Metabolic hormones in bariatric surgery and reward behaviour

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Abstract

The World Health Organization (WHO) defines obesity as a condition in which body fat is increased to the extent that health and well-being are impaired. Obesity and type-2 diabetes are two of the leading healthcare challenges facing this generation. Bariatric surgery is the most effective therapeutic option for morbid obesity. A systematic review has concluded that surgery is superior to conventional treatment in reducing weight. However, the review failed to show the superiority of one surgical method over others. It is thought that the re-routing of food through an anatomically altered and/or shorter gastrointestinal tract leads to an increased delivery of incompletely digested nutrients to the ileum and colon. This leads to over-stimulation of the specialized entero-endocrine L cells. Others argue that the exclusion of an inhibitory factor from the foregut may mediate the rapid improvement in diabetes. Several studies have shown a blunted hind gut hormone (PYY and GLP-1) response in the morbidly obese patients that is reversed by Roux-en-Y gastric bypass (RYGBP) and sleeve gastrectomy (SG). Recent studies on patients undergoing bariatric surgery have revealed a key role for PYY, GLP-1 and acyl-ghrelin in regulating appetite, bodyweight and glucose homeostasis. A correlation between changes in gut hormone secretion and weight loss has not yet been shown in humans, but has been shown in rats after RYGBP. This discrepancy may be related to study design and sample processing, as not all studies have measured the active forms of the circulating hormone, and standardized for collection of blood samples. Some have compared post-surgical changes in gut hormones against control groups, not their pre-operative state, making it difficult to draw conclusions on individual physiological changes and corresponding correlations to anthropometry. Further, no study to date has found correlation between change in active gut hormones and change in perception of hunger and satiety.

In my study, RYGBP and SG led to a differential change in hunger, prospective food consumption and satiety. RYGBP had a more pronounced influence on prospective food consumption and hunger, despite non-significant changes in acyl-ghrelin. As RYGBP led to a more pronounced PYY3-36, GLP-1 and amylin response, it would be expected to alter satiety more. SG by contrast led to a more pronounced and significant decline in acyl-ghrelin, but only mediated a lesser change in hunger in comparison to RYGBP. However, my study does provide a link between the change in gut hormones and measures of appetite and satiety. My study also confirms gut hormone changes that occur after RYGBP and SG correlate to a decline in appetite and an increase in satiety, and therefore mediate weight loss. I also compared the change in hunger, prospective food consumption and satiety from baseline, and confirm a significant

decrease in Δ hunger and Δ prospective food consumption, and a significant increase in Δ satiety after RYGBP and SG.

There is equivalent excess weight loss (%EWL) after both RYGBP and SG at 6 weeks and 12 weeks after surgery. Despite starting with a lower BMI, the SG group lost similar BMI points to the RYGBP group at 6 weeks and at 12 weeks after surgery. This is in keeping with other recent short term and long term human studies. RYGBP and SG led to equivalent fat mass loss and decline in plasma leptin. RYGBP led to a pronounced hind gut hormone response, and SG led to a similar but less pronounced hind gut response. SG alone led to a significant decline in acyl-ghrelin. The amylin response after RYGBP and SG are divergent. In our study patients continued to lose weight from the first post-operative study point at 6 weeks to the second study point at 12 weeks, however there was no significant change in the fasting or meal stimulated insulin, PYY3-36, acyl-ghrelin, GLP-1 and amylin response from 6 to 12 weeks, apart from acyl-ghrelin in the RYGBP group, where acyl-ghrelin did increase between these time points. I also explored the role of insulin/ amylin ratio in appetite and weight loss. It is thought that an increased ratio of amylin/ insulin expression may act as a marker for beta cell dysfunction. Hyperglycaemia is thought to lead to the hypersecretion of amylin relative to insulin, and increase the amylin /insulin ratio in insulin-resistance. In the RYGBP group changes in PYY3-36 and insulin: amylin ratio correlates to weight loss. In the SG group change in PYY3-36, acyl-ghrelin, GLP-1 and amylin correlate to weight loss after surgery. RYGBP and SG seem to utilize different mechanisms to engender weight loss. The outcome after SG is dependent on the hormonal changes that ensue, whereas RYGBP may mediate its effects through neuro-anatomical changes associated with surgery. My findings, like those of others recently, lend support to the hind gut mediating the effects of weight loss after RYGBP and SG surgery.

The resolution of type 2 diabetes occurs immediately after RYGBP and SG. RYGBP and SG markedly improved glucose homeostasis by improving insulin secretion through the augmented GLP-1 response, weight loss and the decrease in acyl-ghrelin secretion seen after SG, leading to improved insulin sensitivity. These changes in insulin secretion and insulin resistance are seen early after surgery before any substantial weight loss has occurred. My study confirms RYGBP and SG to be equally efficacious as metabolic surgical options. The disparity in GLP-1 response after RYGBP and SG is further complicated by the GLP-1 stimulated insulin release displaying a threshold phenomenon. Thus the GLP-1 response after RYGBP and SG did not lead to equivalent glucose-dependent insulin secretion. The GLP-1 stimulated amylin response also showed a threshold phenomenon. However, there did not seem

to be any difference between the two groups. In our study there was a decline in HOMA IR after RYGBP and SG. The decline after SG showed a trend towards statistical significance. This discrepancy can partly be explained by the significant decline in acyl-ghrelin seen only after SG but not RYGBP. The duodenal exclusion hypothesis is unlikely to be a viable explanation given our results on sleeve gastrectomy, which occur in spite of a functional duodenum. The differential insulin/ amylin ratio after RYGBP and SG is noteworthy. In our study, there was a significant decrease in insulin: amylin ratio after RYGBP. Insulin secretion was not significantly altered after RYGBP. However there was an increase in amylin secretion after RYGBP leading to a decrease in insulin: amylin ratio at 6 and 12 weeks after surgery. There was a significant increase in meal stimulated insulin secretion after SG. This led to lower insulin: amylin ratio after SG. The lower amylin seen after SG may also contribute to the improved glucose homeostasis after SG, and further compensate for the relatively lower GLP-1. However, relative increase in amylin secretion did not adversely influence glucose homeostasis after RYGBP. The contrasting alteration in ratio did not correlate to satiety, prospective food consumption or weight loss. In our study GLP-1 secretion did show a positive correlation to amylin secretion in both groups, before and after surgical intervention.

It is known that some patients fail to lose weight after RYGBP and SG, but the mechanisms behind this failure have yet to be explored. One patient in our SG group was noted to have lost no further weight between 3 and 12 months following surgery. This patient had a three month meal stimulated amylin, Δ PYY3-36 and Δ acyl-ghrelin curve below the baseline curve for the respective hormones. This was in sharp contrast to all the other patients in the SG group. In other words a poor hormone response after surgery predicts failure to respond after SG. This altered meal stimulated response could be utilized to fast-track patients predicted to fail to a second stage procedure.

My second study suggests that an individual's metabolic state influences their monetary decisions. The risk-sensitive monetary decisions were influenced by both long-term metabolic signals indexing energy stores and short-term metabolic signals that index energy gains. At the neurobiological level, my results suggest an overlap between food and monetary reward. This has significant implications for all decisions that incorporate risk and monetary reward. In other words an individual's body mass index and his nutritional intake could alter risky behaviour.

