and provides access to the stomach [316]. A g-Cath™ EZ Delivery catheter with suture anchors (USGI, PA, USA) is then used to create a series of plications or gastric folds in the fundus (seven to nine plications) and in the antrum body on the front face (three plications) [314](Figure 4.2).

Normally, ingested food stimulates gastric stretch receptors, resulting in relaxation of the fundus and an increase in stomach volume to accommodate the meal without causing pressure. Anchored plications may partially affect the function of the fundus and restrict its ability to relax, by accelerating the activation of gastric stretch receptors in response to food, thereby causing an early feeling of fullness. Antral plications may further prolong satiety and decrease hunger by slowing down gastric emptying [317, 318].

Compared to open and laparoscopic bariatric surgery, POSE is considered a safer procedure and is effective in achieving up to 45% EWL with no scarring or pain, and no major complications such as bleeding, leakage, wound infection or anastomotic stricture [314, 315]. However, it is not known whether the POSE procedure affects gastrointestinal hormone levels and improves metabolic parameters as seen after RYGB, or whether it has any effect on specifically improving insulin sensitivity.

Therefore, the hypotheses of this study were:

- The POSE procedure produces weight loss comparable to RYGB and also induces beneficial metabolic changes.

To explore these hypotheses, the aims of this study were to compare changes in:

- weight prior to and at 1 week, 2 months and 6 months after POSE and RYGB, and
- systemic glucose, insulin, lipids, GLP-1, leptin and adiponectin levels prior to and at 1 week, 2 months and 6 months after POSE and RYGB.

Figure 4.1 Primary obesity surgery endoluminal (POSE) (adapted from Southampton Hospital)

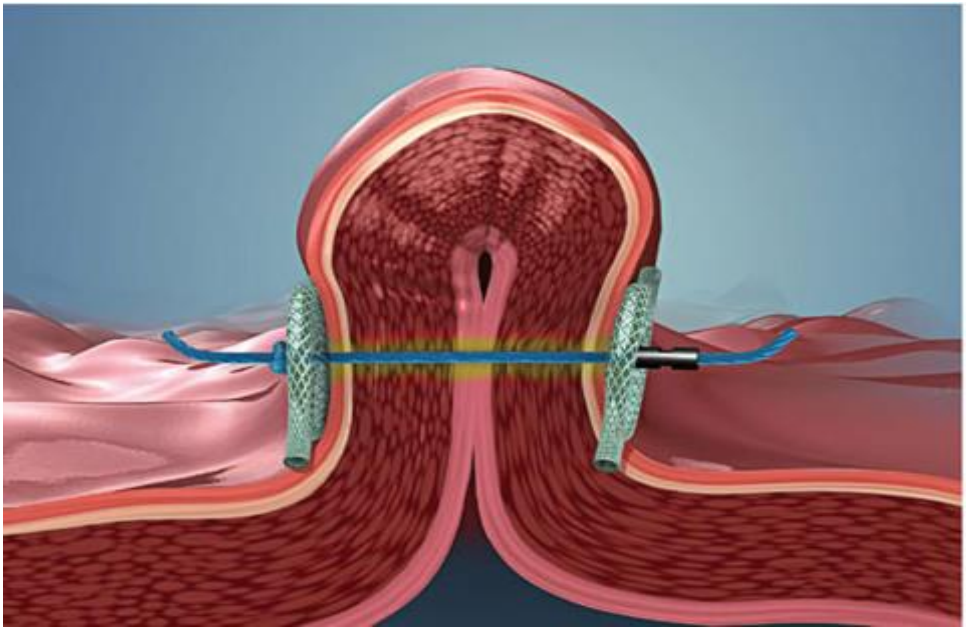

Figure 4.2 g-Cath™ EZ suture anchor holding plicated gastric tissue [249]

4.2 Methods

Study population

Sixteen patients awaiting weight-reducing surgery, either POSE or RYGB, were recruited. Group 1: six POSE patients; Group 2: ten RYGB patients matched for age and gender with the POSE patients.

Subjects were asked to visit the outpatients' clinic at the Whittington Hospital on four separate occasions: 1) 10 days prior to surgery, and then at 2) 1 week, 3) 2 months and 4) 6 months after surgery. Subjects attended the outpatients' clinic in the morning after an overnight fast.

Height (m), weight (kg), BMI (kg/m^2), blood pressure (mm Hg) and pulse (beats per minute) were recorded at each visit.

A cannula was inserted into the forearm vein, and fasting blood samples were collected for measurement of plasma glucose, lipids, insulin, leptin, adiponectin and GLP-1.

Subjects consumed a standard 388.6-kcal liquid mixed meal (17.4 g of fat, 40 g of carbohydrate and 18 g of protein), and additional blood samples for GLP-1, glucose and insulin were collected 30, 120 and 180 minutes after the meal.

Blood samples for GLP-1 were taken and immediately mixed with 50 μL of DPP-IV inhibitor.

Blood samples were centrifuged at 3000 rpm for 15 minutes at 23 °C and stored at –80 °C until analysis.

Assay

Glucose, total cholesterol, LDL, HDL and triglycerides were assayed as mentioned in section **2.2.1**. Insulin, leptin and adiponectin were determined by ELISA (sections **2.2.1** and **2.2.2**). GLP-1 was measured using ELISA (Millipore, Billerica, MA, USA), as detailed in section **2.2.4**.

HOMA was calculated as mentioned earlier in section **2.2.1**.

4.3 Results

4.3.1 Patients' characteristics

Patients' characteristics are shown in Table 4.1. All patients were female. The POSE group included two type 2 diabetes mellitus patients (one was taking metformin 1 g three times a day and gliclazide 30 mg twice a day, and the other one was on dietary control therapy. The RYGB group included two type 2 diabetes mellitus patients (one on metformin 3 g a day and pioglitazone 50 mg a day, the other patient on metformin 1 g three times a day). The anti-diabetic medications were reduced gradually post-RYGB until they were stopped at 6 months; however, the diabetic patients continued to require anti-diabetic medications post-POSE.

The groups were matched for age; mean age (±SD) for POSE versus RYGB groups was 50.0 (± 14.8) versus 50.9 (± 6.9) years, p = NS. Prior to surgery, the weight and BMI of the POSE patients was lower but did not reach statistical significance (POSE vs RYGB, weight (±SD) kg, BMI (±SD) kg/m^2: POSE 111.66 (± 11.05) kg and 41.32 (± 2.84) kg/m^2, RYGB 126.37 (± 32.03) kg and 47.41 (± 10.15) kg/m^2; p = 0.3). Systolic and diastolic blood pressure and pulse were comparable in both groups (p = NS). However, compared to POSE patients, RYGB patients were hyperinsulinaemic (fasting insulin, median and interquartile range 4.96 (2.36–6.48) versus 9.8 (8.37–13.9) mIU/ml, respectively).

Table 4.2 shows the fasting levels of adipokines and GLP-1 prior to surgery. Leptin, adiponectin and GLP-1 were comparable in both groups (p = NS).

Variables	POSE (n = 6)	RYGB (n = 10)	p†
Age (years)	50.0 (± 14.8)	50.9 (± 6.9)	0.9
BMI (kg/m^2)	41.3 (± 2.8)	47.4 (± 10.1)	0.2
Weight (kg)	111.6 (± 11.0)	126.3 (± 32.1)	0.3
Pulse (units)	84.0 (± 14.8)	85.0 (± 11.0)	0.9
SBP (mm Hg)	137.5 (± 14.8)	129 (± 11.7)	0.1
DBP (mm Hg)	84.5 (± 8.7)	83.9 (± 8.0)	0.9
Total cholesterol (mmol/L)	4.1 (± 0.37)	4.3 (± 1.13)	0.6
Triglycerides (mmol/L)	0.92 (± 0.68)	1.36 (± 0.8)	0.19
LDL cholesterol (mmol/L)	2.03 (± 0.35)	2.4 (± 1.02)	0.4
HDL cholesterol (mmol/L)	1.6 (±0.8)	1.2 (± 0.3)	0.05
Glucose (mmol/L)	8.98 (± 4.61)	5.81 (± 1.29)	0.06
Insulin (mIU/L)	4.96 (2.36–6.48)	9.8 (8.37–13.9)	0.005
HOMA	1.44 (0.47–2.7)	2.51 (1.88–3.81)	0.08

Table 4.1 Patients' characteristics

SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment – insulin resistance, HDL: high-density lipoprotein, LDL: low-density lipoprotein, p†: p value, POSE: primary obesity surgery endoluminal, RYGB: Roux-en-Y gastric bypass. Data are shown as mean (± SD) or median (interquartile ranges).

Variables	POSE (n = 6)	RYGB (n = 10)	p†
Leptin (ng/ml)	65.0 (49.9–114.2)	78.4 (50.0–120.0)	0.7
Adiponectin (μg/ml)	7.2 (5.4–10.5)	6.3 (3.9–8.7)	0.4
GLP-1 (pM)	5.4 (5.3–5.6)	5.0 (4.2–5.9)	0.5

Table 4.2 Fasting levels of adipokines and GLP-1 prior to surgery

RYGB = Roux-en-Y gastric bypass, GLP-1: glucagon-like peptide 1, p†: p value. Data are shown as median (interquartile range).

4.3.2 Weight-loss outcome

The changes in weight and BMI before and after (1 week, 2 months and 6 months) the procedures are shown in Figures 4.3 and 4.4. Both groups had lost weight significantly at 2 months. However, RYGB patients continued to lose weight at 6 months, compared to POSE patients. At 6 months, the total body weight loss (TBWL) in the POSE group (14.16 ± 9.2 kg) was less than that in the RYGB group (36.1 ± 10.47 kg). In addition, the percentage of excess weight loss (%EWL) was greater in the RYGB group (53.47 ± 14.06%) than in the POSE group (26.6 ± 16.6%).

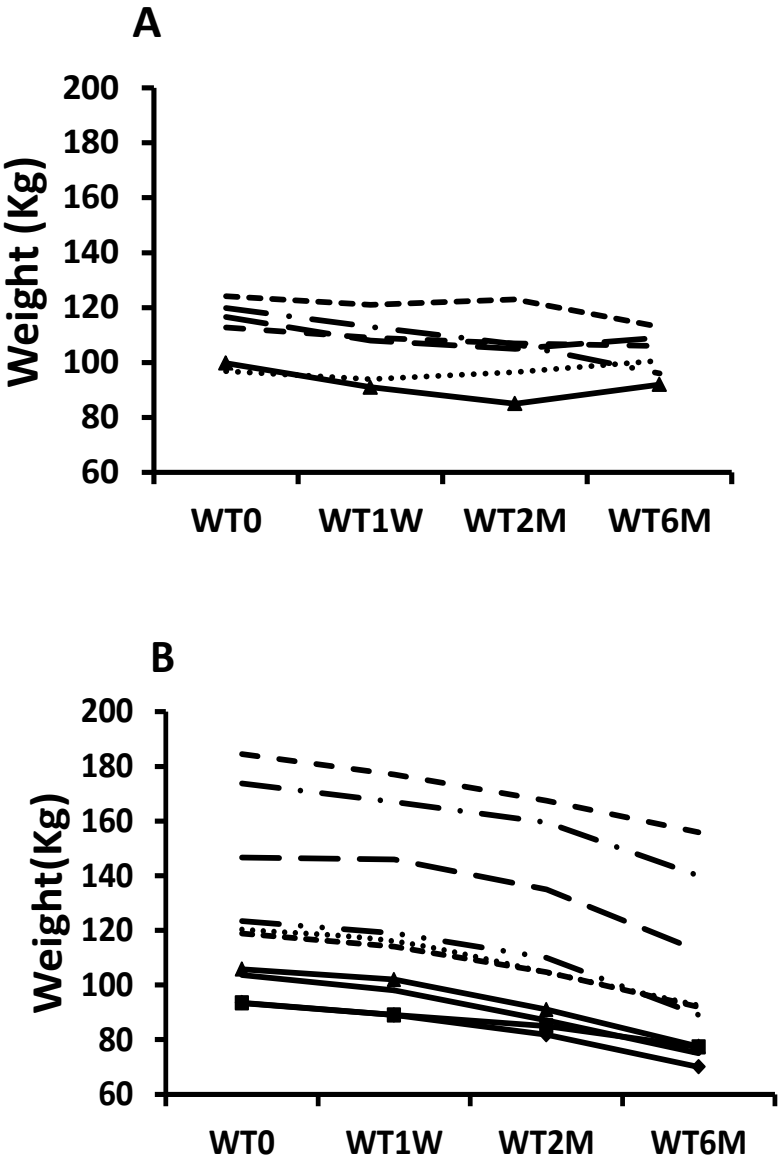

Figure 4.3 Changes in weight before and 1 week, 2 months and 6 months after POSE (A) and RYGB (B)

Weight was recorded for both POSE and RYGB patients before and 1 week, 2 months and 6 months after surgery. Both groups had lost significant weight at 1 week and 2 months after the procedures; however, more weight reduction was seen at 6 months in RYGB patients than in POSE patients, some of whom regained weight. POSE: primary obesity surgery endoluminal, RYGB: Roux-en-Y gastric bypass, WT0: 1 week before surgery, WT1W: 1 week after surgery, WT2M: 2 months after surgery, WT6M: 6 months after surgery.

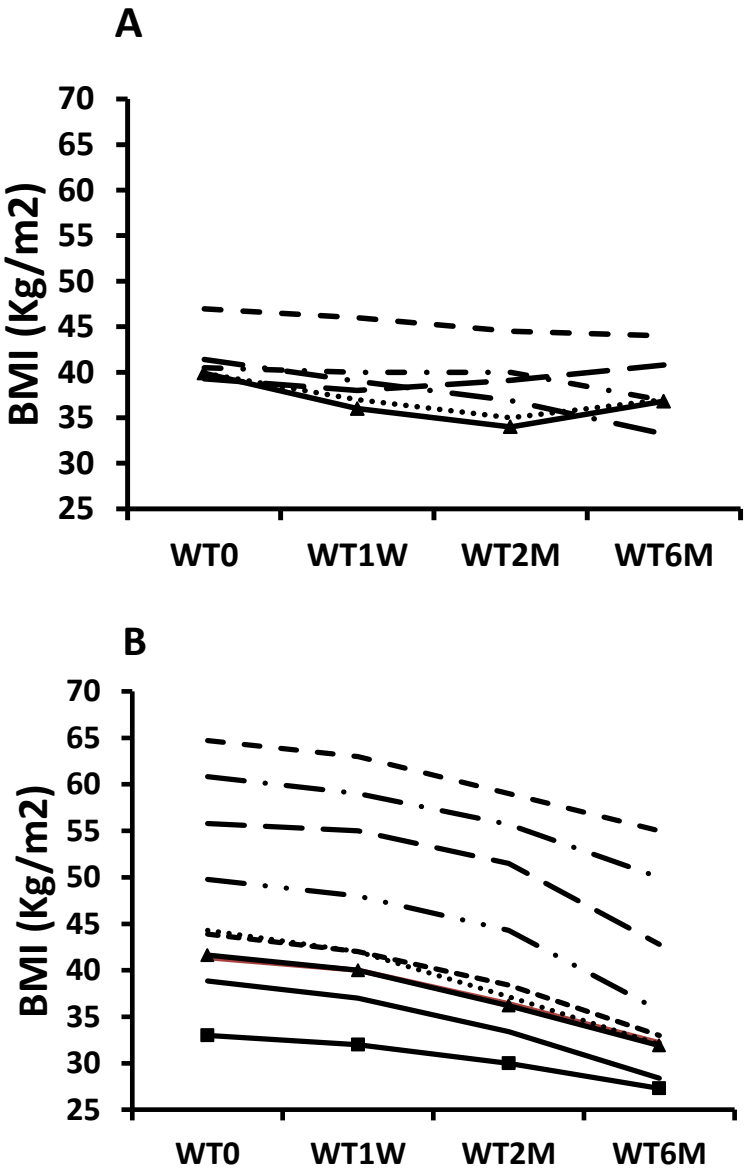

Figure 4.4 Changes in BMI before and 1 week, 2 months and 6 months after POSE (A) and RYGB (B)

BMI were recorded for both POSE and RYGB patients before and 1 week, 2 months and 6 months after surgery. Both groups had significantly lower BMI at 1 week and 2 months after the procedures; however, a greater reduction in BMI was seen at 6 months in RYGB patients than in POSE patients, some of whom regained weight. POSE: primary obesity surgery endoluminal, RYGB: Roux-en-Y gastric bypass, BMI: body mass index, WT0: 1 week before surgery, WT1W: 1 week after surgery, WT2M: 2 months after surgery, WT6M: 6 months after surgery.

4.3.3 Changes in blood pressure, heart rate and lipid profile

There were no significant differences in SBP at 1 week, 2 months and 6 months after POSE and RYGB compared to the baseline ($p > 0.05$). There were no significant differences in DBP at 1 week, 2 months and 6 months (82.3 ± 7.09, 81.83 ± 11 and 83.5 ± 6 mm Hg, respectively) after POSE compared to the baseline (84.5 ± 8.6 mm Hg). Moreover, there were no significant differences in DBP at 1 week, 2 months and 6 months (81 ± 9, 87.7 ± 9 and 85 ± 22 mm Hg, respectively) after RYGB compared to the baseline (83.9 ± 8 mm Hg).

There were no significant differences in heart rate at 1 week, 2 months and 6 months after surgery in either POSE or RYGB groups (POSE 91 ± 20, 79 ± 18 and 82 ± 11 beats; RYGB 94 ± 15, 81 ± 11 and 83 ± 10 beats, respectively) compared to the preoperative baseline (84.3 ± 14.8 and 85.7 ± 11.7 beats, respectively).

Table 4.3 shows the circulating lipid levels before and after POSE and RYGB procedures. In POSE and RYGB patients, total cholesterol decreased significantly ($p < 0.05$) at 1 week (3.7 ± 0.5 and 3.8 ± 0.7 mmol/L, respectively) compared to preoperative levels (4.1 ± 0.3 and 4.3 ± 1.1 mmol/L, respectively). However, at 2 and 6 months, the concentrations were comparable to the baseline in both groups. HDL showed a significant increase ($p < 0.05$) in both groups at 1 week postoperatively compared to the baseline, but this significance disappeared at 2 and 6 months. LDL did not show any significant changes after surgery in either group. Triglyceride levels increased in both groups at 1 week after surgery compared to preoperative levels but were only significant in the POSE group ($p = 0.01$). At 2 and 6 months postoperatively, there were no significant changes in triglyceride levels compared to preoperative levels.

	POSE	RYGB
Pre-op total cholesterol (mmol/L)	4.1 (± 0.3)	4.3 (± 1.13)
Post-op (1 w) total cholesterol (mmol/L)	3.7 (± 0.5)	3.8 (± 0.7)
Post-op (2 m) total cholesterol (mmol/L)	3.9 (± 0.5)	4.4 (± 0.9)
Post-op (6 m) total cholesterol (mmol/L)	4.4 (± 0.9)	4.4 (± 1.0)
Pre-op LDL (mmol/L)	2.03 (± 0.35)	2.4 (± 1.02)
Post-op (1 w) LDL (mmol/L)	1.9 (± 0.4)	2.0 (± 0.6)
Post-op (2 m) LDL (mmol/L)	1.9 (± 0.3)	2.6 (± 0.7)
Post-op (6 m) LDL (mmol/L)	2.7 (± 0.7)	2.5 (± 0.7)
Pre-op HDL (mmol/L)	1.6 (± 0.8)	1.2 (± 0.3)
Post-op (1 w) HDL (mmol/L)	1.1 (± 0.4)	0.9 (± 1.0)
Post-op (2 m) HDL (mmol/L)	1.5 (± 0.4)	1.2 (± 0.2)
Post-op (6 m) HDL (mmol/L)	1.6 (± 0.6)	1.4 (± 0.4)
Pre-op triglyceride (mmol/L)	0.92 (± 0.68)	1.36 (± 0.8)
Post-op (1 w) triglyceride (mmol/L)	1.6 (± 0.4)	1.4 (± 0.5)
Post-op (2 m) triglyceride (mmol/L)	1.0 (± 0.3)	1.4 (± 0.6)
Post-op (6 m) triglyceride (mmol/L)	1.1 (± 0.6)	1.3 (± 0.9)

Table 4.3 Circulating lipid levels before and after (1 week, 2 months and 6 months) POSE and RYGB surgery

Pre-op = preoperatively, Post-op = postoperatively, w = week, m = month. Data are expressed as mean and standard deviation.

4.3.4 Glucose, insulin and GLP-1 secretions

POSE patients had higher fasting glucose levels than RYGB patients (8.98 ± 4.61 and 5.81 ± 1.29 mmol/L, respectively; $p = 0.06$). In both groups, fasting glucose levels did not differ significantly between preoperative and 1 week, 2 month or 6 month postoperative levels. There were no significant changes in postprandial glucose levels (30, 120 and 180 minutes) postoperatively at 1 week, 2 months and 6 months compared to preoperative levels in either POSE or RYGB groups (Table 4.4, Figures 4.5 and Figure 4.6).

In the POSE group, there was no significant change in postoperative basal insulin levels or HOMA at 1 week, 2 months and 6 months compared to the preoperative level; however, in RYGB patients, significant decreases in basal insulin levels were apparent at 1 week and 6 months postoperatively compared to the preoperative level ($p < 0.05$). HOMA also decreased significantly postoperatively in RYGB patients compared to the baseline ($p < 0.05$; Table 4.5, Figures 4.7 and 4.8).

Prior to surgery, postprandial insulin levels (30, 120 and 180 minutes) were comparable between POSE and RYGB patients (Table 4.6). Postprandial insulin response at 30, 120 and 180 minutes showed no significant difference before and 1 week, 2 months and 6 months after the POSE procedure (Figures 4.9 A, 4.10 A, 4.11 A and 4.12 A). No significant difference in 30-minute postprandial insulin response showed at 1 week, however, a significant drop in 120-minute postprandial insulin response ($p = 0.02$) was shown at 1 week after the RYGB procedure (Figure 4.10 B). The early (30 minute) postprandial insulin response increased, and the late (120 minute) postprandial insulin response decreased following RYGB surgery, and reached significance ($p < 0.05$) at 2 and 6 months (Figures 4.11 B & 4.12 B). The AUC0-180 of insulin in response to the meal was not changed post-POSE compared to preoperatively (Figure 4.13 A and Table 4.7). However, it was increased post-RYGB at 2 and 6 months compared to preoperatively but did not reach significance ($p > 0.05$) (Figure 4.13 B and Table 4.7).

Fasting and postprandial (30, 120 and 180 minute) GLP-1 levels were comparable between POSE and RYGB patients preoperatively (Figure 4.14). No changes were seen in postoperative fasting and postprandial GLP-1 levels compared to the baseline in POSE patients (Figures 4.15 A, 4.16 A and 4.17 A). In addition, the AUC0-180 of GLP-1 in response to the meal did not differ before and after (1 week, 2 months and 6 months) the POSE procedure (Figure 4.18 A). However, postprandial GLP-1 at 30 minutes showed significant elevation ($p < 0.05$) post-RYGB procedure at 1 week, 2 months and 6 months compared to the baseline (Figures 4.15 B, 4.16 B and 4.17 B). Consistently, there was significant elevation in the AUC0-180 of GLP-1 in response to the meal after (1 week, 2 months and 6 months) RYGB compared to the preoperative level (Figure 4.18 B).

	POSE	RYGB
Pre-op glucose (mM)		
Fasting	8.98 (± 4.6)	5.8 (± 1.3)
Postprandial 30 m	9.9 (± 5.0)	6.5 (± 1.4)
Postprandial 2 h	11.1 (± 7.8)	6.3 (± 1.2)
Postprandial 3 h	9.1 (± 7.8)	5.7 (± 0.8)
Post-op (1 week) glucose (mM)		
Fasting	8.6 (± 4.2)	6.4 (± 2.9)
Postprandial 30 m	8.7 (± 6.1)	8.7 (± 3.1)
Postprandial 2 h	9.2 (± 7.3)	6.1 (± 3.1)
Postprandial 3 h	9.3 (± 6.8)	6.7 (± 3.2)
Post-op (2 month) glucose (mM)		
Fasting	7.8 (± 3.2)	5.6 (± 1.7)
Postprandial 30 m	9.1 (± 5.2)	7.2 (± 1.9)
Postprandial 2 h	10.2 (± 6.8)	4.5 (± 0.5)
Postprandial 3 h	9.3 (± 6.3)	5.0 (± 1.1)
Post-op (6 month) glucose (mM)		
Fasting	6.2 (± 2.0)	5.7 (± 2.2)
Postprandial 30 m	8.4 (± 4.5)	8.4 (± 3.9)
Postprandial 2 h	8.2 (± 7.4)	5.5 (± 3.7)
Postprandial 3 h	5.5 (± 1.2)	5.5 (± 3.0)

Table 4.4 Fasting and postprandial glucose levels before and after (1 week, 2 months and 6 months) POSE and RYGB procedures

Data are expressed as mean and standard deviation. Pre-op = preoperatively, post-op = postoperatively, m = minute, h = hour.

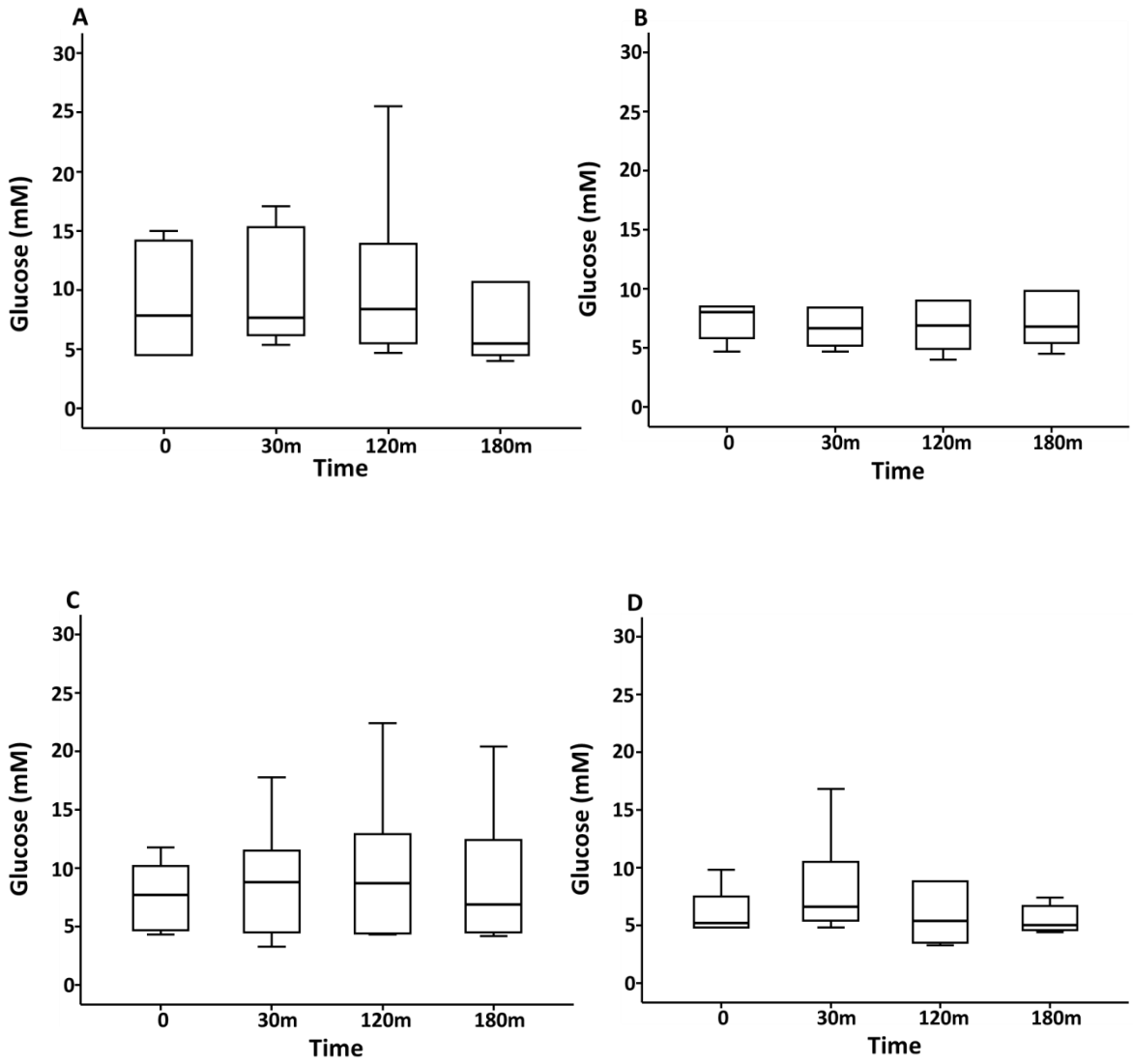

Figure 4.5 Fasting and postprandial (30, 120 and 180 minutes) glucose levels prior to (A) and 1 week (B), 2 months (C) and 6 months (D) after POSE surgery

There were no significant changes in fasting and postprandial glucose levels before and after (1 week, 2 months and 6 months) the POSE procedure. Data are expressed as median and interquartile range.

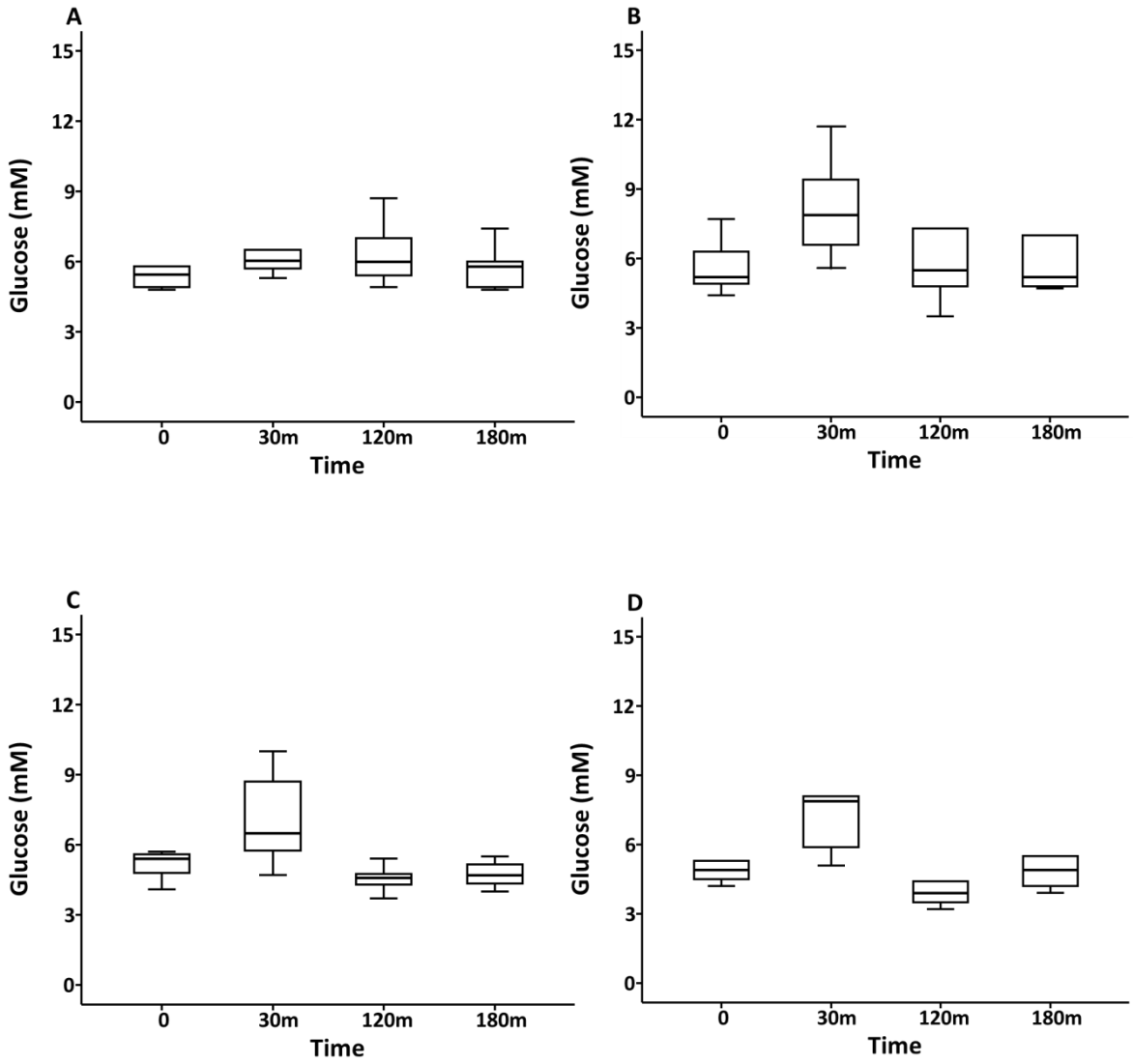

Figure 4.6 Fasting and postprandial (30, 120 and 180 minutes) glucose levels prior to (A) and 1 week (B), 2 months (C) and 6 months (D) after RYGB surgery

There were no significant changes in fasting and postprandial glucose levels before and 1 week, 2 months and 6 months after the RYGB procedure. Data are expressed as median and interquartile range.

	POSE	RYGB
HOMA-IR		
Pre-op	1.4 (0.4–2.7)	2.5 (1.8–3.8)
1 w post-op	1.9 (0.9–2.7)	1.6 (1.0–2.6)
2 m post-op	1.1 (0.6–3.4)	2.2 (1.1–3.1)
6 m post-op	0.9 (0.4–1.5)	1.2 (0.5–1.7)
Fasting insulin (mIU/L)		
Pre-op	4.9 (2.3–6.4)	9.8 (8.3–13.9)
1 w post-op	5.7 (3.5–7.0)	6.9 (4.5–8.2)
2 m post-op	5.0 (2.7–7.6)	9.2 (5.5–13.9)
6 m post-op	4.1 (1.6–6.0)	5.5 (2.6–6.2)

Table 4.5 HOMA-IR and fasting insulin levels before and after POSE and RYGB procedures

Data are expressed as median and interquartile range. HOMA-IR = homeostatic model assessment – insulin resistance, pre-op = preoperatively, post-op = postoperatively, w = week, m = month.

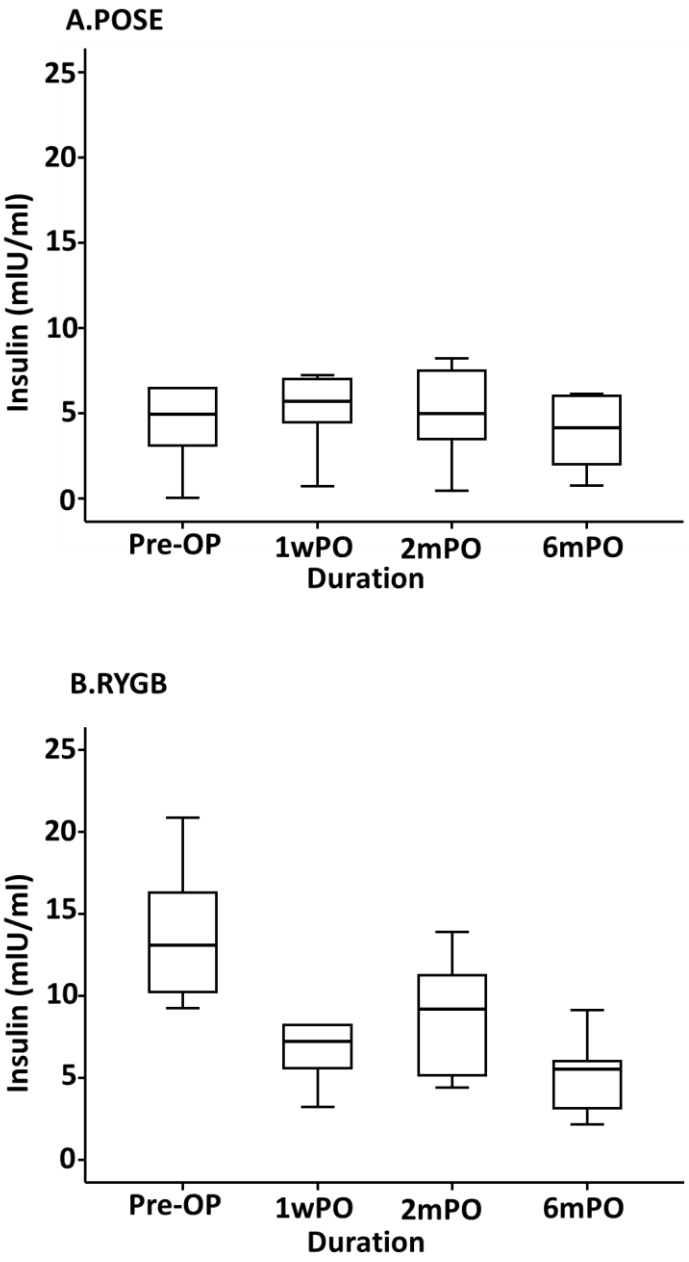

Figure 4.7 Fasting insulin level prior to and following (1 week, 2 months and 6 months) POSE (A) and RYGB (B) procedures

Fasting insulin plasma levels showed no significant changes before and after (1 week, 2 months and 6 months) the POSE procedure ($p > 0.05$). However, there was a significant decrease in fasting insulin levels at 1 week and 6 months after the RYGB procedure compared to the preoperative level ($p < 0.05$). Data are expressed as median and interquartile range. Pre-op: preoperatively, 1wPO: 1 week postoperatively, 2mPO: 2 months postoperatively, 6mPO: 6 months postoperatively.