Chapter 1

Introduction

1 Introduction

1.1 Obesity

The World Health Organization (WHO) defines obesity as a condition in which body fat is increased to the extent that health and well-being are impaired (WHO 1998). The operational definition of obesity is based on BMI. Obesity, defined as a body mass index (BMI = weight in kg/ height m^2) of above 30 (WHO 2000). The currently used cut-off points for overweight (i.e., 25 kg/m^2) and obesity (i.e., 30 kg/m^2) are based on morbidity and mortality data in relation to BMI from population studies in Caucasians (WHO 1998). It is a leading cause of death worldwide (Kopelman PG 2000). Obesity is set to overtake infectious disease as the most significant contributor to poor health worldwide (Kopelman PG 2000, Ogden CL et al 2004).

1.2Classification of obesity

A classification of obesity into four subclasses of obesity proposed: obesity 1 (30–34.9 kg/m^2); obesity 2 (35–39.9 kg/m^2); extreme obesity (>40 kg/m^2); and super obesity (>50 kg/m^2) (Leff and Heath 2009). This classification also fits in well with the guidelines for obesity surgery (Leff and Heath 2009).

Body mass index (kg/m^2)	Classification
18.5-24.9	Normal weight
25.0-29.9	Overweight
30.0-34.9	Obesity type I
35.0-39.9	Obesity type II
≥40.0	Morbid obesity/obesity type III
≥50.0	Super obesity

Figure-1 Classification of obesity based on body mass index thresholds (Leff and Heath 2009).

Worryingly the trend in morbid obesity accelerated above that of non-morbid obesity between 2000 and 2005. There was a 24% increase in obesity rates, but a 50% increase in extreme obesity (BMI >40), and an even greater 75% increase in severe obesity (BMI>50) (Sturm 2007). This trend will lead to an increase in healthcare utilization costs, as healthcare costs for the morbidly obese are 81% above those for the non-obese population and 47% above costs for the non–morbidly obese population. (Flegal et al 2002, Arterburn et al 2005)

1.3 Obesity prevalence

In England and Wales One in four adults are obese, 32% of women and 46% of men are overweight , with a BMI of >25 but <30 kg/m2 (the NHS information centre 2010) The direct cost of treating obesity and overweight individuals is estimated to be over three billion pounds per annum in the UK (Allender S et al 2007).

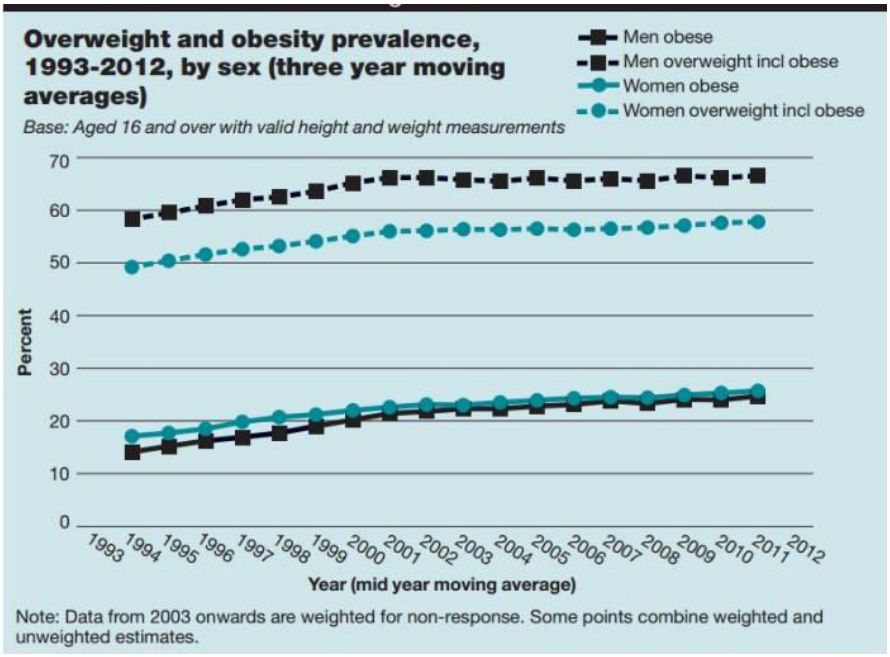


Figure 2; Obesity rates in England from 1993 to 2012- Health Survey for England 2012.

Women had a significantly higher rate of obesity in the early nineties, but the rates did converge with no significant difference by 2006. There was a 68% increase in the overall trend from 1993 to 2006.

1.4 Economic costs of obesity

In 2005 over 871,000 prescription items were dispensed for the treatment of obesity. This compares with 127,000 in 1999 (NHS Information centre, England, 2006). The Foresight Report forecasts that by 2050, 60% of men and 40% of women could be clinically obese. Without action, obesity-related diseases will cost the UK economy £45 billion a year, including £6.5 billion to the NHS in treatment costs (Foresight Report 2007).

1.5 Mortality associated with obesity

Approximately 30,000 deaths annually in the UK are attributable to obesity (National Audit Office, 2001). There has been a substantial recent increase in mortality ascribed

to obesity in the U.K national data (Haslam and James, 2005). However this was not consistent in all regions of England (Marie Duncan et al 2010). It is not yet clear if this represents a geographical variation in the contribution of obesity to mortality, in certification practice, or both. It seems likely that this reflects the increase in the prevalence of obesity. However, other factors, such as increased clinical awareness of, and willingness to certify obesity may have played a role too. Approximately 300,000 deaths in the USA are attributed to obesity (Allison et al 1999), where obesity is set to overtake smoking as the main preventable cause of premature death (Mokdad et al 2004). A number of prospective studies in Caucasian and Asian populations have demonstrated an increase in mortality with a BMI >30, but not with a BMI within the range of 18.5 and 25 (Stevens et al 2003). Further, a recent systematic review of over 890000 participants found that each 5 point increase in body mass index (kg/m^2) over 25 was associated with a 30% increase in overall mortality (Hitlock et al 2009).

1.6 Co-morbidities associated with obesity

Obesity is a medical disorder that leads to co-morbidities (Haslam and James, 2005). This association is profoundly important for the affected individuals, but the associated morbidity is also economically damaging for society (Haslam and James, 2005). At least 18 co-morbid conditions are known to be associated with obesity (asthma, coronary artery disease, diabetes mellitus, dyslipidemia, gallstones, gastroesophageal reflux, hypertension, nonalcoholic steatohepatitis or nonalcoholic fatty liver disease, sleep apnea, and urinary incontinence, breast cancer, congestive heart failure, lymphoedema, major depression, osteoarthritis, polycystic ovary syndrome, pseudotumor cerebri, and venous stasis or leg ulcers) (Cremieux et al 2008). Further the risk of developing these co-morbidities is directly correlated to the degree of obesity (figure-4) (Leff and Heath 2009).