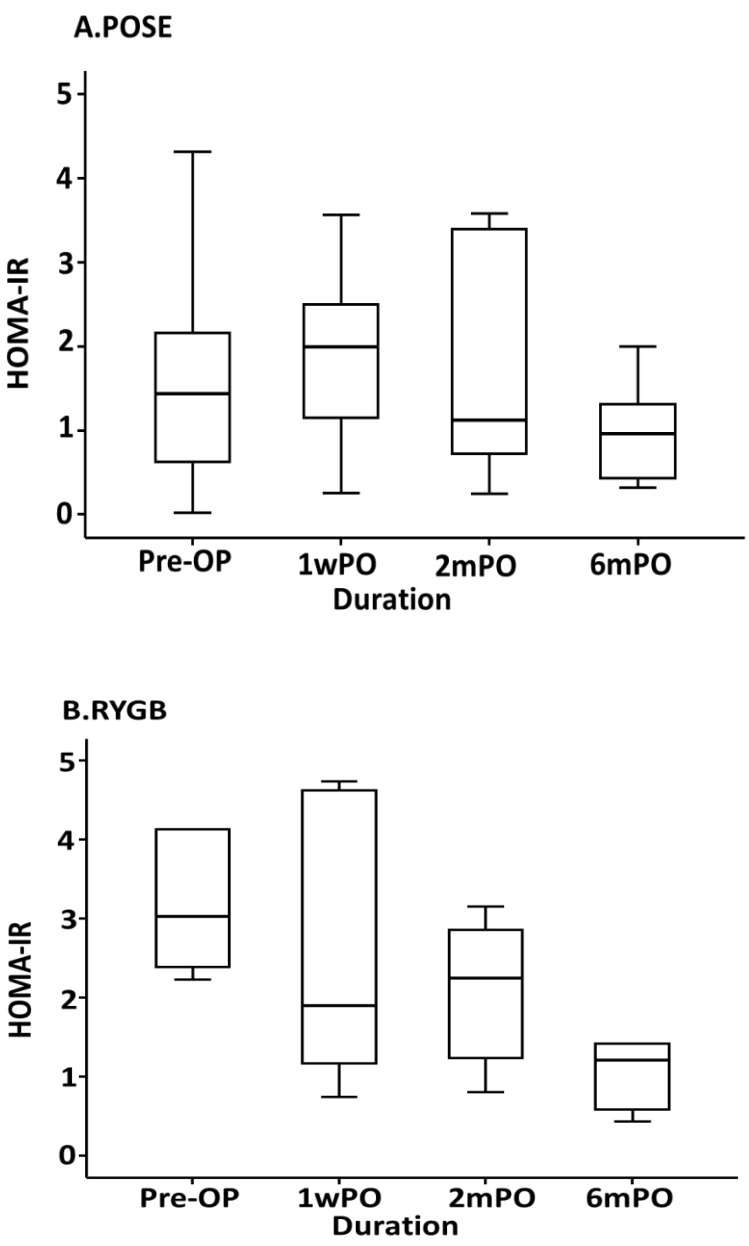

Figure 4.8 HOMA-IR before and after (1 week, 2 months and 6 months) POSE (A) and RYGB (B) procedures

There were no significant changes in HOMA-IR following POSE surgery compared to preoperatively. There was a significant reduction in HOMA-IR at 1 week ($p = 0.04$) and 6 months ($p = 0.02$) after RYGB surgery compared to preoperatively ($p < 0.05$). Data are expressed as median and interquartile range. Pre-op: preoperatively, 1wPO: 1 week postoperatively, 2mPO: 2 months postoperatively, 6mPO: 6 months postoperatively, HOMA-IR: homeostasis model assessment – insulin resistance.

	POSE	RYGB
Pre-op insulin (mIU/L)		
Fasting	4.9 (2.3–6.4)	9.8 (8.3–13.9)
Postprandial 30 m	19.0 (9.1–29.8)	32.6 (21.2–86.6)
Postprandial 2 h	17.8 (3.4–30.4)	27.4 (21.7–35.6)
Postprandial 3 h	5.5 (1.5–12.7)	15 (9.0–22.7)
Post-op (1 week) insulin (mIU/L)		
Fasting	5.7 (3.5–7.0)	6.9 (4.5–8.2)
Postprandial 30 m	27.4 (4.6–31.9)	51.2 (26.6–100.2)
Postprandial 2 h	15.5 (4.1–27.1)	9.1 (5.9–23.9)
Postprandial 3 h	9.0 (2.7–15.9)	6.8 (4.4–11.6)
Post-op (2 month) insulin (mIU/L)		
Fasting	5.0 (2.7–7.6)	9.2 (5.1–13.9)
Postprandial 30 m	21.3 (9.1–44.2)	137 (74.2–228.5)
Postprandial 2 h	11.5 (2.2–25.7)	7.9 (4.1–20.4)
Postprandial 3 h	4.1 (1.5–12.1)	7.8 (3.4–8.8)
Post-op (6 month) insulin (mIU/L)		
Fasting	4.1 (1.7–6.0)	5.5 (2.6–6.2)
Postprandial 30 m	25.5 (3.4–36.1)	117.4 (117.4–147)
Postprandial 2 h	8.1 (1.7–32.0)	5.1 (3.6–10.0)
Postprandial 3 h	3.8 (1.3–13.0)	4.4 (2.8–7.1)

Table 4.6 Fasting and postprandial insulin levels before and after (1 week, 2 months and 6 months) POSE and RYGB procedures

Data are expressed as median and interquartile range. Pre-op = preoperatively, post-op = postoperatively, m = minute, h = hour.

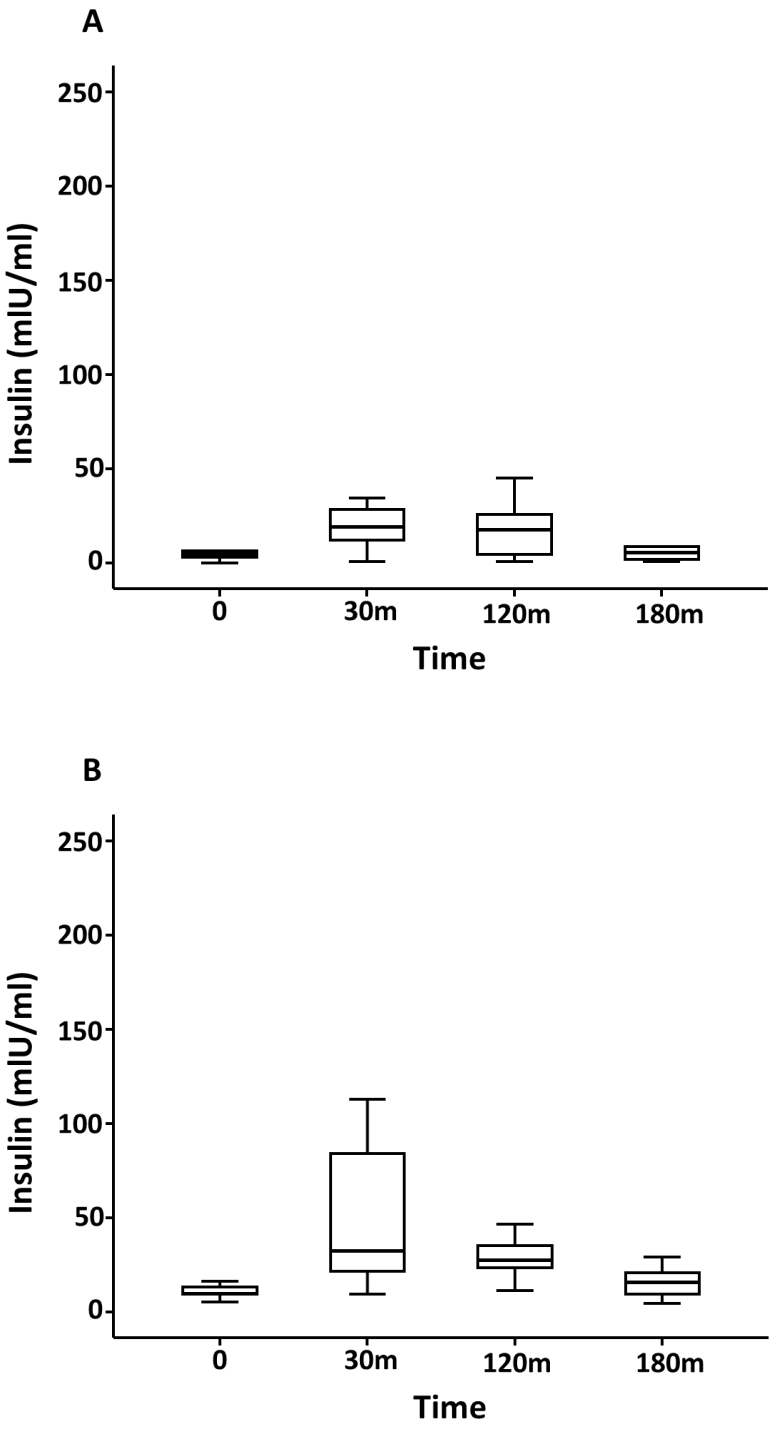

Figure 4.9 Preoperative insulin responses to a meal in POSE (A) and RYGB (B) patients

In the POSE and RYGB groups, 30-minute levels of postprandial insulin compared to fasting were significantly elevated ($p = 0.028$ and 0.005, respectively). Further, 120-minute postprandial levels were comparable to 30-minute postprandial

levels in both groups ($p > 0.05$). The delta changes of insulin levels from baseline to 30-minute postprandial, and from 30 to 120 minutes postprandial in POSE patients were comparable to those in RYGB patients ($p > 0.05$). Data are expressed as median and interquartile range. m = minute.

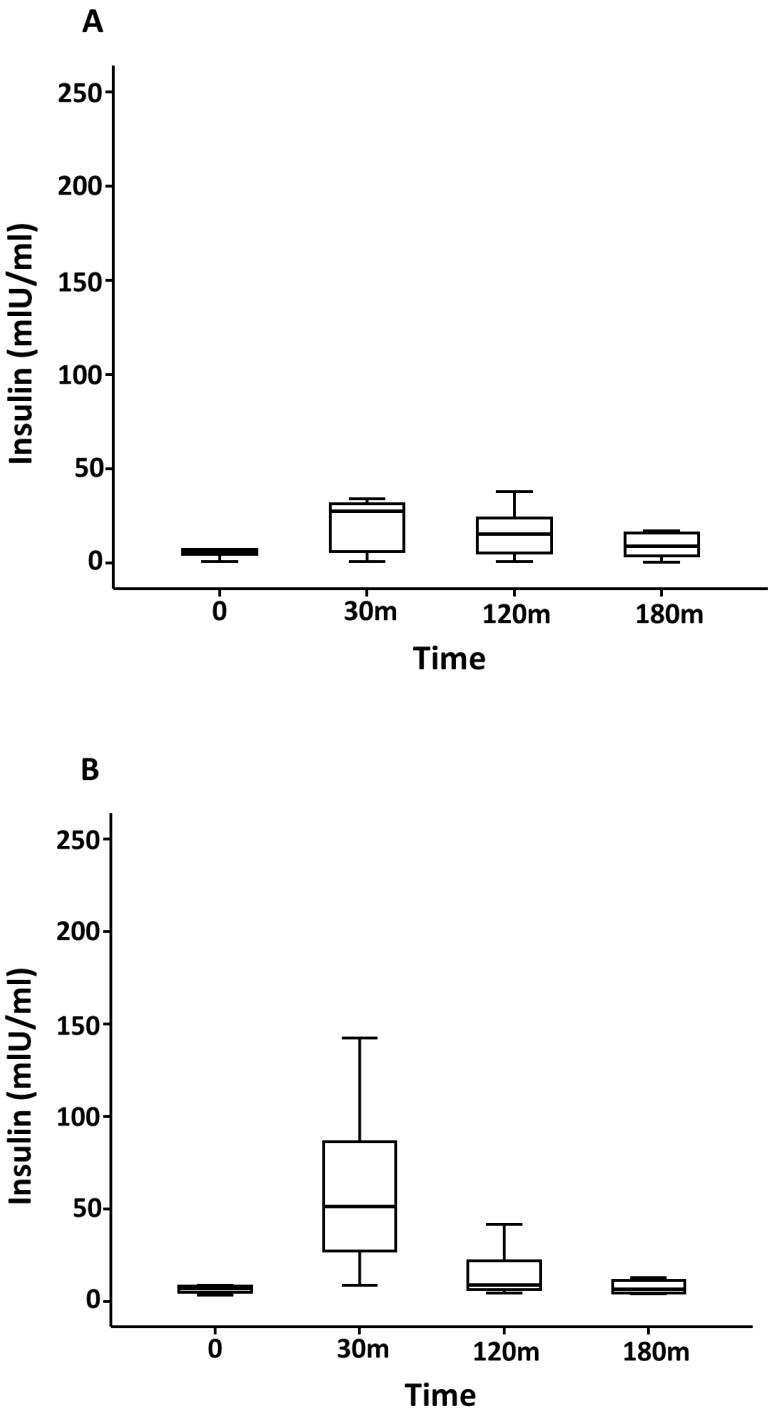

Figure 4.10 One-week postoperative insulin response to a meal in POSE (A) and RYGB (B) patients

There were no significant changes in postprandial (30, 120 and 180 minute) insulin levels 1 week after POSE compared to preoperative levels. One week after RYGB, 30-minute postprandial insulin levels were comparable to preoperative

levels; however, 120-minute postprandial insulin levels decreased significantly ($p = 0.02$) compared to preoperative levels. The delta change of insulin levels from baseline to 30-minute postprandial was comparable between POSE and RYGB; however, the delta change from 30 to 120 minutes was significantly greater in RYGB compared to POSE ($p = 0.02$). Data are expressed as median and interquartile range.

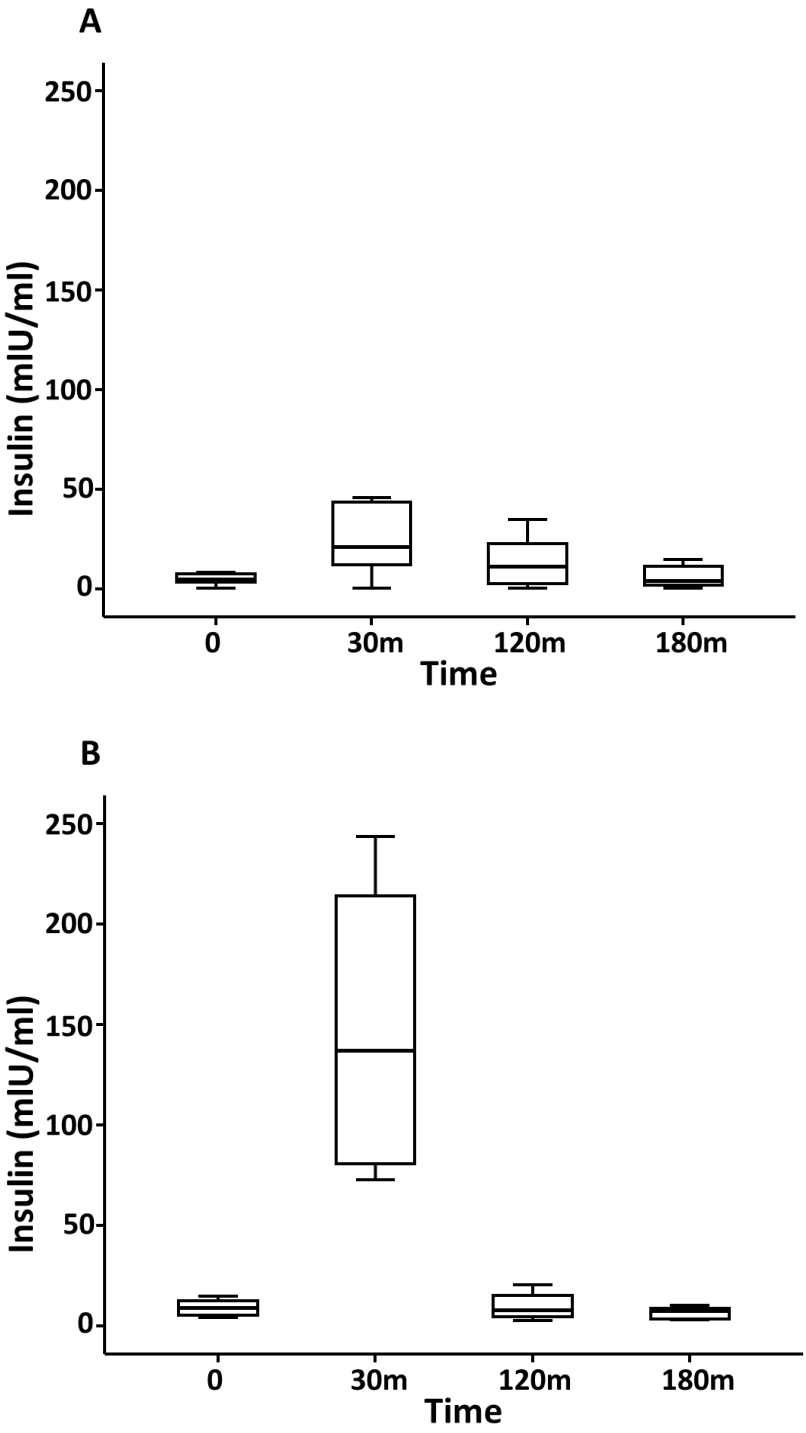

Figure 4.11 Two-month postoperative insulin responses to a meal in POSE (A) and RYGB (B) patients

There were no significant changes in postprandial (30, 120 and 180 minute) insulin levels 2 months after POSE compared to preoperative levels. Two months

after RYGB, 30-minute postprandial insulin levels increased significantly ($p = 0.02$), and 120-minute postprandial insulin levels decreased significantly ($p = 0.04$) compared to preoperative levels. The delta changes of insulin levels from baseline to 30-minute postprandial and from 30 to 120 minutes postprandial for RYGB were significantly greater than those for POSE ($p = 0.001$). Data are expressed as median and interquartile range.