Relative risk	Diseases associated with metabolic consequences (indirect association)	Diseases associated with excess weight (direct association)
Greatly increased risk (>3)	Type 2 diabetes, gallbladder disease, hypertension, dyslipidaemia, insulin resistance, non-alcoholic fatty liver	Sleep apnoea, breathlessness, asthma, social isolation, depression, daytime sleepiness/fatigue
Moderately increased risk (2-3)	Coronary heart disease, stroke, gout	Osteoarthritis, respiratory disease, hernia, psychological problems
Slight increased risk (1-2)	Cancer, impaired fertility, polycystic ovaries, skin complications, cataract	Varicose veins, musculoskeletal problems, backache, stress incontinence, oedema/cellulitis

Figure-3; Co-morbidities associated with obesity. Data adapted from guidance from the National Institute for Health and Clinical Excellence (Leff and Heath 2009)

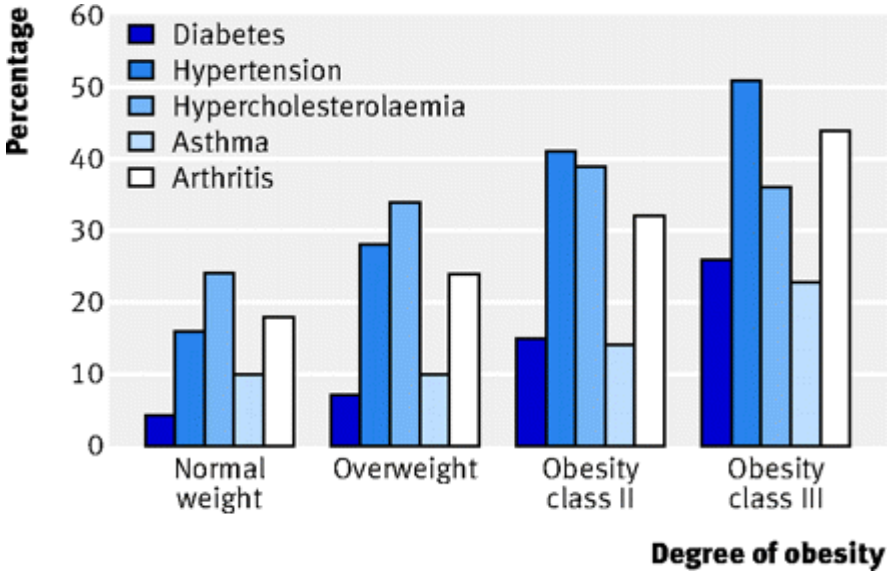


Figure-4; Proportion of people with a major co-morbidity, by degree of obesity (Leff and Heath 2009)

1.7 Type-2 diabetes mellitus

A dramatic rise in the incidence of T2DM has paralleled the rise in obesity. Diabetes mellitus is a metabolic disease characterized by insufficient insulin and/or resistance to the actions of insulin in the target tissues, resulting in chronic hyperglycaemia. A diagnosis of type-2 diabetes mellitus (T2DM) is made from either fasting blood glucose of greater than or equal to 7mmol/L or a two hour plasma glucose above 11.1 mmol/ L during an oral glucose tolerance test. More recently a haemoglobin A1c above 6.5% has also been utilized to diagnose diabetes. The elevated glucose leads to excessive glycation of molecules, β-cell damage, cardiovascular dysfunction, blindness, nerve demyelination and nephropathy (Alberti and Zimmet, 1998). T1DM is thought to result, for the most part, from autoimmune destruction of insulin-producing pancreatic β- cells resulting in an absolute insulin deficiency and is generally not associated with obesity. The failure to respond normally to insulin is called “insulin resistance”. This coupled with the inability to produce enough insulin to overcome this resistant state leads to T2DM (Lazar 2005). However, a number of other causes including genetic defects in β-cell development, β-cell function, and dysfunctional insulin action at target sites, infection, drug-induced and gestational diabetes have also been identified. The majority of T2DM is associated with obesity resulting in a concurrent global T2DM pandemic (WHO, 2008). It is estimated that the prevalence of T2DM will rise from 171 million in 2000 to over 350 million by 2030 globally. It is also estimated that diabetes related deaths will rise by more than 50% worldwide in the next decade (WHO, 2008).

1.8 Obesity and T2DM

The prevalence of T2DM in obese population is 5-10 times that of the normal population (reviewed by Diamond J 2003). The current epidemics of these two conditions are seemingly related (Mokdad et al 2003*).* Conventional wisdom links T2DM to obesity by virtue of the insulin resistance that arises from an excess of body fat (reviewed by Diamond J 2003, Kahn S E et al 2006, and O’Rahilly S 2009). Adipose tissue is now recognized as an endocrine organ that communicates with the brain and peripheral tissues through hormones to regulate appetite and metabolism (Kershaw and Flier, 2004). Obesity is associated with biochemical resistance to both insulin and leptin (Porte Jr. 2001). The brain is known to utilize input from insulin, leptin, and nutrient-related signals to regulate body fat content and hepatic insulin sensitivity. It is thought that impaired neuronal signaling by these afferent signals causes hyperphagia, weight gain, and hepatic insulin resistance (reviewed by Schwartz and Porte Jr. 2005). This led to a model based on brain insulin resistance (reviewed by Schwartz and Porte Jr. 2005). The association of diet-induced obesity (DIO) with both higher serum levels

of insulin and leptin and increased activation of inflammatory signaling pathways raises the possibility that these two alterations are causally linked. Disruption of inflammatory pathways in neurons protects against DIO and insulin resistance (reviewed by Thaler and Schwartz, 2010). Inflammation, mitochondrial dysfunction and endoplasmic reticulum stress are three of the proposed theories to explain the aetiology of T2DM in the presence of obesity (O'Rahilly S 2009).

1.9 Regulation of food intake and energy homeostasis

Adequate food and water are a pre-requisite for an organism's survival. Energy balance encompasses the exquisite matching of energy intake and energy expenditure to maintain homeostasis. Ingested nutrients must provide a supply of adequate fuel, essential to bodily functions including somatic maintenance, thermogenesis, metabolic processes, muscle action and reproduction. Constant supply of fuel to all body tissues is achieved through the maintenance of blood glucose concentrations. The homeostatic pathway is concerned with regulating an organism's energy balance to maintain growth, repair, reproduction and somatic maintenance. Although energy intake is a highly regulated process excess energy can be stored as body fat for future use. The regulation of energy balance requires integration of information on acute nutrient status as well as body energy stores; a process that is achieved through signalling of circulating hormones and metabolites upon neural circuits. Furthermore, a host of environmental cues and genes influence all aspects of energy balance (Lenard and Berthoud, 2008). Galen hypothesized that stomach contractions regulated appetite. It was thought that the physical contents of the abdominal cavity determined appetite (Mayer and Thomas, 1967). This 'peripheral control' hypothesis was challenged by the theory of 'central control'. The presence of centres within the brain that regulate feeding was proposed as an alternative (Anand BK and Brobeck JR 1951, Stellar, 1954). With further investigation and technological advances, the concept of brain regions involved in appetite regulation was replaced with the identification of discrete neuronal sub-populations involved in feeding behaviour and bodyweight regulation (Schwartz et al 2000). The procurement of food and food intake is regulated by a complex neuro-endocrine network (Lenard NR and Berthoud HR 2008). The neural network regulating food intake can be divided into homeostatic and non-homeostatic pathways (Gao Q and Horvath TL 2008). The non-homeostatic pathway is thought to mediate the rewarding aspects of food (Lenard NR and Berthoud HR 2008, Gao Q and Horvath TL 2008). The two pathways are thought to interact to govern feeding behaviour (Morton GJ et al 2006). More recently, a number of studies have begun to explore the

importance of non-hypothalamic and cortical regions in feeding behaviour (Berthoud, 2007).

Despite large daily fluctuations in food intake and energy expenditure, bodyweight remains relatively stable over time (Seeley RJ and Woods SC 2003). It is well documented that a reduction in bodyweight results in increased appetite and food intake, whilst an increase in bodyweight through experimental manipulation (with resultant fat deposition) can proportionally reduce appetite. Interestingly, bodyweight and adiposity resolve to baseline levels when *ad libitum* food intake is resumed (Bray, 1991; Sims et al, 1973; Weigle, 1994). Maintenance of bodyweight requires adjustment of both energy intake and energy expenditure. Energy expenditure comprises thermogenesis, resting metabolic rate and physical activity, and compensatory changes in these modalities occur in situations of both energy deficit and energy excess (Leibel et al, 1995). From an evolutionary standpoint, this control mechanism is likely to exist not only to maintain energy stores to avoid starvation, but also prevent excessive fat accumulation to avoid predation (Mercer and Speakman, 2001). Several hypotheses have been presented to account for the maintenance of bodyweight. The most widely accepted of these is the 'lipostatic' theory. This proposes that humoral signals released by body fat stores are responsible for conveying information regarding the quantity of body fat to the CNS in order to modulate appetite, maintain and/or restore bodyweight (Kennedy, 1953). The lipostatic theory suggests that changes in bodyweight alter secretion of humoral factors signaling an alteration in energy balance. An afferent humoral factor would need to fulfill a set of criteria. They must firstly be in proportion to body fat. Secondly, signal transduction mechanisms mediating the effect should be located within CNS regions demonstrated to be involved in bodyweight regulation. Finally, administration of the signal, either into the circulation or directly into the brain should alter both food intake and bodyweight (McMinn et al 2000). Insulin and leptin have both been classified as lipostatic signals that accurately reflect and regulate bodyweight status.

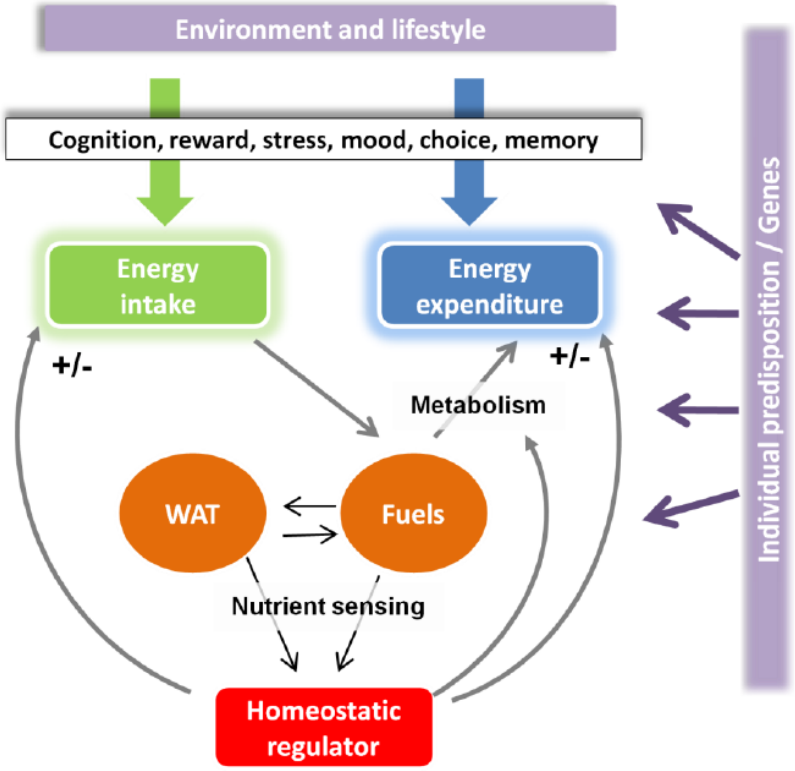


Figure-5; The control of energy balance is a complex process requiring bidirectional integration of information regarding nutrient status and body fat stores with homeostatic circuits in the central nervous system. A homeostatic regulator is thought to modulate energy intake and expenditure. Environment and genetics further influence the control of homeostatic processes. (WAT = white adipose tissue) (Adapted from Lenard and Berthoud, 2008)

1.10 The homeostatic pathway

1.10.1 Hypothalamus

Early case reports of obesity in patients with pituitary adenoma linked the hypothalamus to obesity (Frohlich A 1901). Animal model studies initially utilized systematic anatomical lesions in the hypothalamus, to identify areas that influence food intake (Anand BK and Brobeck JR 1951). More recent *in vivo* studies have led to the identification of specific populations of neurons in the hypothalamus that govern food intake (Morton GJ et al 2006, Lenard NR and Berthoud HR 2008, Gao Q and Horvath TL 2008). The hypothalamus acts as a primary integrator of nutritional information, with

populations of neurons directly and differentially sensitive to leptin, insulin, ghrelin, PYY3-36 and circulating metabolites including glucose, fatty acids, and amino acids (Morton GJ et al 2006, Lenard NR and Berthoud HR 2008, Gao Q and Horvath TL 2008). It contains structures involved in integrating both satiety and hunger pathways including the arcuate nucleus (ARC), ventromedial nucleus (VMN) and lateral hypothalamic area (LHA) (Schwartz et al 2000, Stellar 1954). It is the primary centre for regulation of food intake and energy metabolism. In addition, the hypothalamus receives afferent information from the brainstem. Information regarding nutrient status and energy stores is relayed via nervous afferents or hormones to regions involved in homeostatic control of feeding and also project to areas of the brain that integrate the rewarding features of food intake- reward and limbic pathways (Lenard NR and Berthoud HR 2008)

The ARC is a circumventricular collection of neuronal cell bodies, superior to the base of the third ventricle in the brain, possessing a modified blood brain barrier, allowing access to circulating nutrients as well as peptides and hormones (Brightman and Broadwell 1976). First order neurons in the ARC are thought to respond to humoral signals which project to second order neurons in the paraventricular nucleus (PVN), VMN, LHA and dorsomedial nucleus (DMN) (Elmquist et al 1999). Electrical stimulation of the VMN suppresses food intake whilst ablation induces profound hyperphagia and the subsequent development of obesity. Conversely, stimulation or lesioning of the LHA induces the opposite responses (Stellar 1954).

The neurons in the lateral hypothalamus responsible for food intake seem to be constrained by tonic inhibition that can be relieved by activation of the reward pathway, and thought to promote motor programs to stimulate feeding behaviour (Kelley et al 2005). Lateral hypothalamic area neurons may also attenuate the response to satiety signals, increasing the amount of food consumed during a meal. These considerations support the view that the lateral hypothalamic area may act as an integrative neural site for homeostatic, satiety and reward-related neural input, and collectively activates feeding behaviour (Morton G et al 2006).

1.10.2 Brainstem

Neural afferents from the GI tract and abdominal viscera are relayed to the CNS via the vagus nerve which terminates in the nucleus tractus solitarius (NTS) at the base of the brainstem (Grill 2006). The brainstem has been proposed to integrate and relay this information to the hypothalamus. Similar to the ARC nucleus, the NTS has an

incomplete blood brain barrier at the area postrema, allowing access for circulating factors. Interestingly, the NTS has well-established reciprocal connections with several other regions in the brain involved in energy balance, suggesting a role for the brainstem as a primary integration centre of meal-related sensory input (Berthoud, 2002).

1.11 Non-homeostatic regulation of food intake- the reward pathway

The hypothalamus and brainstem are crucial to energy homeostasis. The strongest evolutionary pressure driving the development of this system was the deficiency of food for survival, resulting in the development of robust mechanisms to defend against the lower limits of adiposity (Zheng and Berthoud, 2008). Food intake is not just driven by homeostatic mechanisms but also by the rewarding value of food in the current calorie abundant environment (Berridge KC 1996). Several authors have recently questioned if the homeostatic pathway plays a primary role in our current calorie abundant environment (Volkow ND and Wise RA 2005, Palmiter RD 2007, Lenard NR and Berthoud HR 2008). Hence brain regions involved in the processing of the psychological features of appetite, such as liking (pleasure), wanting (motivational value-cognitive incentives/ explicit desire), hedonic value (objective affective reactions) and reward, as well as the memories of these features, have been under investigation recently (Berthoud, 2003, Berridge KC 2009). Neuronal tracing studies demonstrate the hypothalamus to be well connected to many other regions in the brain, resulting in a complex circuit that allows adaptation and coordination in an unpredictable environment (Berthoud, 2002). The lateral hypothalamus is thought to play an integrative role in feeding behaviour. The lateral hypothalamus is known to potently stimulate food intake, and is inter-connected to homeostatic and reward pathways, leading some to suggest that it may play a mediators role in promoting consumption of palatable food (Kelley et al 2005).