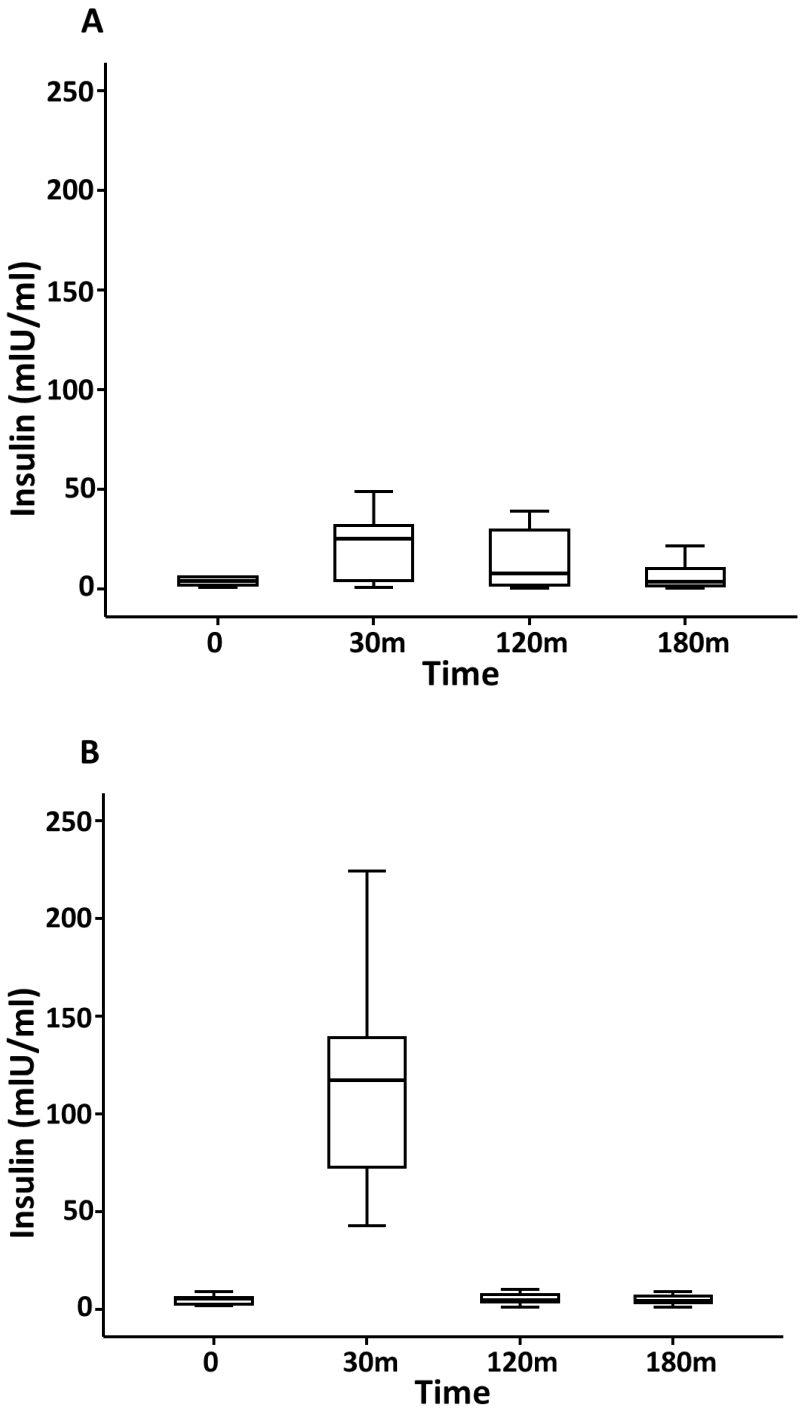

Figure 4.12 Six-month postoperative insulin responses to a meal in POSE (A) and RYGB (B) patients

There were no significant changes in postprandial (30, 120 and 180 minute) insulin levels 6 months after POSE compared to preoperative levels. Six months after RYGB, 30-minute postprandial insulin levels increased significantly ($p = 0.05$), and 120-minute postprandial insulin levels decreased significantly ($p =$

0.04) compared to preoperative levels. The delta changes of insulin levels from baseline to 30-minute postprandial and from 30 to 120 minutes postprandial for RYGB were significantly greater than those for POSE ($p = 0.002$ and 0.001, respectively). Data are expressed as median and interquartile range.

	POSE	RYGB
AUC insulin		
Pre-op	2826 (± 1786)	5819 (± 2898)
1 w post-op	2847 (± 1841)	5894 (± 4010)
2 m post-op	2755 (± 2144)	10285 (± 5317)
6 m post-op	2736 (± 2078)	7583 (± 3865)

Table 4.7 Area under the curve for insulin response to a meal before and after (1 week, 2 months and 6 months) POSE and RYGB surgery

Data are expressed as mean and standard deviation. AUC = area under the curve, pre-op= preoperatively, post-op = postoperatively, w = week, m = month.

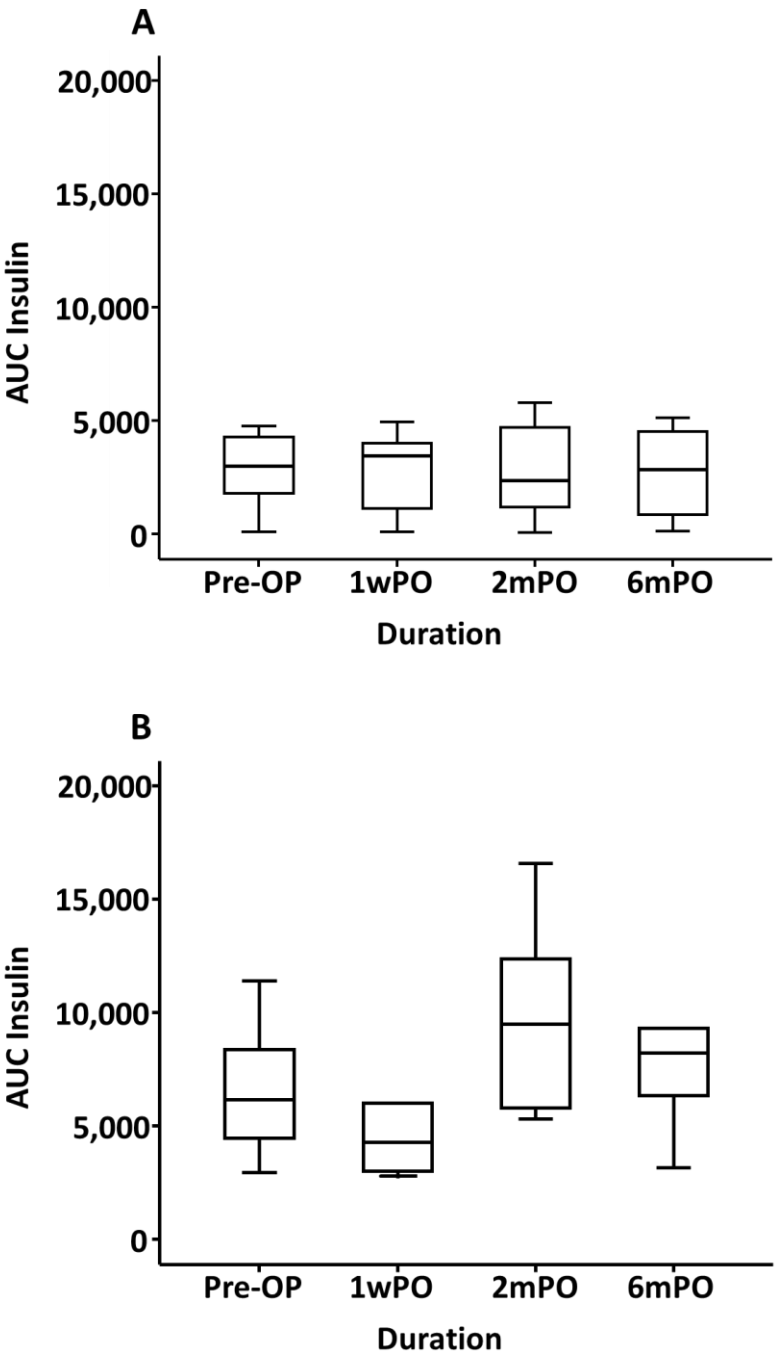

Figure 4.13 Area under the curve for insulin response to a meal before and after (1 week, 2 months and 6 months) POSE (A) and RYGB (B) surgery

The AUC for insulin response to the meal did not differ before and after (1 week, 2 months and 6 months) POSE surgery (A). However, the AUC for insulin response increased after RYGB at 2 and 6 months compared to preoperatively (B) but did not reach significance ($p = 0.09$ and 0.06, respectively). Data are expressed as median and interquartile range. Pre-op: preoperatively, 1wPO: 1 week

postoperatively, 2mPO: 2 months postoperatively, 6mPO: 6 months postoperatively.

	POSE	RYGB
Pre-op GLP-1 (PM)		
Fasting	5.3 (5.2–5.6)	4..9 (4.2–5.9)
Postprandial 30 m	10.0 (7.0–12.8)	7.0 (6.6–8.2)
Postprandial 2 h	9.8 (7.0–12.8)	6.2 (5.2–9.4)
Postprandial 3 h	6.5 (6.1–7.7)	5.9 (4.4–7.1)
Post-op (1 week) GLP-1 (PM)		
Fasting	5.4 (5.2–5.9)	5.3 (4.5–6.6)
Postprandial 30 m	10.4 (7.6–13.0)	79.4 (13.3–99.2)
Postprandial 2 h	8.5 (6.3–10.5)	10.6 (6.8–26.5)
Postprandial 3 h	6.0 (5.8–10.4)	6.3 (5.2–14.4)
Post-op (2 month) GLP-1 (PM)		
Fasting	5.6 (3.8–7.1)	4.5 (4.1–6.1)
Postprandial 30 m	8.8 (7.7–13.8)	100.5 (81.5–111.6)
Postprandial 2 h	6.5 (5.3–14.7)	11.1 (5.3–15.8)
Postprandial 3 h	5.7 (4.6–16.8)	5.4 (4.5–8.5)
Post-op (6 month) GLP-1 (PM)		
Fasting	4.3 (4.1–5.3)	4.3 (4.0–4.3)
Postprandial 30 m	8.1 (6.8–10.2)	83.6 (71.9–91.7)
Postprandial 2 h	5.6 (4.4–6.5)	6.3 (5.2–13.9)
Postprandial 3 h	4.9 (4.2–5.8)	4.4 (4.0–5.1)

Table 4.8 Fasting and postprandial GLP-1 levels after (1 week, 2 months and 6 months) POSE and RYGB procedures

Data are expressed as median and interquartile range. Pre-op = preoperatively, post-op = postoperatively, m = minute, h = hour.

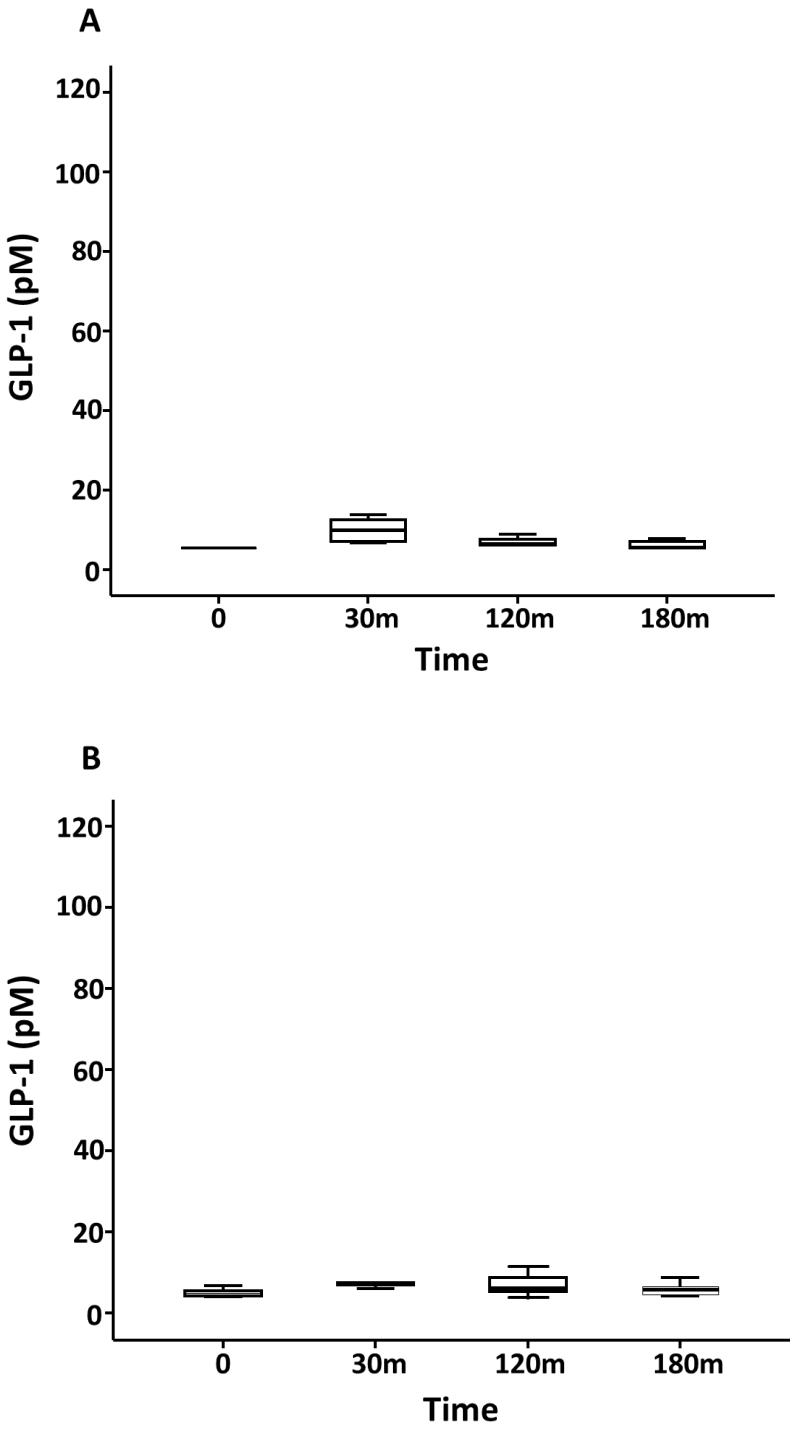

Figure 4.14 Preoperative fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels for POSE (A) and RYGB (B) procedures

Fasting and postprandial (30, 120 and 180 minute) GLP-1 levels were comparable between POSE and RYGB patients preoperatively. Data are expressed as median and interquartile range.

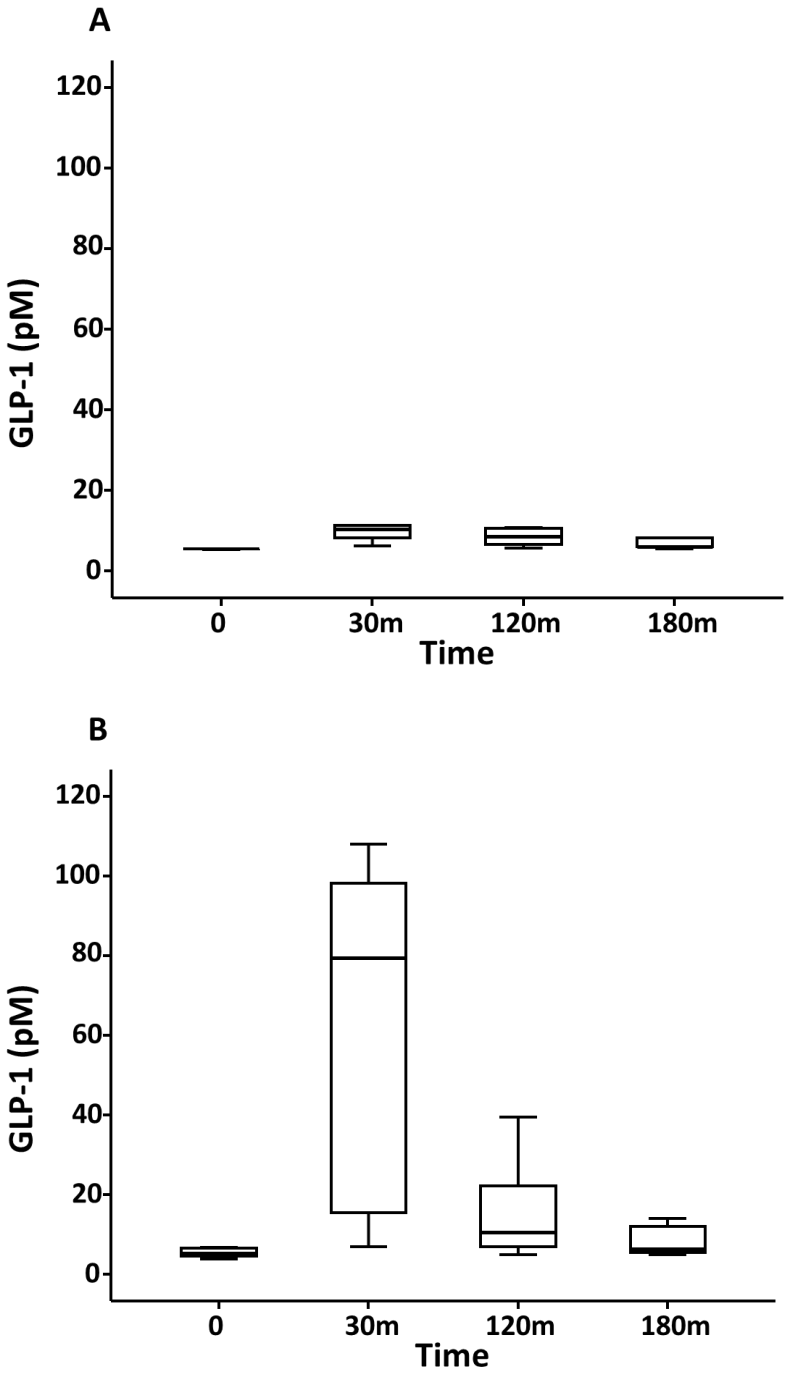

Figure 4.15 One-week postoperative fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels for POSE (A) and RYGB (B) procedures

There were no significant changes in fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels 1 week after POSE compared to preoperative levels ($p > 0.05$). One week after RYGB, there were no significant changes in fasting and 120- and 180-minute postprandial GLP-1 levels compared to preoperative levels;

however, 30-minute postprandial GLP-1 increased significantly ($p = 0.05$). The delta change of GLP-1 levels from baseline to 30 minutes postprandial for RYGB was significantly greater than that for POSE ($p = 0.02$). Data are expressed as median and interquartile range.

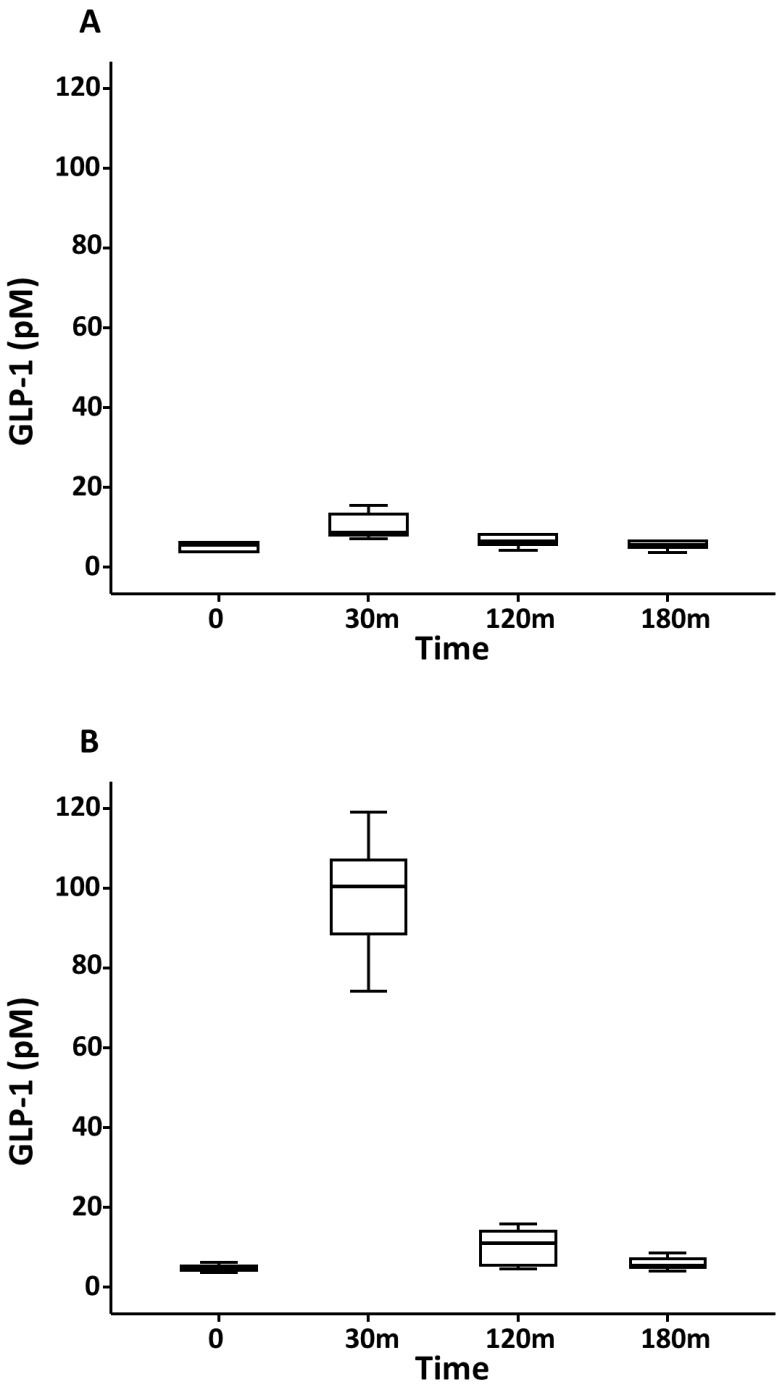

Figure 4.16 Two-month postoperative fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels for POSE (A) and RYGB (B) procedures

There were no significant changes in fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels 2 months after POSE compared to preoperative levels ($p > 0.05$). Two months after RYGB, there were no significant changes in fasting and 120- and 180-minute postprandial GLP-1 levels compared to preoperative levels;

however, 30-minute postprandial GLP-1 increased significantly ($p = 0.01$). The delta change of GLP-1 levels from baseline to 30 minutes postprandial for RYGB was significantly greater than that for POSE ($p = 0.001$). Data are expressed as median and interquartile range.

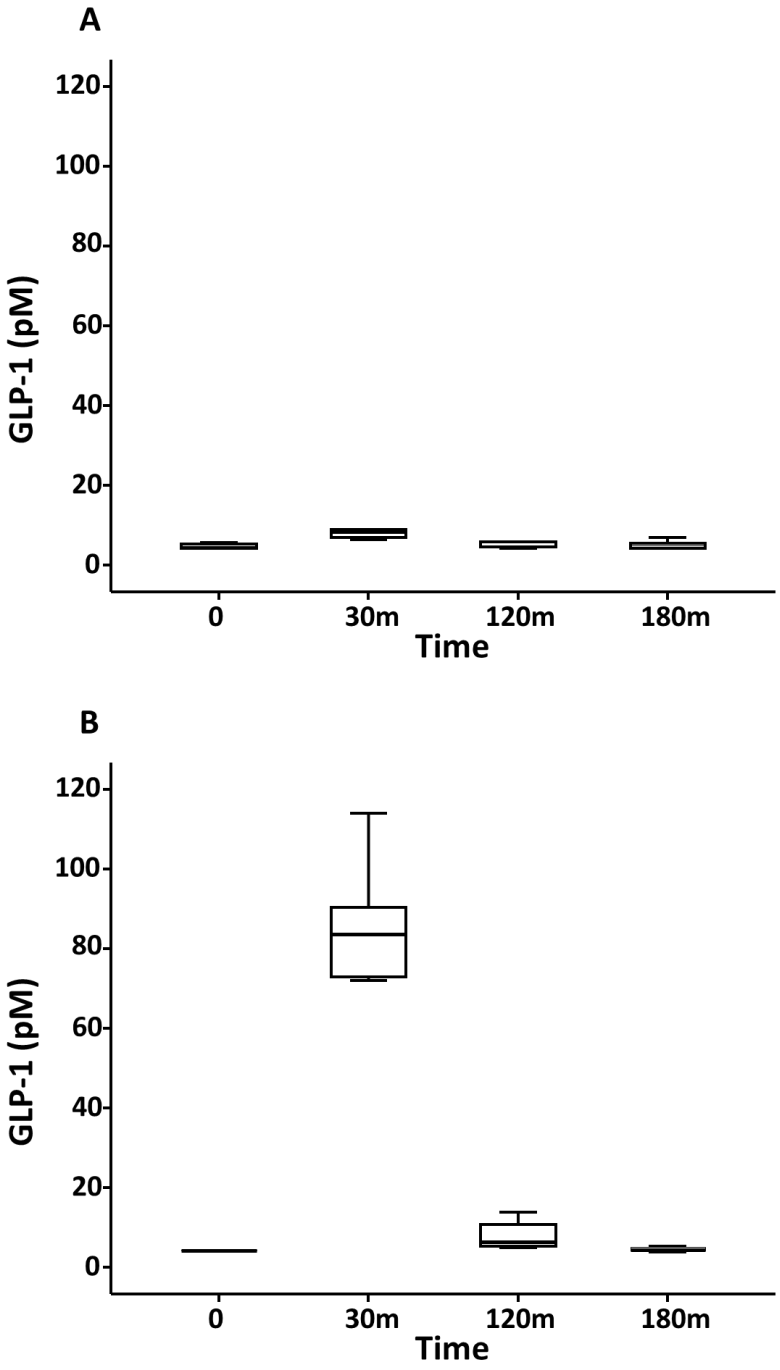

Figure 4.17 Six-month postoperative fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels for POSE (A) and RYGB (B) procedures

There were no significant changes in fasting and postprandial (30, 120 and 180 minutes) GLP-1 levels **6** month**s after** POSE compared to preoperative levels ($p > 0.05$). **Six** month**s** after RYGB, **there were** no significant changes in fasting and 120**-** and 180**-**minute postprandial GLP-1 levels compared to preoperative levels**;** however, 30**-**minute postprandial GLP-1 increased significantly ($p = 0.01$). The delta change of GLP-1 levels

from baseline to **30-minute** postprandial **for** RYGB was significantly greater than that **for** POSE ($p = 0.001$). Data are expressed as median and interquartile range.

	POSE	RYGB
AUC GLP-1		
Pre-op	1383 (± 184)	2043 (± 279)
1 w post-op	1614 (± 397)	5497 (± 3712)
2 m post-op	1893 (± 1566)	7224 (± 1063)
6 m post-op	1184 (± 303)	5733 (± 1683)

Table 4.9 Area under the curve for GLP-1 response to meal before and after (1 week, 2 months and 6 months) POSE and RYGB surgery

Data are expressed as mean and standard deviation. AUC = area under the curve, pre-op = preoperatively, post-op = postoperatively, w = week, m = month.

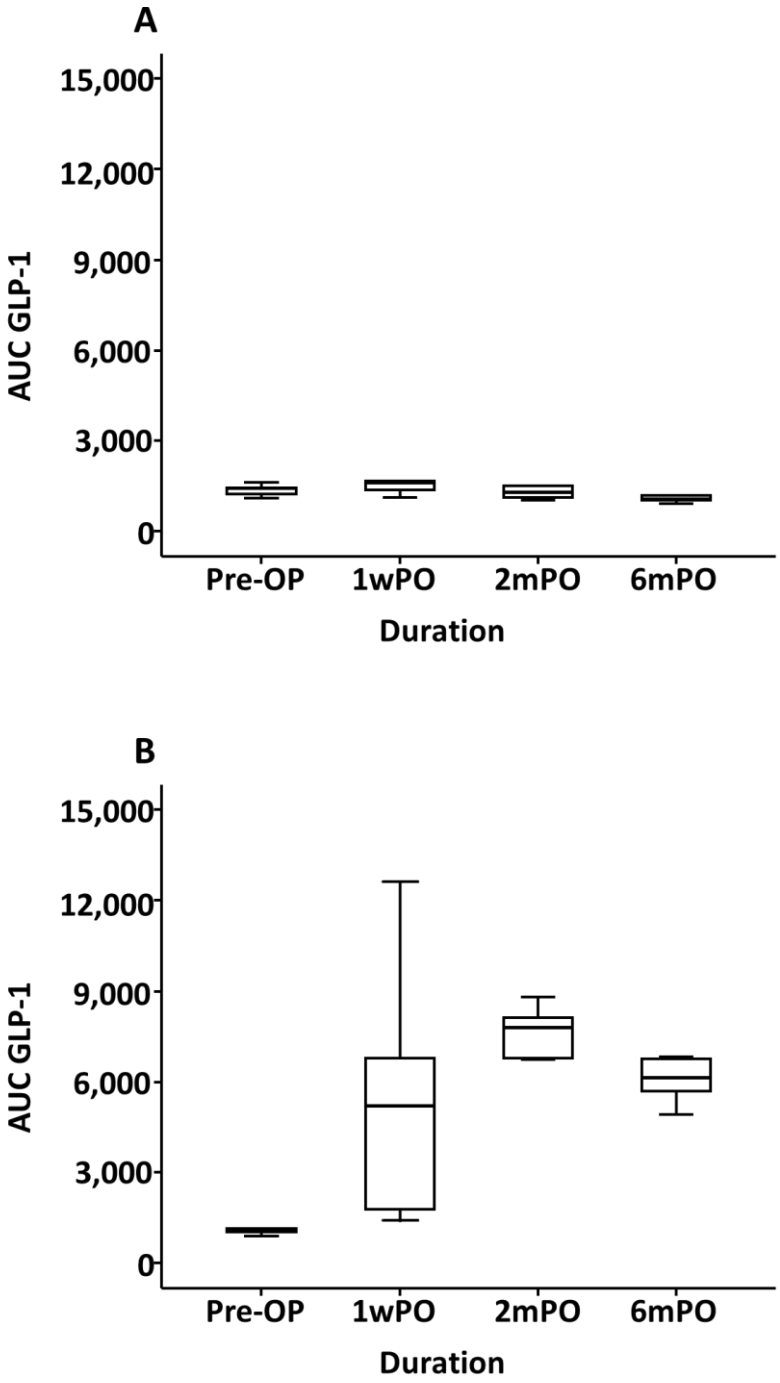

Figure 4.18 Area under the curve for GLP-1 response to meal before and after (1 week, 2 months and 6 months) POSE (A) and RYGB (B) surgery

The area under the curve for GLP-1 response did not differ before and after (1 week, 2 months and 6 months) POSE surgery (A). However, the AUC for GLP-1 response increased significantly after RYGB surgery (1 week: $p = 0.01$; 2 months: $p = 0.03$; 6 months: $p = 0.04$) compared to preoperatively (B). Data are expressed

as median and interquartile range. Pre-op: preoperatively, 1wPO: 1 week postoperatively, 2mPO: 2 months postoperatively, 6mPO: 6 months postoperatively.

4.3.5 Adipokine concentrations

Table 4.10 shows circulating leptin and adiponectin levels before and after (1 week, 2 months and 6 months) POSE and RYGB procedures. Leptin levels were reduced significantly at 1 week after POSE and RYGB surgeries compared to the preoperative values ($p = 0.028$ and $p = 0.005$, respectively). Further significant reduction was seen in RYGB patients at 2 and 6 months postoperatively. Two and six months after the POSE procedure, leptin levels were also significantly reduced compared to the baseline, but the reduction was greater at 1 week postoperatively (Figure 4.19 A and B).

Adiponectin levels showed no significant difference before and after the POSE procedure (Figure 4.20 A). For RYGB, a trend of increasing adiponectin was seen at 2 and 6 months postoperatively compared to the baseline level (Figure 4.20 B).

	POSE	RYGB
Leptin (ng/ml)		
Pre-op	65.0 (49.9–114.2)	78.4 (50.0–120.0)
1 w post-op	131.0 (21.4–78.0)	39.7 (32.6–51.7)
2 m post-op	49.2 (18.4–73.2)	32.5 (27.1–42.3)
6 m post-op	46.4 (21.3–71.3)	31.8 (8.2–59.3)
Adiponectin (μg/ml)		
Pre-op	7.2 (5.3–10.5)	6.3 (3.9–8.6)
1 w post-op	6.2 (5.0–10.6)	5.9 (4.8–10.2)
2 m post-op	8.5 (5.4–12.7)	8.0 (6.3–14.7)
6 m post-op	7.6 (6.3–9.5)	16.4 (6.6–28.1)

Table 4.10 Leptin and adiponectin levels prior to and following (1 week, 2 months and 6 months) POSE and RYGB procedures

Data are expressed as median and interquartile range. Pre-op = preoperatively, post-op = postoperatively, w = week, m = month.

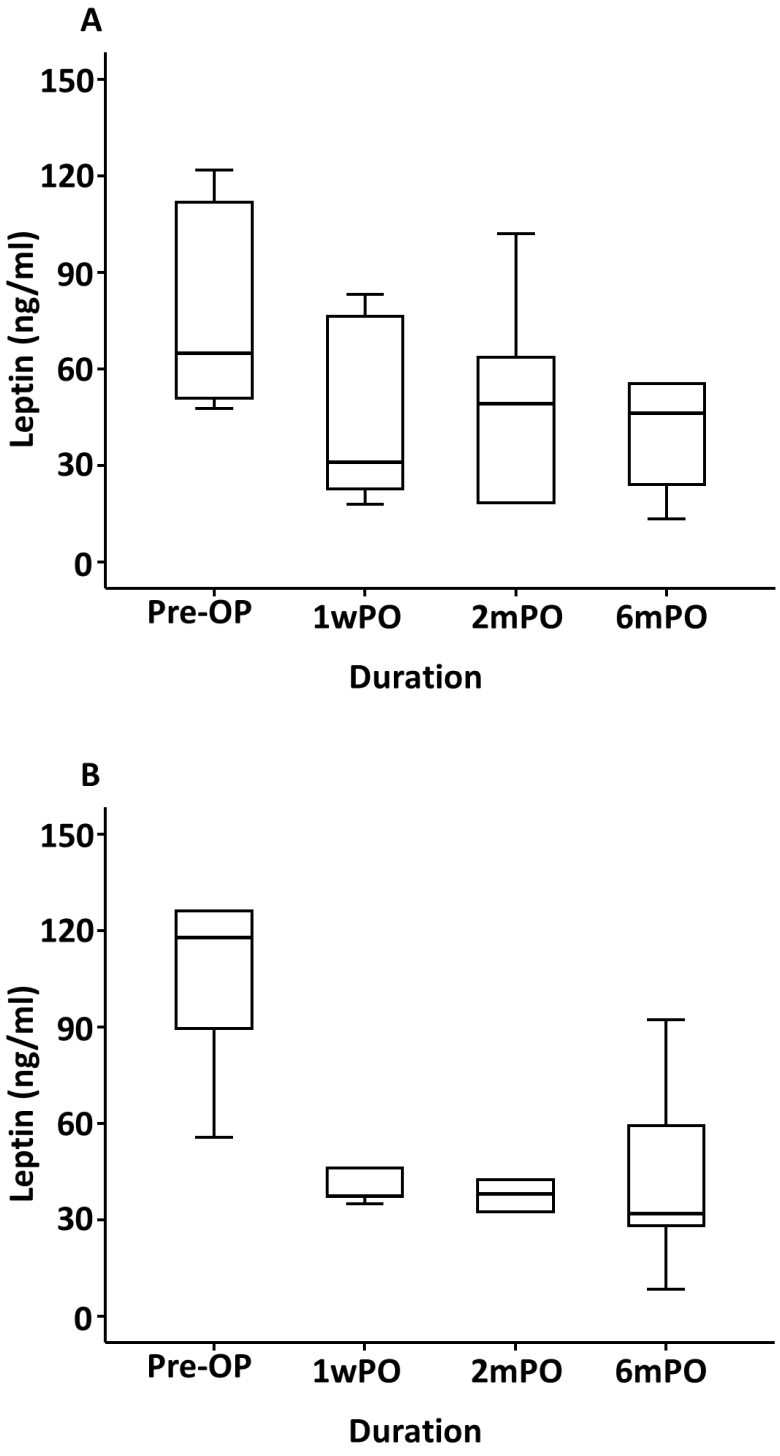

Figure 4.19 Leptin levels before and after (1 week, 2 months and 6 months) POSE (A) and RYGB (B) surgery

Leptin concentration decreased significantly 1 week after POSE ($p = 0.02$) and RYGB ($p = 0.005$) procedures, and a further reduction occurred at 2 ($p = 0.018$) and 6 ($p = 0.018$) months after the RYGB procedure. Although a significant decrease in leptin level appeared at 1 week and 2 months ($p < 0.05$) compared to the preoperative level, this significance had disappeared at 6 months ($p= 0.07$) in POSE patients. Data are expressed as median and interquartile range. Pre-op: preoperatively, 1wPO: 1 week postoperatively, 2mPO; 2 months postoperatively, 6mPO: 6 months postoperatively.