The ventral tegmental area (VTA) and its projection to the nucleus accumbens (NAc), which forms part of the reward pathway have been known to mediate the rewarding effects of drug addiction (Volkow ND and Wise RA 2005). This pathway is associated with motivation and hedonic behaviour, and recently has also been shown to mediate the rewarding aspects of food (Volkow ND and Wise RA 2005, Lenard NR and Berthoud HR 2008, Lutter M and Nestler EJ 2009). A parallel between obesity and drug addiction has been drawn, in that both are ingestion habits pursued to catastrophic ends, leading some to claim that the reward pathway may play a part in the aetiology of obesity (Volkow ND and Wise RA 2005, Stoeckel LE et al 2008, Lutter M and Nestler

EJ 2009). Preliminary evidence for this comes from functional magnetic resonance imaging studies where a differential activation pattern in normal weight and obese individuals is seen. Obese women presented with images of high-calorie food showed increased activation in brain regions that mediate reward and emotion (Stoeckel LE et al 2008). Also imaging studies have begun to demonstrate that circulating appetite signals can modulate brain activity in the reward pathway (Malik et al 2008, Batterham et al 2007). The “priming” effect of a small amount of palatable food on binge eating parallels the ‘priming’ effect of drugs in addiction behaviour, where even a small dose tends to elicit a strong ‘craving’ and compulsion for further use, hence some argue that food can be thought of as a drug that can lead to dependence (Davis et al 2004).

The concept that reward perception is subject to homeostatic regulation derives from evidence that food deprivation strongly augments the reward value of addictive drugs including heroin, amphetamine and cocaine (Carroll et al 1979 and Stuber et al 2002). One mechanism to explain this effect proposes that metabolic signals leptin and insulin tonically inhibit brain reward circuitry and that, by lowering circulating levels of these hormones, energy restriction increases the sensitivity of reward circuits (Fulton et al 2000 and Figlewicz et al 2004). Consistent with this hypothesis, centrally administered insulin or leptin diminish food reward (Figlewicz et al 2004). This has led to some authors proposing that energy restriction may decrease inhibitory neuronal input and in-turn increase the animal’s response to rewarding stimuli as an adaptive mechanism motivating animals threatened by caloric insufficiency to seek and obtain palatable foods (Figlewicz et al 2003).

1.12 Reward pathway and feeding behaviour

It is clear that not everyone exposed to a calorie abundant environment over eats (Engelmann JB 2006). The role of the reward pathway in feeding behaviour is the subject of much investigation at present (Lowe and Levine 2005). Food consumption is known to stimulate the reward pathway and lead to motivated behaviour in animals and humans (Hoebel et al 1989, Berridge 1996, Schultz 1998, Bassareo and Di Chiara 1999, Volkow et al 2002, Dawe S and Loxton N 2004). Further, high calorie foods are more rewarding (Cummings DE and Foster KE 2003). The current ease of access to palatable energy-dense food is considered an environmental factor predisposing to obesity (Volkow and Wise 2005). Palatable foods are able to override homeostatic signals, and stimulate brain reward systems independent of their caloric value (reviewed by Kenny PJ 2011).

The effects of palatable food consumption on brain reward systems have been directly assessed in laboratory animals. Animals given prolonged access to palatable food went on to gain significant amounts of weight (reviewed by Kenny PJ 2011). Further, studies on animals exposed to an environment with an abundance of food, point to feeding behaviour being driven by the rewarding value of food and not energy homeostasis (Berridge 1996 and Berridge 2004). A diet-induced reward deficit is noted in these rats, and may reflect an adaptive response to overstimulation by palatable food (reviewed by Kenny PJ 2011). Animal studies also suggest that the reward pathway in the brain can be dysregulated by starvation and intermittent access to palatable food (Carr 2007).

On the one hand some propose a theory based on a lack of neuro-transmission in the reward pathway, known as the Reward Deficiency Syndrome (RDS) to explain a range of addictions to alcohol, cocaine, and pathological gambling (Bowirrat and Oscar-Berman 2005). Obesity and drug addiction are thus thought to be reward deficiency syndromes (Wang et al 2001, 2002, and 2003). It is proposed that individual's compensate for this reward deficiency by frequent food consumption (Blum et al 2000). It is thought that the reward pathway does not differentiate between rewarding experiences provoked by natural re-inforcers like food, illicit drugs like cocaine, or behaviours like gambling (Kelley et al 2005). A high rate of co-morbidity is observed for drug addiction and obesity (Wolfe and Maisto 2000). However, eating is not known to produce the neuro-adaptive effects known to be produced by drugs of abuse, that lead to withdrawal effects, central to drug addiction (Rogers and Smit, 2000). Adiposity (leptin) does correlate to feeding behaviour in obese and underweight individuals (Adami et al 2002, Prittwitz et al 1997). The development of leptin resistance in the reward pathway may play a role in the dysregulation of feeding behaviour and compulsive overeating in obesity (reviewed by Kenny PJ 2011).

The converse of RDS with enhanced neuro-transmission in the reward pathway promoting appetitive response to primary re-inforcers such as food has also been linked to obesity (Volkow e al. 1999, Cohen et al 2005, Davis et al 2007). An Individual with high reward sensitivity is thought to have a reactive reward pathway that encourages hyperphagia (Pickering and Gray 2001). It is thought that an individual's personality and personality trait may predispose him/ her to overeating and obesity (Ryden et al 2003, Dawe S et al 2004, Davis C et al 2004 and Beaver JD et al 2006). An individual's reward drive does predict relative body weight in normal and overweight populations (Bulik et al 2003, Davis et al 2004, Dawe and Loxton 2004, Franken and Muris 2005). Further, behavioural studies have shown a link between reward sensitivity, feeding behavior, body weight and binge eating through greater sensitivity

towards food-related cues (Franken and Muris 2005, Dawe and Loxton 2001, S Dawe & N J Loxton 2004, D.J. Mela 2006). Individuals with higher reward sensitivity were shown to display enhanced activity in brain regions implicated in food reward in response to palatable foods (Beaver et al 2006). This study linked personality trait to over eating (Beaver JD et al 2006), and could give us an insight into neurobiological factors that contribute to over eating and obesity (Beaver JD et al 2006). Other recent functional imaging studies (Rothemund et al 2007 and Stoeckel et al 2008) did also show that food cues are related to hedonic responses in obese individuals. Other functional imaging studies have shown that monetary reward (Elliott et al 2000, Ernst et al 2004 and Matthews et al 2004) is also mediated through the reward pathway.

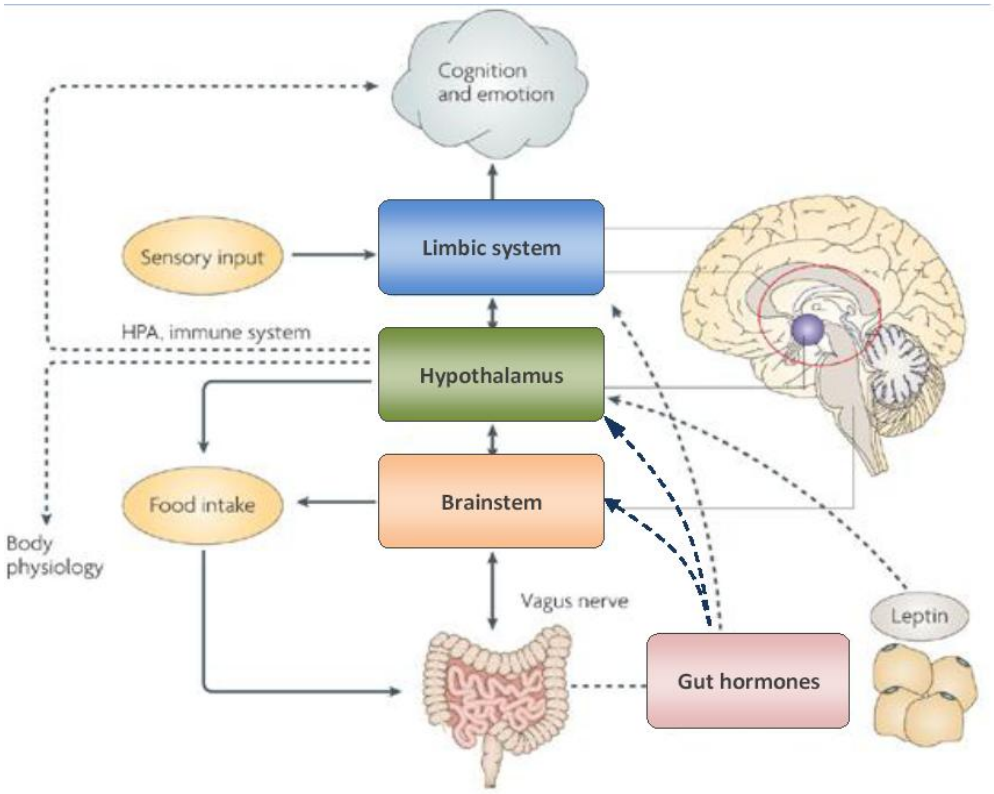


Figure-6; Complex neural circuitry governs many aspects of energy balance. Information from the periphery is conveyed by circulating hormones and vagal afferents to the caudal brainstem, hypothalamus and cortico-limbic brain regions. Cortico-limbic regions integrate meal-related sensory input and nutrient information with internal emotional factors. Together, these circuits regulate ingestive behaviour and bodyweight. (Adapted from Gomez-Pinilla, 2008)

The addiction model may result in social stigmatization from the labeling of obese individuals as food addicts. This may lead to adverse outcomes as obese individuals already feel subjected to societal stigmas and bias (Boroni MAP et al 2012). This could lead to a sense of lack of control or choice over their behavior leading to a disease label and hampering change. The suggestion of addictive foods also shifts the focus away from promoting healthy behaviour and onto particular types of food (Boroni MAP et al 2012).

In studying the aetiology and treatment of obesity, it is important to remember the contribution of energy expenditure (Cizza G and Rother KI 2012). In humans this is determined by basal metabolic rate, diet-induced thermogenesis, physical activity and non-exercise activity thermogenesis (Cizza G and Rother KI 2012). The latter is an important determinant of total energy expenditure and is thought to be under the control of neuro-peptides including leptin, and account for 100 to 700 kcal/day, this in part genetically determined (Cizza G and Rother KI 2012). The role of sleep in obesity has also been studied, with chronic sleep deprivation linked to obesity. Acute sleep deprivation increased Cortisol, decreased GH and leptin and increased ghrelin (Cizza G and Rother KI 2012) leading to appetite and insulin resistance (Cizza G and Rother KI 2012).

The role of external organisms in human physiology has also taken on new perspectives with evidence confirming that microbes can influence host physiology, and the role of gut microbiota in the development of obesity has received much attention recently (Boroni MAP et al 2012). Obese and lean subjects have different microbiota composition profile, with those of obese subjects having capacity to harvest more energy from the diet through lipogenic pathways (Boroni MAP et al 2012). Further, microorganisms are also able to influence lipoprotein lipase activity, and triglyceride content of adipose tissue. In turn the dietary composition (fatty acids, carbohydrates, micronutrients, prebiotics, and probiotics) can modulate gut microbiota (Boroni MAP et al 2012). Obese twins had reduced bacterial diversity, and altered metabolic pathways. A study to test fecal transplantation as a viable treatment option for obesity is currently under way (Boroni MAP et al 2012).

Further, recent reviews have challenged the gut hormone mediated reversal of T2DM (Knop FK and Taylor R 2013). An acute negative calorie balance has been proposed as the only pre-requisite for reversal of type T2DM (Knop FK and Taylor R 2013). Taylor and colleagues argue that plasma glucose is normalised within days of a low calorie diet (Knop FK and Taylor R 2013). In our study the pre-operative mixed meal

study and the gut hormone changes observed does not support this hypothesis, as patients were still glucose intolerant and hyper-insulinaemic despite being on a liver reducing diet. Critics of gut hormone mediated changes, whilst accepting the contribution of incretins, note that the proportional effect of these changes is yet to be quantified. It is also difficult to rule out the role of energy restriction in most studies. The role of GLP-1 mediated improvement in β-cell function following bariatric surgery is also hotly debated as this is thought to be responsible for the delayed improvement in glucose homeostasis (Knop FK and Taylor R 2013). The difficulty in teasing out the mechanisms leading to the improvement in liver glucose handling either through reduction in liver fat content, or gut hormones will need to be addressed. It also remains to be seen if incretins or pancreatic fat content mediates the improved long term changes in glucose homeostasis (Knop FK and Taylor R 2013).

1.13 Pathogenesis of obesity

Modern molecular genetics has been deployed to obtain mechanistic insights into the pathophysiology of obesity (O'Rahilly 2009). Two cardinal features of obesity are energy intake in excess of requirement and the biological defence of an elevated level of body fat mass (reviewed by Thaler and Schwartz 2010). The search for an aetiological factor for the current obesity pandemic has led some authors to point to an evolutionary advantage. They propose that prolonged periods of famine were common in early human hunter-gatherer communities. Therefore genes that favour economical use of energy will be selected for, as they offer a survival advantage. These genes involved in economical use and storage of energy are called "thrifty" genes (Zimmet and Thomas, 2003). Thrifty genes would promote an increase in adipose tissue as an efficient storage of energy resource. The current calorie abundant environment and sedentary lifestyle is thought to lead to mal-adaptation leading to the twin epidemics of obesity and diabetes (Zimmet and Thomas 2003) (reviewed by Lazar et al 2005). Proponents of the thrifty gene hypothesis point out that a low BMI is known to be associated with amenorrhoea. Leptin replacement reverses this amenorrhea in leptin-deficient females with low bodyweight (Welt et al 2004). This provides the mechanistic explanation for the link between body fat and reproductive capacity seen in epidemiological studies (Frisch and McArthur 1974). This promotes survival of nutritionally fit individuals (reviewed by Lazar et al 2005). This contrasts with the "thrifty phenotype" hypothesis (Neel 1962). He noted that fetal malnutrition results in tissue adaptations favouring efficient use and storage of nutrients in-utero. This is thought to predispose to obesity and T2DM later in life in the setting of adequate nutrition (Hales and Barker 1992). They proposed that epigenetic memory from the prenatal

environment as a putative mechanism. This epigenetic regulation is thought to be mediated by energy dependent modification to enzymes (Jenuwein and Allis 2001, Blander and Guarente 2004). In the thrifty phenotype model, selective pressures for genes that protect from early malnutrition then promote obesity and diabetes under modern conditions by preserving glucose for use by the brain during these periods. This is also thought to lead to insulin resistance in peripheral tissues (reviewed by Lazar et al 2005).