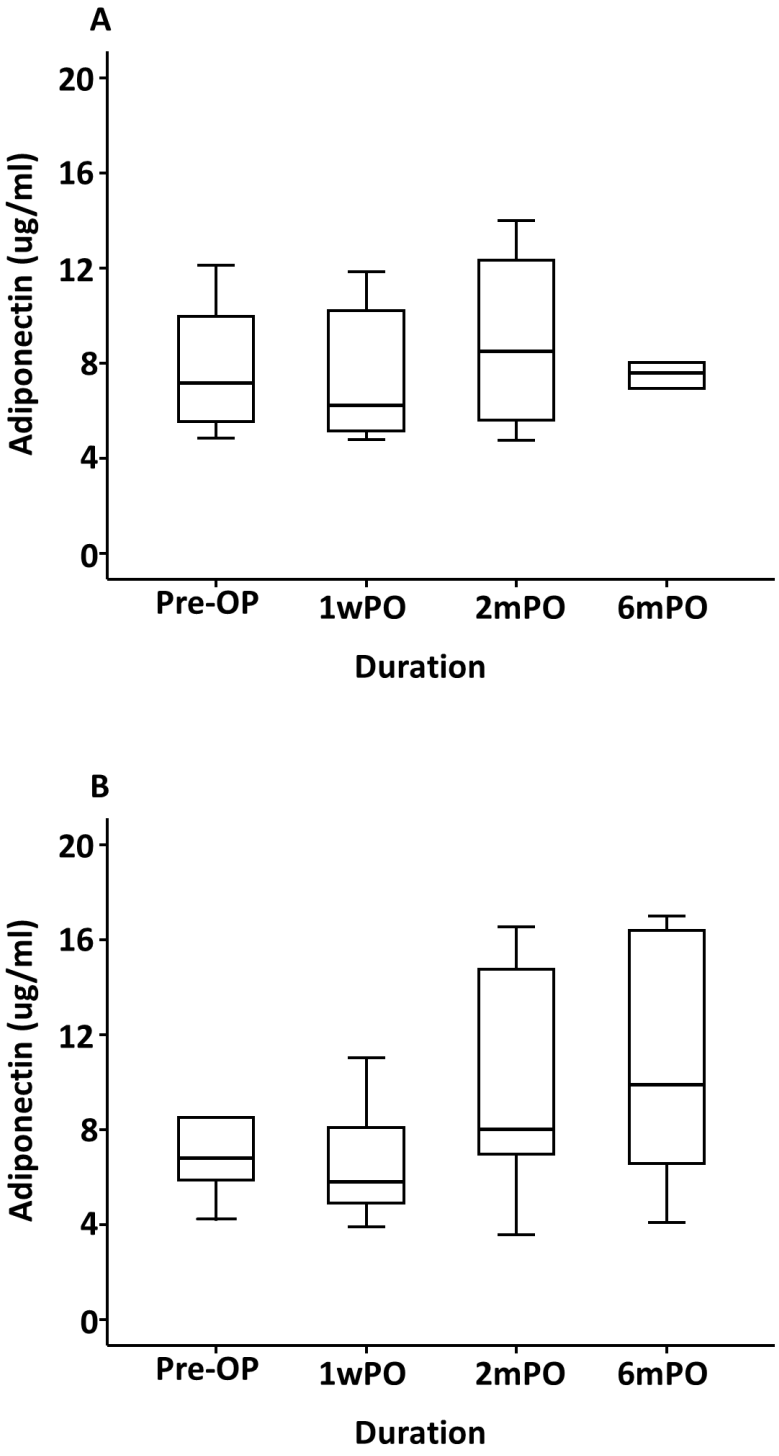

Figure 4.20 Adiponectin levels before and after (1 week, 2 months and 6 months) POSE (A) and RYGB (B) surgery

Adiponectin levels for POSE (A) and RYGB (B) patients. The postoperative level at 1 week, 2 months and 6 months did not differ from the preoperative level in POSE and RYGB patients ($p > 0.05$). Data are expressed as median and interquartile

range. Pre-op: preoperatively, 1wPO: 1 week postoperatively, 2mPO: 2 months postoperatively, 6mPO: 6 months postoperatively.

4.4 Discussion

POSE is a new less invasive and incisionless surgical weight-loss technique. This study investigated the difference between the outcome of POSE and RYGB surgeries with regard to weight loss and changes in GLP-1 and metabolic markers. Significant weight loss was recorded even at 1 week, and increased at 2 and 6 months after RYGB. This result is consistent with other studies that showed steady weight loss after RYGB which was maintained beyond 6 months up to 10 years [319]. Conversely, the weight loss after POSE was less than that seen after RYGB and had reached a plateau at 2 months postoperatively. This result conflicts with those reported by Espinos et al., who showed effective weight loss at 6 months following the POSE procedure [314]. Also, EWL was greater in the study of Espinos et al. This difference in weight-loss result can be explained by the difference in BMI; in this study, the pre-surgical BMI was greater (mean 41.3 kg/m^2) than that of Espinos et al. (mean 36.7 kg/m^2). In addition, the sample size was greater in the study of Espinos et al. (n = 45) than in this study (n = 6), with two diabetic patients included in this study. It seems that POSE may be more effective in patients with a lower BMI and without complications.

No significant improvement was seen in fasting and postprandial glucose levels after POSE at any of the time-points in either the non-diabetic or diabetic patients. However, at 6 months, the fasting glucose level was reduced but not significantly; this reduction could be secondary to weight loss. At 6 months, one diabetic patient reported that her anti-diabetic medication dose had been reduced 2 months after having the POSE procedure. Also, neither fasting nor postprandial insulin responses were affected by POSE. Furthermore, no improvement in fasting or postprandial active GLP-1 levels was recorded, perhaps explaining the lack of changes in insulin response postoperatively.

The only other data on patients being studied after POSE was reported in abstract form [320]. They showed significant improvement in glucose/insulin ratio at 2 and 6 months after the POSE procedure. The study also showed that in the postprandial state, orexigenic ghrelin was reduced significantly, and anorexigenic

PYY increased at 2 and 6 months post-POSE surgery. The authors concluded that significant weight loss was achieved by an increase in the satiety response at 2 and 6 months after POSE [321].

We show that, while there was no change following RYGB in fasting and postprandial glucose level, fasting insulin concentrations were lower, and insulin sensitivity improved. Furthermore, in these patients, postprandial GLP-1 levels increased significantly from the first week, and stayed elevated at 2 and 6 months, perhaps explaining the improvement in insulin secretion and sensitivity. The two diabetic patients who underwent RYGB reduced their anti-diabetic medications at 1 week and 2 months, and had stopped taking any medication by 6 months after surgery. This is in accordance with previous studies showing early improvement in glycaemia and insulin sensitivity, and remission of diabetes after RYGB surgery, even before achieving significant weight loss, perhaps related to an acute increase in postprandial GLP-1 secretion [322, 323]. Thus, unlike the results observed for RYGB, the early improvement in insulin and glycaemia independent of weight loss that could be partially due to acute alteration in gut hormones such as GLP-1 was not seen after the POSE procedure, though late improvement in fasting glucose was observed (6 months) secondary to weight reduction.

Leptin decreased significantly 1 week after POSE, but this was attributed to weight loss brought about by the low calorie intake of the patients who were on a liquid diet for 2 weeks until discomfort from the surgical procedure disappeared. However, leptin stayed lower than pre-surgery levels at 2 and 6 months, suggesting some loss of fat mass. Adiponectin levels remained unchanged following POSE, reflecting the lack of effect on insulin sensitivity.

After RYGB, the leptin level was also significantly decreased after 1 week, and the suppression was greater at 2 and 6 months. Adiponectin levels increased gradually after RYGB but did not reach significance. This is in accordance with a previous study [324, 325]. No changes in lipid parameters were apparent after either type of surgery.

In conclusion, our study revealed that although the POSE procedure is a less invasive and risky technique than RYGB, it is not as effective as RYGB with regard

to weight loss, improved glycaemic homeostasis and insulin secretion, and an increase in postprandial GLP-1 hormone. Further studies are required in a larger sample cohort and for longer periods post-surgery to carefully compare the advantages of this new procedure to RYGB in its ability to facilitate weight loss along with beneficial effects on metabolism. The POSE procedure may be more effective for healthy obese patients with a BMI between 30 and 35 with no comorbidities.

Chapter 5

Post-absorptive and postprandial GLP-1 in an Arab female population

5.1 Introduction

The prevalence of obesity has increased significantly throughout the world during the last three decades. The countries of the Middle East in particular have the highest incidence of obesity and type 2 diabetes globally [326-328].

In Saudi Arabia, only 19.9% of Saudis attending primary healthcare clinics have a normal body weight, while 49.9% are obese. This rate reflects a rise from that reported in the late 1990s (35.6%) [329]. In Kuwait, adolescent obesity in 2002 was 19.9%, already exceeding that of the United States (15.3%) [330, 331]. Kuwait also has the highest rate of type 2 diabetes among the Arab countries [332]. The specific reasons for this phenomenon have not as yet been completely elucidated.

An imbalance between energy intake and expenditure is an important influence contributing to obesity, so any factor that affects this balance could result in excess weight [333]. Lack of exercise and a sedentary lifestyle may thus contribute to the high rates of obesity. Rising income levels since the discovery of oil have led to a sedentary lifestyle, with an increase in the usage of motor vehicles, and diets high in fat and carbohydrates [334].

Whether obesity in this region is predominantly due to lower quality of food and sedentary lifestyle, or whether there may be other physiological mediators to this rise is of intense current research. A possible candidate that may explain to some extent the increased susceptibility to obesity and its associated metabolic diseases is the hormones of the gut–brain axis, such as GLP-1. Whether GLP-1 is secreted to a lesser extent either post-absorptively or postprandially and therefore affects satiety and insulin sensitivity in these individuals needs to be investigated. The current hypothesis is that impaired secretion of postprandial GLP-1 contributes to obesity of people living in the Middle East.

The aim of this study was to investigate fasting and postprandial GLP-1 secretion in normal-weight, overweight and obese individuals living in countries of the Middle East.

5.2 Methods

Forty female subjects of mixed ethnicity without documented metabolic diseases were recruited for this study. Twenty-seven Arab subjects living in Qatar were studied at the Clinical Research Centre (CRC) of the ADLQ. Thirteen subjects of mixed ethnicity (Caucasians, South Asians, Arabs and Africans) living in London were concomitantly studied at the CRC at UCL. All subjects provided written informed consent, and the studies were approved by the relevant national ethics committees.

Subjects attended in the morning after an overnight fast of at least 8–10 hours. Blood pressure, pulse, height (m) and weight (kg) were measured; body fat was measured by bioimpedance, and expressed as a % of body weight. BMI was calculated (kg/m^2).

Blood was collected during fasting and 30 and 120 minutes after consuming a standard 388.6-kcal liquid mixed meal (17.4 g of fat, 40 g of carbohydrate and 18 g of protein). The fasting samples were used to measure GLP-1, insulin, proinsulin, total cholesterol, LDL, HDL, triglycerides, glucose, adiponectin and leptin, whereas the postprandial samples were used to measure GLP-1, insulin, proinsulin and glucose. A specific DPP-IV inhibitor was added to the blood tubes that were used to determine GLP-1.

Active GLP-1 (Millipore, Billerica, MA, USA), insulin, glucose, lipids and adipokine levels were determined as mentioned previously in sections **2.2.1**, **2.2.2** and **2.2.4**. Proinsulin was also measured by ELISA (Mercodia, Sweden).

All assays had inter- and intra-assay coefficients of variation (CV) less than 10%. Normality of results was tested; parametric tests were used for normally distributed data, and non-parametric analysis used for skewed data (see section **2.5**).

5.3 Results

5.3.1 Subjects' characteristics

The subject characteristics of the population are shown in Table 5.1. Subjects in both groups were euglycaemic and normoinsulinaemic in the basal state. However, subjects living in Doha were significantly hyperinsulinaemic in the postprandial state at 30 and 120 minutes, compared to the London cohort ($p < 0.001$), despite being significantly younger; this difference in postprandial insulin levels between the two groups is illustrated in Figure 5.1.

The lipid profile of the London cohort showed greater levels of total cholesterol as well as of HDL, but no differences in triglycerides and LDL.

Of note were the especially high levels of systemic leptin in the Qatari cohort ($p < 0.001$), suggesting a high body fat mass, although both groups had comparable BMI ($p = 0.61$).

The cohort living in Doha were then investigated further by grouping them into those of normal-weight, overweight and those who were obese by WHO criteria.

Table 5.2 shows the characteristics of Arab subjects living in Qatar. Subjects were divided into three categories: 12 subjects of normal weight (mean (± SD), BMI = 21.4 ± 2 kg/m^2), eight overweight subjects (BMI = 27 ± 1.6 kg/m^2) and seven obese subjects (BMI = 32.5 ± 1.6 kg/m^2). Subjects were matched for age in the normal-weight group (mean (± SD), 29.5 ± 5.5 years), overweight group (27.1 ± 4.4 years) and obese group (30 ± 5.3 year). Obese subjects had the highest body fat percentage (mean (± SD), 42.1 ± 3.2%) compared to overweight (36.2 ± 2.7%) and normal-weight (29.4 ± 4.7%) subjects. All subjects were normotensive, euglycaemic and normolipidaemic. The obese subjects were hyperinsulinaemic (insulin and HOMA-IR median and interquartile range were 15.5 (5.8–19.8) and 3.1 (1.2–4.22) mIU/L, respectively), whereas normal-weight and overweight subjects were normoinsulinaemic (insulin median and interquartile range: 4.8 (3.5–5.2) and 5.2 (4.2–6.1) mIU/L, respectively; HOMA-IR median and interquartile range: 0.97 (0.67–1.16) and 1.12 (0.85–1.38), respectively).

Variables	Living in London (n = 13)	Living in Qatar (n = 27)	p value
Age (years)	28.9 ± 5.1	38.2 ± 13.5	0.02
Weight (kg)	66.4 ± 14.7	64.3 ± 14.3	0.67
BMI (kg/m^2)	25.9 ± 4.9	24.0 ± 5.8	0.61
Glucose (mmol/L)	4.6 ± 0.3	5.0 ± 0.7	0.05
Insulin (mIU/L)	5.1 (4–7)	5.7 (3.6–7.1)	0.9
PP 30 min insulin (mIU/L)	50.5 (35–63)	20.2 (13.2–28.7)	< 0.001
PP 120 min insulin (mIU/L)	31.2 (23.6–53.1)	3.8 (2.7–7.0)	< 0.001
HOMA-IR	1.12 (0.8–1.4)	1.2 (0.7–1.5)	0.82
Total cholesterol (mmol/L)	3.6 ± 0.7	4.7 ± 1	0.001
Triglyceride (mmol/L)	0.60 (0.4–0.8)	0.6 (0.5–1)	0.42
HDL cholesterol (mmol/L)	1.22 ± 0.29	1.79 ± 0.73	0.01
LDL cholesterol (mmol/L)	2.1 ± 0.5	2.6 ± 0.9	0.1
Leptin (ng/L)	30.8 (17.5–53)	5.9 (3.8–11.5)	< 0.001

Table 5.1 Subjects' characteristics

Data are expressed as mean ± standard deviation or median (interquartile range). PP: postprandial, BMI: body mass index, HOMA-IR: homeostatic model assessment – insulin resistance, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

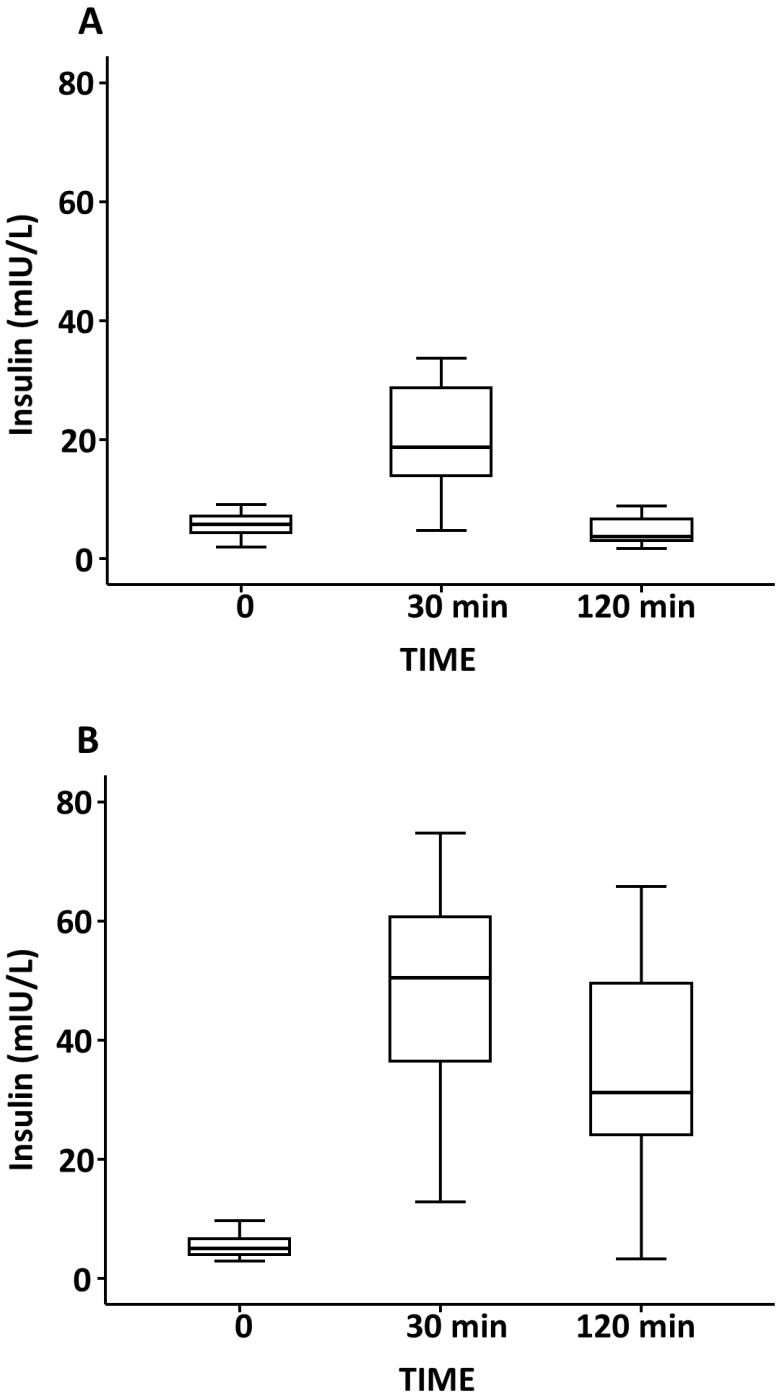

Figure 5.1 Fasting and postprandial (30 and 120 minutes) insulin level in subjects living in London (A) and subjects living in Qatar (B)

Fasting insulin levels were within the normal range and were comparable between the two groups ($p = 0.9$). However, postprandial insulin levels were significantly higher in the group living in Qatar, especially at 120 minutes ($p < 0.001$). Data are expressed as median and interquartile range. min: minutes.