It is proposed that genetic polymorphisms affect the central sensing and control of energy balance through an alteration in appetite and satiety to mediate the adverse outcome (reviewed by O'Rahilly 2009). The inheritance of several polymorphisms with small differences in expression can make populations more or less susceptible to obesity and diabetes (Diamond 2003). Leptin is thought to be one such candidate gene. Rodents and humans with one functional copy of the leptin gene have increased body fat (Farooqi et al 2001). Genome-wide association studies are beginning to identify the common genetic variation that underpins difference in adiposity across the normal population. *FTO* were the first to emerge as unequivocally associated with human obesity (O'Rahilly 2009). *FTO* is highly expressed in hypothalamus, where its expression is regulated by feeding and fasting O'Rahilly 2009). Recently four reported genome-wide linkage studies have identified several loci that show positive evidence for linkage to the pro-opiomelanocortin (POMC) gene in which a complete loss-of-function causes monogenic obesity in mice and humans (reviewed by Barsh GS et al 2000). The pro-opiomelanorcotin neurons in the hypothalamus are known play a significant role in appetite regulation. It is proposed that the genetic determinants of inter-individual variation predisposing to obesity are likely to be multiple with most single variants producing only a moderate effect (reviewed by Barsh GS et al 2000). Further, rare forms of monogenic obesity stem from genetic defects in leptin or melanocortin signalling pathways (Farooqi and O'Rahilly 2006). Mutations in the melanocortin-4 receptor account for up to 4% of cases of severe obesity. Common monogenic forms of human obesity seem to increase the 'set point' at which body adipose stores stabilize in an individual (reviewed by O'Rahilly 2009). Some argue that susceptibility to obesity is determined largely by genetic factors, but the environment determines phenotypic expression (Barsh GS et al 2000). Animal studies indicate difference in adiposity among inbred strains can be magnified by a high-fat diet (reviewed by Barsh GS et al 2000). This has led to calls for public health efforts to prevent obesity be focused on recognition and counseling of susceptible individuals (Barsh GS et al 2000). The determinants of BMI do vary between ethnic groups (reviewed by Barsh GS et al 2000).

High fat diet is one such environmental factor. Animal studies identify hypothalamic inflammation as a reversible mediator of high fat diet (HFD) induced weight gain (Thaler JP and Schwartz MW 2010). Inflammatory changes are detectable in the brain of HFD-fed animals (Zhang X et al 2005), and manifested to an even greater degree in animals lacking leptin signaling (Zhang X et al 2008). This has led some authors to propose a causal role for hypothalamic inflammation in HFD-induced obesity. Neuron-specific disruption of inflammatory pathways protects against DIO, hypothalamic leptin resistance, and systemic insulin resistance (Zhang X et al 2008). Further, over expression of a dominant-negative inflammatory marker in hypothalamic neurons reduce food intake and weight gain during HF feeding, and neuronal expression of a constitutively active inflammatory marker increases food intake (reviewed by Thaler and Schwartz 2010), suggesting that hypothalamic inflammation is both necessary and sufficient for weight gain during HF feeding (reviewed by Thaler and Schwartz 2010). Interventions that limit hypothalamic inflammatory signaling can prevent obesity from developing, implicating the latter as cause rather than just a consequence of obesity (reviewed by Thaler and Schwartz 2010). In the hypothalamus inflammatory signaling leads to insulin and leptin resistance.

HF feeding also induces inflammatory signaling in peripheral tissues, resulting in peripheral insulin resistance (Thaler JP and Schwartz MW 2010). A low-grade chronic inflammation is known to accompany excess visceral adiposity. This is accompanied by increased circulating levels of inflammatory cytokines (Thaler JP and Schwartz MW 2010). Leptin and insulin signal through common downstream pathways including the insulin receptor substrate protein (IRS), phosphatidylinositol-3 (PI3) kinase and (mitogen activated protein kinase) MAPK pathways. It is thought that inflammatory pathways interfere with these signal transduction mechanisms and lead to central and peripheral resistance to leptin and insulin (Thaler JP and Schwartz MW 2010). Other mechanisms such as up-regulation of suppressor cytokine signaling, and the unfolded protein response may also contribute to high fat diet induced hypothalamic inflammation and leptin/ insulin resistance (Thaler JP and Schwartz MW 2010). It is not yet clear if hypothalamic inflammation results from consumption of HFD irrespective of dietary composition (Thaler JP and Schwartz MW 2010).

1.14 Metabolic signals modulate neural pathways

The central pathways rely on metabolic signals from the periphery to assay an organism's energy stores. These metabolic signals can be divided into, those that relay information on long term energy stores in adipocytes (leptin and insulin) and those that

relay information on short term energy gains from food intake (ghrelin, insulin, PYY, GLP-1, amylin) (Morton GJ et al 2006, Murphy KG and Bloom SR 2006). A number of hormones released from the GI tract with receptors in areas of the brain characterised for their involvement in appetite and bodyweight regulation have been investigated for their roles in energy balance (Chaudhri et al 2006). These GI peripheral signals relaying information regarding nutrient status also appear to be essential for appetite regulation (Murphy KG and Bloom SR 2006). Gut hormones are sensitive to ingested nutrients. Hunger and satiety and therefore energy intake is partly mediated by changes in circulating gut hormone levels (Murphy KG and Bloom SR 2006). Ghrelin, PYY, amylin, GLP-1 act on homeostatic pathways to maintain energy homeostasis (Murphy KG and Bloom SR 2006). Leptin, insulin and ghrelin are known to act on both the hypothalamic homeostatic centres and the dopamine reward pathway. They stimulate (ghrelin) or inhibit (leptin and insulin) dopaminergic signalling, and alter the subjective reward value attached to food (Palmiter RD 2007). Several areas of the gastrointestinal tract have also been implicated in relaying these signals to the brain. Nervous afferents arising from stomach mechanoreceptors and chemoreceptors are transmitted via vagal afferent nerves to the hindbrain where visceral input is integrated. In addition, satiety is thought to be dependent upon nutrient passage into the small intestine (Sepple and Read, 1989).

1.14.1.1 Leptin

The cloning of leptin and the characterization of its molecular pathways in the hypothalamus has led to the elucidation of the mechanisms that govern energy homeostasis (Schwartz et al 2000, Morton et al 2006). A set of experiments in spontaneously occurring strains of obese (*ob/ob*) and diabetic (*db/db*) mice demonstrated the existence of a humoral factor regulating food intake. The coupling of circulation between normal and obese *ob/ob* mice, led to a reduction in food intake and bodyweight in the *ob/ob* mice. The authors suggested a deficiency in a humoral lipostatic factor in *ob/ob* mice (Coleman, 1973). A 167 amino acid polypeptide produced primarily by adipocytes in proportion to body fat mass is incorrectly synthesized in *ob/ob* mice (Zhang et al 1994). The obesity displayed in *ob/ob* mice can be reversed with exogenous leptin treatment (Halaas et al 1995). However studies in *db/db* mice shown them to be leptin unresponsive. The *db/db* gene was characterized as the leptin receptor (Chen et al 1996). Leptin serves as a circulating signal of energy stores by providing feedback inhibition to hypothalamic orexigenic pathways (reviewed by Thaler and Schwartz 2010). Circulating leptin levels correlate strongly with adiposity in both rodents and humans (Maffei et al 1995). Adipose tissue is now recognized to be an endocrine organ that communicates with the brain and peripheral tissues by

secreting hormones regulating appetite and metabolism (Kershaw and Flier 2004). These functions appear to be modulated by the location of the adipose tissue (visceral versus subcutaneous) (Das et al 2004), by the size of the average adipocyte in the tissue (Weyer et al 2001), and by adipocyte metabolism of glucose (Abel et al 2001). Mutations in the leptin gene can also cause severe obesity in humans, and can be improved with recombinant leptin therapy in children and adults (Farooqi et al1999, Licinio et al 2004). Administration of leptin leads to a reduction in fasting induced hyperphagia in rodents (Ahima et al 1996). Chronic administration leads to reduced food intake and decreased adiposity (Halaas et al 1995).