Variables	Normal weight (n = 12)	Overweight (n = 8)	Obese (n = 7)
Age (years)	29.5 ± 5.5	27.1 ± 4.4	30 ± 5.3
Weight (kg)	54.1 ± 5.7	68.4 ± 5.6	85.2 ± 11.3
BMI (kg/m^2)	21.4 ± 2.0	27 ± 1.6	32.5 ± 1.6
Body fat %	29.4 ± 4.7	36.2 ± 2.7	*42.1 ± 3.2
SBP (mm Hg)	108.4 ± 11.5	108 ± 7.8	116 ± 6.1
DBP (mm Hg)	70 ± 5.8	70.2 ± 7.3	74.1 ± 7.8
Glucose (mmol/L)	4.53 ± 0.4	4.74 ± 0.29	4.53 ± 0.14
Insulin (mIU/L)	4.8 (3.5–5.2)	5.2 (4.2–6.1)	*15.5 (5.8–19.8)
HOMA-IR	0.97 (0.67–1.16)	1.12 (0.85–1.38)	*3.1 (1.2–4.22)
Total chol (mmol/L)	3.6 ± 0.63	3.3 ± 0.82	3.9 ± 0.75
Triglyceride (mmol/L	0.52 (0.46–0.66)	0.53 (0.36–0.8)	0.83 (0.67–1.07)
HDL chol (mmol/L)	1.28 ± 0.29	1.31 ± 0.32	1.03 ± 0.22
LDL chol (mmol/L)	2.1 ± 0.4	1.82 ± 0.52	2.47 ± 0.56
Leptin (ng/ml)	21.3 (13.4–33.0)	31.8 (28.3–43.8)	*61.4 (29.3–91.5)
Adiponectin (□g/ml)	7.9 (7.6–10.3)	7.8 (5.4–16.9)	8.6 (4.2–11.3)

Table 5.2 Characteristics of the cohort living in Qatar

Data are expressed as mean ± standard deviation or as median (interquartile range). BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment – insulin resistance, HDL: high-density lipoprotein, LDL: low-density lipoprotein, chol: cholesterol.
*denotes $p < 0.05$ comparing the obese group to all other groups.

5.3.2 Glucose, insulin, proinsulin and GLP-1 parameters in a cohort living in Qatar

The fasting and postprandial glucose levels in the three groups were comparable and were within normal range (Figure 5.2 A, B and C).

Fasting insulin was significantly higher in obese subjects (median (interquartile range) was 15.5 (5.8–19.8) mIU/L) compared to normal-weight and overweight subjects ($p < 0.05$). The postprandial insulin response to the meal at 30 minutes increased significantly ($p < 0.05$) compared to baseline, and this elevation remained even at 120 minutes in all three groups. There were no significant differences in postprandial response at 30 and 120 minutes between the three groups (Figure 5.3 A, B and C).

The fasting proinsulin levels in the three groups were comparable (normal-weight, overweight and obese groups' median (interquartile range) were 8.15 (5.75–12.37), 8.4 (5.97–12.22) and 10.7 (6.1–14.5) pmol/L, respectively). Postprandial proinsulin levels had increased significantly ($p < 0.05$) at 30 minutes in all groups, and remained elevated even at 120 minutes (Figure 5.4 A, B and C).

Fasting GLP-1 levels were comparable in all three groups; the median (interquartile range) of the normal-weight group was 2.0 (1.9–2.0) pmol/L; for the overweight group it was 1.8 (1.7–2.2) pmol/L, and for the obese group was 2.1 (1.9–2.4) pmol/L. Postprandial GLP-1 levels had increased significantly ($p < 0.05$) at 30 and 120 minutes compared to baseline in all three groups. However, there were no significant differences in postprandial GLP-1 response to the meal between either normal-weight and obese groups or normal-weight and overweight groups ($p > 0.05$) (Figure 5.5 A, B and C).

Variables	Normal weight	Overweight	Obese
Fasting glucose (mmol/L)	4.5 (± 0.4)	4.7 (± 0.3)	4.5 (± 0.1)
30 m PP glucose (mmol/L)	4.7 (± 0.48)	5.1 (± 0.7)	5.2 (± 0.8)
120 m PP glucose (mmol/L)	4.1 (± 0.37)	4.6 (± 0.5)	4.5 (± 0.7)
Fasting insulin (mIU/L)	4.8 (3.5–5.3)	5.2 (4.2–6.2)	15.5 (5.8–19.9)
30 m PP insulin (mIU/L)	49.1 (30.5–54.2)	50.0 (32.4–57.0)	74.7 (38.6–111.6)
120 PP insulin (mIU/L)	28.0 (21.2–35.8)	33.4 (25.6–50.3)	53.5 (23.6–65.8)
Fasting proinsulin (PM)	8.2 (5.7–12.4)	8.4 (6.0–12.2)	10.3 (6.1–14.5)
30 m PP proinsulin (PM)	36.5 (14.8–43.4)	23.7 (19.5–29.3)	26.8 (13.1–51.9)
120 m PP proinsulin (PM)	33.7 (27.4–59.3)	43.6 (21.4–66.1)	41.1 (22.1–66.3)
Fasting GLP-1 (PM)	2.0 (1.9–2.9)	1.8 (1.7–2.2)	2.1 (1.9–2.4)
30 m PP GLP-1 (PM)	8.9 (3.7–11.2)	7.2 (4.7–8.6)	10.7 (6.0–30.1)
120 m PP GLP-1 (PM)	7.5 (6.6–10.1)	4.9 (2.7–8.3)	7.0 (2.9–12.1)

Table 5.3 Fasting and postprandial (30 and 120 minutes) levels of glucose, insulin, proinsulin and GLP-1 in the normal-weight, overweight and obese groups from Qatar

Data were expressed as mean ± standard deviation or as median (interquartile range). m = minute, PP = postprandial.

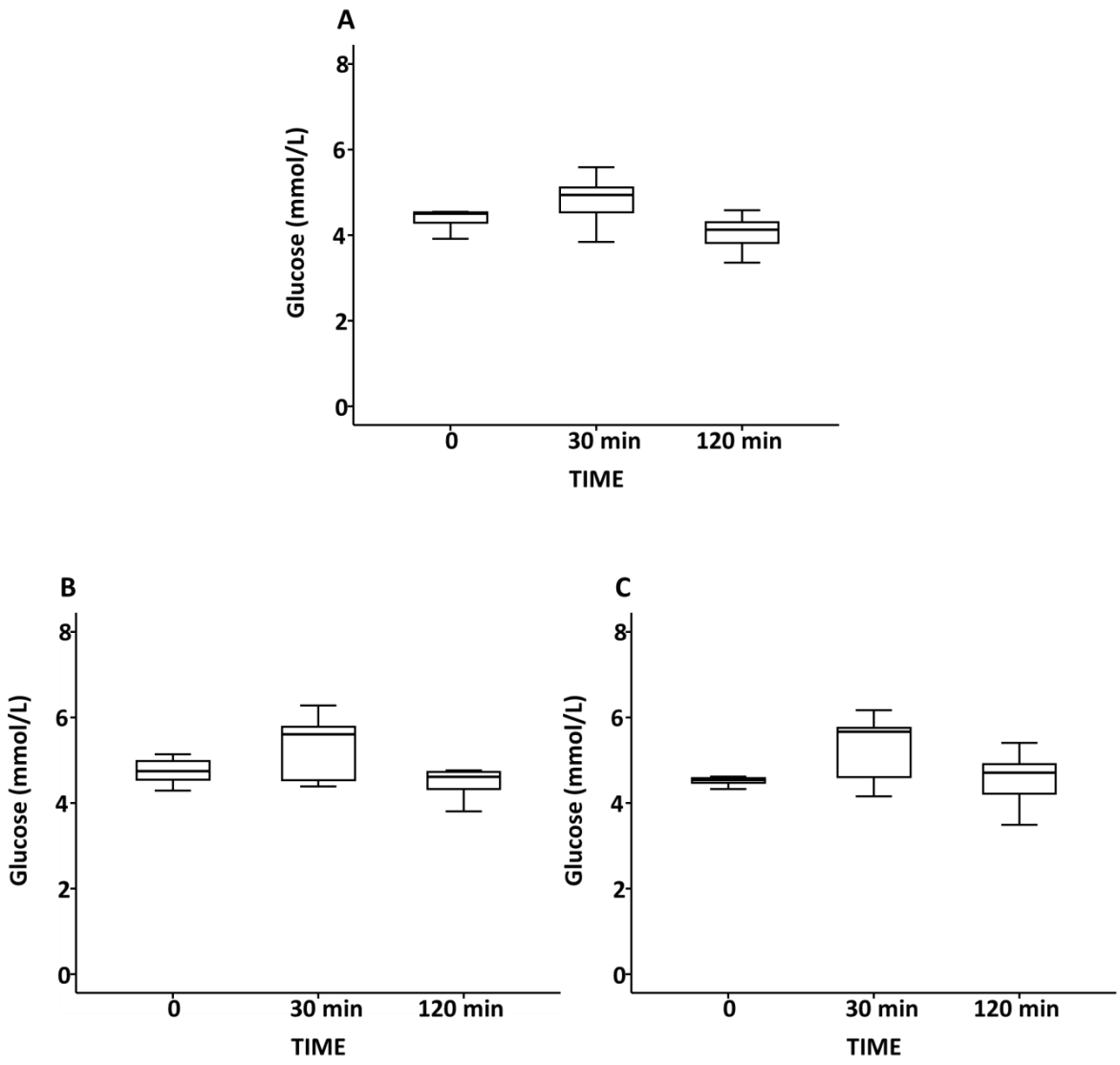

Figure 5.2 Fasting and postprandial glucose levels in normal-weight (A), overweight (B) and obese (C) groups

Normal fasting and postprandial glucose levels were compared in the three groups, and no significant differences were apparent in the basal and postprandial glucose levels among the three groups (A, B and C). Data are expressed as median and interquartile range. Min = minute.

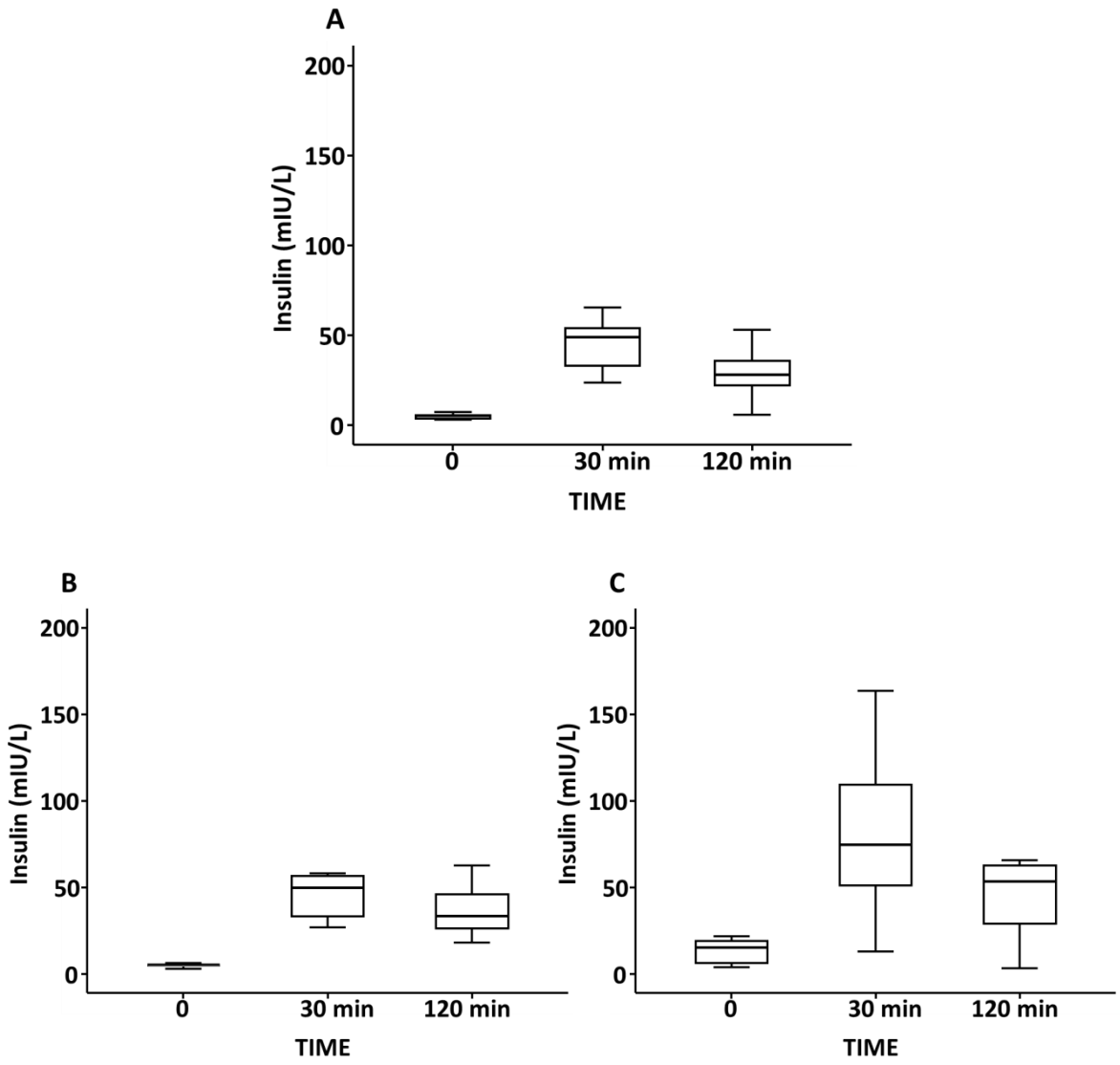

Figure 5.3 Fasting and postprandial insulin levels in normal-weight (A), overweight (B) and obese (C) groups

The obese group had significantly elevated basal insulin levels compared to normal and overweight groups ($p = 0.005$) (A, B and C). The postprandial insulin response increased significantly at 30 minutes ($p < 0.05$) in the three groups, and this increase remained significant at 120 minutes in all groups (A, B and C). Data are expressed as median and interquartile range. Min = minute.

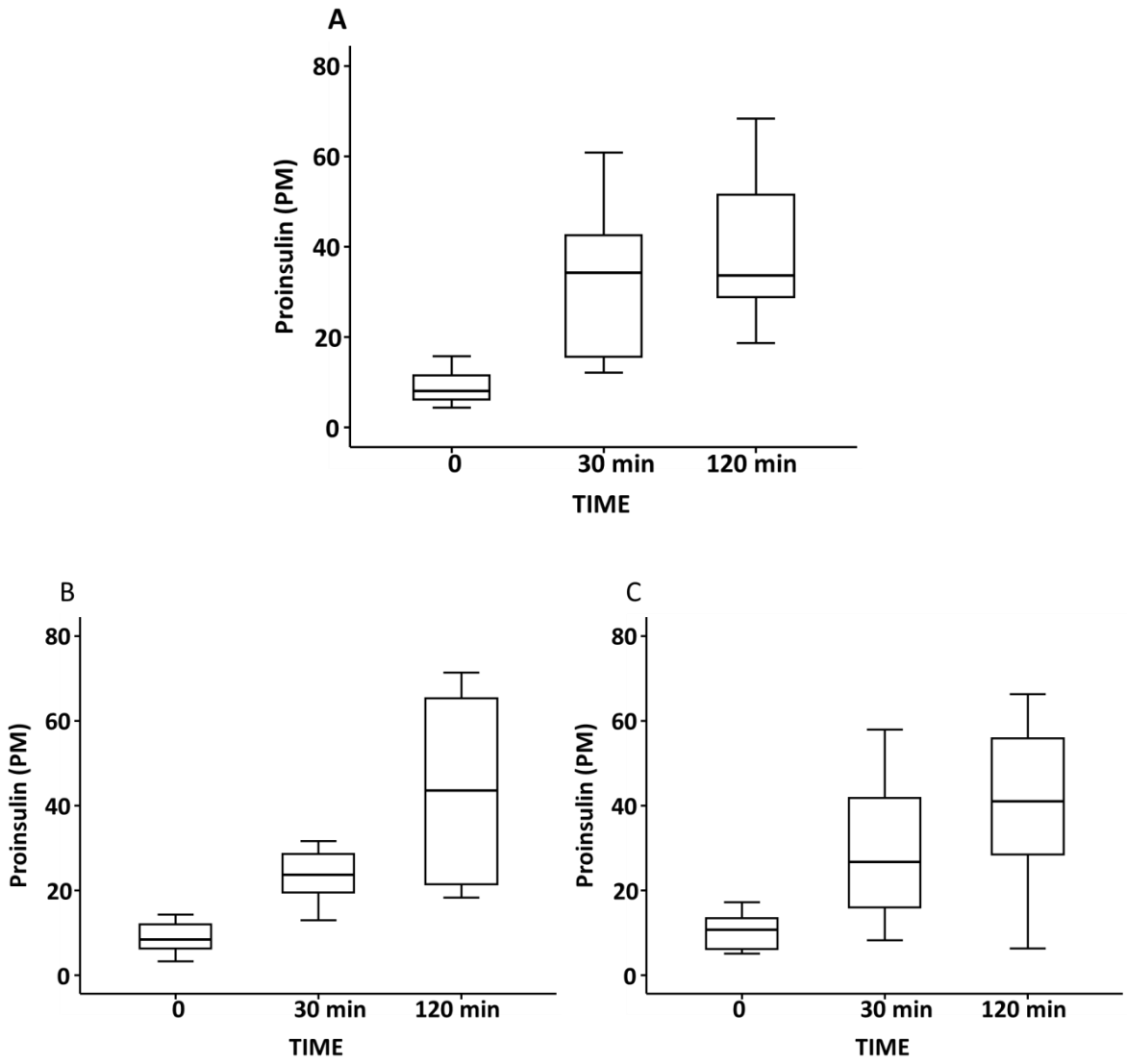

Figure 5.4 Fasting and postprandial proinsulin levels in normal-weight (A), overweight (B) and obese (C) groups

Fasting and postprandial proinsulin was comparable between the three groups ($p > 0.05$). Postprandial proinsulin increased significantly at 30 minutes and remained elevated at 120 minutes. Data are expressed as median and interquartile range. Min = minute.

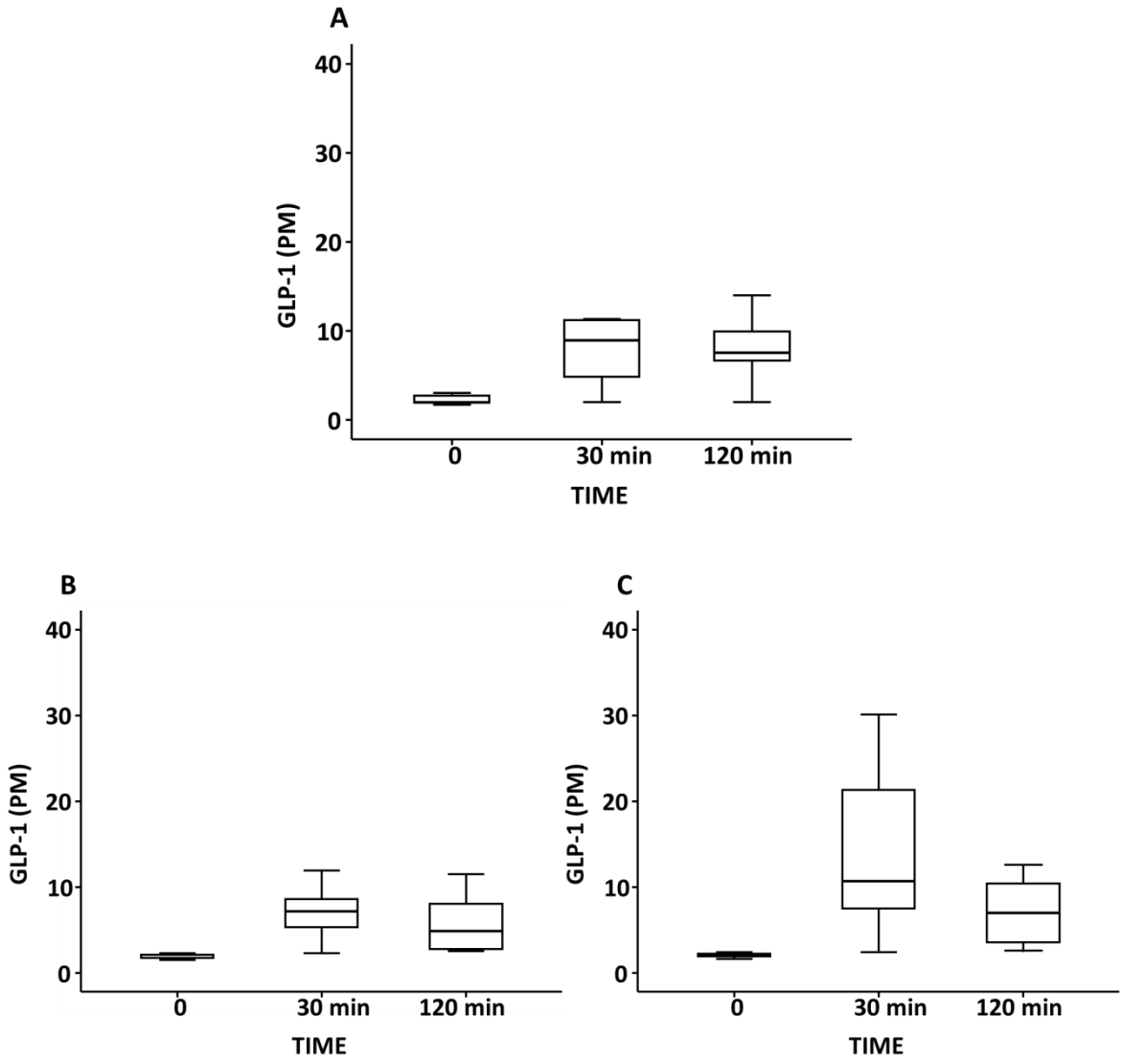

Figure 5.5 Fasting and postprandial GLP-1 levels in normal-weight (A), overweight (B) and obese (C) groups

There were no significant differences in basal and postprandial GLP-1 levels between the three groups ($p > 0.05$). Data are expressed as median and interquartile range. Min = minute.

5.3.3 Adipokine level in the cohort living in Qatar

Figure 5.6 A shows the leptin levels in the three different weight groups of the cohort living in Qatar. Leptin concentrations were significantly higher in the obese group compared to normal-weight and overweight groups ($p = 0.01$). However, adiponectin levels were comparable for the three groups (Figure 5.6 B).

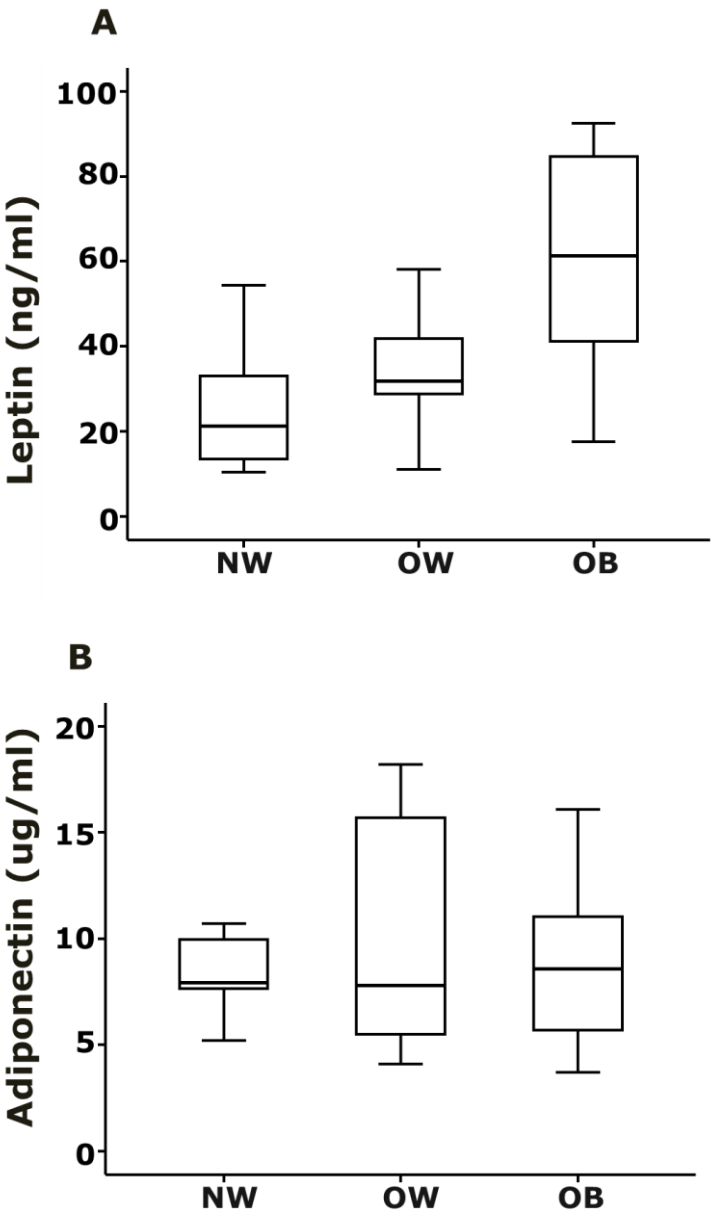

Figure 5.6 Adipokine levels in normal-weight, overweight and obese groups

Leptin level (A) was significantly greater in obese groups compared to normal-weight and overweight groups ($p = 0.01$). However, there were no significant differences in adiponectin level between the three groups (B). Data are expressed as median and interquartile range. NW: normal weight, OW: overweight, OB: obese.

5.4 Discussion

The prevalence of obesity in the Middle Eastern region was the highest worldwide in 2013 [335]. In Qatar, the obesity rate among females was 32% in 2010 [336]. Whether there is any lesion in the gut–brain axis that would affect the satiety mechanism and lead to hyperphagia in that particular group of individuals had not been investigated previously in this region. In this study, GLP-1 hormone, a mediator that is involved in the satiety process, was investigated in normal-weight, overweight and obese female subjects living in Qatar.

However, no differences in either basal or postprandial GLP-1 secretion were found between the three groups. Therefore, impairment in postprandial GLP-1 secretion was not found in these obese subjects, and the phenomenon of weight gain seems not to be related to a disorder in triggering satiety by postprandial GLP-1 secretion in response to caloric intake. This of course does not rule out the involvement of other gut peptides such as GIP.

In contrast with this result, it was reported that attenuated postprandial GLP-1 secretion in response to oral carbohydrate but not fat intake is found in obese individuals compared to lean individuals living in England [140]. The conflict in the two results might be explained by the differences in BMI of obese subjects; BMI was lower in the current study (32.5 ± 1.6 kg/m^2) compared to that of Ranganath et al. (40.1 ± 8 kg/m^2). Moreover, in the current study, a mixed meal consisting of 40 g of carbohydrate, 17.4 g of fat and 18 g of protein was used as a meal challenge test, whereas Ranganath's group used a carbohydrate- (100 g) and fat-rich (37.8 g) meal for the challenge.

As no physiological defect in satiety index or in the mediator postulated, GLP-1, could be found in the current study, the susceptibility to obesity and diabetes in the Middle East region could be explained by a sedentary lifestyle, lack of activity and high caloric intake. Supporting this suggestion is the difference that was found in the 2-hour insulin response between the two groups of subjects living in Qatar and those living in London. The two groups had a comparable fasting insulin level. However, the postprandial insulin concentrations were significantly

elevated 30 minutes after the ingestion of a meal, and importantly these levels remained elevated even 120 minutes after meal in the group of people living in Qatar compared to the group living in London, despite their younger age and comparable BMI. Moreover, leptin levels were also higher in the group of people living in Qatar compared with those living in England, despite having comparable BMI; this result supports greater fat mass in the Qatar dwellers, perhaps as a result of the sedentary lifestyle in Qatar, which might explain the high prevalence of obesity and diabetes in this region.

Postprandial hyperinsulinaemia is accompanied by elevated systemic proinsulin, which may further facilitate prolonged hyperinsulinaemia and might be an indicator of ER stress. A study conducted in San Francisco on normal-weight, healthy South Asian and Caucasian subjects to investigate insulin response after a 75 g OGTT found that while fasting insulin was similar in both groups, the postprandial insulin response was greater in South Asians. The 2-hour insulin level was especially greater in Asian women compared to the Caucasians, and it was concluded that South Asians have a defect in insulin-stimulated glucose uptake [337]. A further study was carried out to find out whether the increase in insulin response is accompanied by elevated GLP-1 secretion. A comparison between eight healthy young South Asian and ten Caucasian men with reference to fasting and postprandial glucose, insulin and GLP-1 levels in response to OGTT showed comparable fasting glucose, insulin and GLP and postprandial glucose levels between the two groups. However, the postprandial insulin and GLP-1 responses as well as the AUC for insulin and GLP-1 were greater in South Asians. It was concluded that increased postprandial GLP-1 might be a possible explanation for the higher insulin levels in South Asians [338]. Although the South Asians had similar results as regards the high insulin response to the group of subjects living in Doha in the current study, postprandial GLP-1 levels in this group did not seem to be related to elevated 2-hour postprandial insulin levels.

Two-hour postprandial hyperinsulinemia was also reported in a normal glucose tolerance Nauruan population, and it was concluded that this elevation in postprandial insulin level strongly predicts development of impaired glucose

tolerance and diabetes [339]. Thus, elevated postprandial levels of insulin-like molecules may be an early, sensitive marker for the metabolic defect that precedes diabetes disease in the Middle Eastern population.

Like Qatar, Nauru has also had rapid increases in the rate of obesity and diabetes that accompanied a dramatic and rapid change in the economic status of the island. Due to the discovery of large deposits of phosphate, the income of Nauruans increased, and it became, for a short period of time, one of the countries with the highest per capita incomes in the world. This led to a transition from a traditional to a western diet along with a sedentary lifestyle and high caloric intake, which were correlated with the high incidence of obesity and diabetes in this region [340]. In a similar way, inactivity and sedentary lifestyle could be a contributing factor to high rates of obesity and diabetes in the Middle East. Therefore, early detection of abnormal postprandial insulin response and prevention of progress to metabolic disorder with lifestyle interventions such as LCD, and more importantly exercise and active lifestyle, may yield encouraging health benefits.

Chapter 6

Discussion

This thesis examined the role that GLP-1 might play in three separate scenarios: firstly, in the chronic N&V symptoms experienced by some patients following bariatric surgery; secondly, in patients undergoing a new surgical weight-loss technique; and thirdly, in an obesity-prone population. All three of the studies were novel; obesity is likely to be at least partly mediated by changes in GLP-1 secretion and function and therefore examination of GLP-1 is warranted.

Chronic persistent N&V after RYGB surgery has been found to be associated with an elevation in basal GLP-1 level, and it was concluded that elevated basal GLP-1 might partly contribute to chronic undesirable N&V symptoms experienced by some patients after RYGB surgery. Increased GLP-1 concentrations may therefore also explain similar symptoms seen after other bariatric procedures that result in alteration of gut hormone secretion, such as SG [310] but not GB, as increased GLP-1 secretion is not reported after this surgical technique [247].

In this study, the kit that was used to measure GLP-1 did not detect all forms of active GLP-1. It detected only GLP-1 (7-36); therefore, it would be useful if a similar study could be conducted in the future measuring all active forms of GLP-1 (7-36) and (7-37), as both forms have been found to affect satiety [341, 342]. These patients with elevated GLP-1 might benefit from being treated with a specific GLP-1 antagonist such as exendin (9-39). In the current study, intervention with a generalized GLP-1 antagonist (somatostatin) was tried, but only in one patient; it led to amelioration of N&V symptoms along with a reduction in GLP-1. A larger cohort to confirm both the chronic elevation in fasting GLP-1 and amelioration of N&V symptoms along with a reduction in systemic GLP-1 after treatment with a specific GLP-1 antagonist is required before this can be recommended as an effective management for those patients. However, these preliminary results offer an encouraging, possibly effective, avenue for future exploration in a group of patients who are considered particularly difficult to manage clinically.

This is also the first study to show a direct effect of GLP-1 on adipose leptin secretion. Inhibition of leptin secretion *in vitro* was found after 16 hours but not 4 hours of exposure to GLP-1. After the RYGB procedure, chronic exposure to GLP-

1 might contribute to a reduction in leptin levels. Therefore, the finding that patients with persistent N&V had a lower leptin level compared to patients without N&V after the RYGB procedure could be due to a chronic elevation in fasting GLP-1 levels in this group. How this might alter leptin sensitivity, both centrally and peripherally, requires further investigation. It has been reported in rats that chronic administration of liraglutide significantly reduces fasting circulating leptin levels and body fat percentage more than that seen with caloric restriction [343]. Thus, it will be beneficial to find out in the future whether chronic administration of GLP-1 agonist to obese individuals with hyperleptinaemia could reduce leptin levels and enhance leptin sensitivity, and whether GLP-1 agonists such as exendin-4 and liraglutide could be used as therapeutic medications for weight loss besides their use in type 2 diabetes.

POSE is a novel, minimally invasive and incisionless endoscopic technique for weight loss. Our study revealed that POSE provides a reasonable short-term weight-loss outcome in the morbidly obese; however, it is not as effective as RYGB in the longer term. Moreover, as compared to RYGB, its impact on improved glycaemic homeostasis and insulin secretion, and increased postprandial GLP-1 hormone was all minimal. It might be useful to consider the POSE procedure in obese individuals with a lower BMI (30–35). As it was found that weight loss was reported in the first 2 months following POSE, it might also be useful to consider this technique as a more effective alternative method to LCD for weight reduction preceding bariatric surgery. However, further studies are required in larger sample cohorts and for longer periods post-surgery to carefully compare this new procedure to RYGB, as well as other procedures such as gastric band and sleeve.

Lastly, GLP-1 secretion was examined in a population susceptible to obesity and type 2 diabetes. No attenuation in the secretion of GLP-1 was found in obese individuals that could explain a decrease in satiety and increased caloric intake. An interesting finding was the increased 2-hour insulin response in the young normal-weight women living in Doha with no apparent metabolic disease, compared to those living in London, although the fasting insulin and glucose levels were normal in both groups. That finding could be explained by little physical

activity and a sedentary lifestyle. This finding might also be an early indicator for future development of diabetes, so it would be useful to follow up those individuals identified with postprandial hyperinsulinaemia for a long period of time to monitor development of diabetes. In addition, based on this result, the research group at ADLQ has already started another project that aims to find out the effect of exercise on late postprandial hyperinsulinaemia among individuals living in Doha, and whether a change in lifestyle could ameliorate the postprandial insulin resistance and could be considered as a preventative intervention for development of diabetes.

Finally, all the studies were carried out in female individuals; whether the results would be any different among males also needs to be explored.

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Appendices

	Medication
Group 1 N&V	Citalopram, vitamin B_{12} injection, iron tablet, fluoxeline, lansoprazole, clozapine
Group 2 No N&V	Vitamin B_{12} injection, ramipril, amlopidine, iron tablet, citalopram, vitamin D and calcium
Group 3 MO	Acetazolamide, fluoxeline, lansoprazole, Adalat
Group 4 OW	Atenolol, amlopidine
Group 5 NW	No medication

Appendix 1: Medication of patients in the N&V study.

The five groups of patients (1–5; Chapter 3) differed in terms of treatment as specified in the table. N&V: nausea and vomiting, MO: morbidly obese, OW: obese and overweight, NW: normal weight.

	Medication
POSE	Metformin 1 g, gliclazide 30 mg
RYGB	Metformin 3 g, metformin 1 g, pioglitazone 50 mg

Appendix 2: Medication of patients in the POSE vs RYGB study.

The two groups of patients (POSE and RYGB; Chapter 4) differed in terms of treatment as specified in the table. POSE = primary obesity surgery endoluminal, RYGB= Roux-en-Y gastric bypass.

	Medication
Group 1 NW	No medication
Group 2 OW	No medication
Group 3 OB	No medication

Appendix 3: Medication of subjects in an obesity-prone Arab population study.

The three groups of subjects (1–3; Chapter 4) were not on any medication, as specified in the table. NW: normal weight, OW: overweight, OB: obese.