Obesity is strongly associated with hyperleptinemia in both humans and rodents placed on a high-fat diet (HFD) (reviewed by Thaler and Schwartz 2010). Once obesity is established leptin is relatively ineffective in reducing food intake or body weight. It is postulated that DIO arises at least in part from a failure of key hypothalamic neurocircuits to respond to leptin, and has been compared to the central and peripheral insulin resistance that occurs in this setting (Myers et al 2008, Schenk et al 2008, Shoelson et al 2006). Mechanisms underlying obesity-induced insulin resistance at the cellular level can also impair leptin signalling (Myers et al 2008, Schenk et al 2008, Shoelson et al 2006, Hotamisligil GS 2006, and Wisse et al 2007). However, whether leptin resistance causes common forms of obesity or is a consequence of excess weight gain is still not known (reviewed by Thaler and Schwartz 2010).

Furthermore, leptin levels reflect changes in energy balance independently of modest changes in body fat. A reduction in leptin is observed during short-term fasting and food restriction whilst an increase is seen following re-feeding and overfeeding (Kolaczynski et al 1996, Weigle et al 1997). Human studies have demonstrated dynamic changes in circulating leptin with changes in weight. Weight loss is associated with a significant decrease in leptin and the converse with weight gain (Rosenbaum et al 1996). These changes in leptin were not directly associated with modulation of energy expenditure (Rosenbaum et al 1996). Circulating leptin acts on the hypothalamic homeostatic centres that govern food intake, to regulate energy stores and maintain energy homeostasis (Morton GJ et al 2006). Leptin has a pivotal role in regulating negative feedback to the homeostatic centres, to maintain body energy stores (Morton GJ et al 2006). Peripheral and centrally administered leptin led to intra-cellular second messengers in the reward pathway, altering neuronal firing rate and decreasing food intake, and long-term genetic knockdown of the leptin receptor in the reward pathway led to an increase in food intake, but does not alter body weight (Hommel et al 2006). Others have also shown leptin regulation of dopamine levels in the reward pathway

(Krugel et al 2003). Leptin can modulate behavioral responses to rewarding, novel food by its action on the reward pathway (Schultz and Dickinson, 2000 and Bassareo and Di Chiara, 1999). The metabolic sensing by the reward pathway may provide a mechanism for the well-described increase in drug sensitivity seen during states of food restriction and leptin's ability to reverse this sensitivity (Carr et al 2002 and Shalev et al 2001). It is thought that leptin signals directly to independent, inter-connected brain circuits encompassing the homeostatic and non-homeostatic centre's, to generate an overall behavioral response (Hommel et al 2006). Leptin is thought to diminish the perception of food reward and palatability of food through direct and indirect actions (Morton GJ et al 2006). Functional leptin receptors have been isolated in VTA dopamine neurons (Palmiter RD 2007, Figlewicz DP and Benoit SC 2009). Leptin is known to reduce firing rate in these neurons (Palmiter RD 2007). In addition to its acute inhibitory effect on midbrain dopamine systems, there is accumulating evidence that tonic leptin signaling may also be necessary to maintain appropriate levels of mesostriatal dopamine signalling (reviewed by Kenny PJ 2011). On the one hand, acute activation of leptin receptors in the VTA exerts an inhibitory effect on mesoaccumbens dopamine transmission and can inhibit feeding behavior. On the other, leptin signaling in the midbrain is necessary to maintain appropriate dopamine production and signal transmission (reviewed by Kenny PJ 2011). Furthermore, a recent functional magnetic resonance imaging (fMRI) study assessed the sensitivity of brain regions to exogenous leptin administration before and after weight loss in overweight individuals. It was demonstrated that following weight loss leptin administration reversed neural activity patterns in response to visual food cues in feeding-related brain regions including the brainstem, hypothalamus, as well as the parahipppocampal, inferior and middle frontal gyri (Rosenbaum et al 2008). Thus, it appears leptin influences both behavioural and passive responses to changes in energy stores; a decrease in body fat leads to a decrease in leptin resulting in an increase in food intake; conversely increased adiposity causes a rise in leptin which reduces food intake allowing maintenance of bodyweight. fMRI to assess neural activity in the reward pathway to visual food stimuli was noted to be higher in genetically leptin-deficient adolescents, returning to normal levels with leptin administration. Further, in these leptin-deficient individuals whilst activity in the reward pathway correlate to ratings of liking in the fasted and fed state, the correlation was only noted in the fasted state after leptin treatment, and in normal individuals (Abizaid et al 2006).

1.14.1.2 Leptin Resistance

The switch to a highly palatable, energy-dense diet favours weight gain. DIO in both humans and rodent models is characterized by an increase in the defended level of body fat stores (Levin and Keesey 1998, Rosenbaum et al 2002, Leibel 2008, reviewed by Thaler and Schwartz 2010). Obesity is strongly associated with hyperleptinemia in both humans and rodents placed on a high-fat diet (HFD) (reviewed by Thaler and Schwartz 2010). Acquired leptin resistance is implicated in the predisposition to DIO in rodent models (reviewed by Thaler JP and Schwartz MW 2010). However, the mechanisms responsible for the defence of an elevated adiposity are unclear. Leptin resistance seems to occur as a result of obesity, and reduced sensitivity may in turn contribute to the aetiology of obesity (Heymsfield et al 1999). However, whether leptin resistance causes common forms of obesity or is a consequence of excess weight gain is still not known (reviewed by Thaler and Schwartz 2010).

A small proportion of obese humans have relative leptin deficiency. The vast majority of obese individuals are hyperleptinaemic (Considine et al 1996). Exogenous leptin administration does not affect food intake in rodents with diet induced obesity (DIO) (Van Heek et al 1997). Leptin infusion in obese humans has only moderate effects on bodyweight (Heymsfield et al 1999). However the anorexigenic effects of leptin are retained with central administration in obese rodents (Van Heek et al 1997), suggesting isolated peripheral leptin resistance in obesity. The mechanism underlying leptin resistance is not fully understood. It is thought that leptin transporter complexes that cross the blood brain barrier are saturated in obesity (Banks et al 1999). Once obesity is established leptin is relatively ineffective in reducing food intake or body weight. It is postulated that DIO arises at least in part from a failure of key hypothalamic neurocircuits to respond to leptin, and has been compared to the central and peripheral insulin resistance that occurs in this setting (Myers et al 2008, Schenk et al 2008, Shoelson et al 2006). Mechanisms underlying obesity-induced insulin resistance at the cellular level can also impair leptin signalling (Myers et al 2008, Schenk et al 2008, Shoelson et al 2006, Hotamisligil GS 2006, and Wisse et al 2007). Methods to improve leptin sensitivity have been investigated. The co-administration of the pancreatic hormone amylin with leptin reduces food intake and bodyweight and induces leptin signalling pathways greater than either treatment alone (Roth et al 2008). The ‘chemical chaperones’ 4-phenyl butyric acid and tauroursodeoxycholic acid, implicated in endoplasmic reticulum stress, can potentially act as leptin-sensitizing agents (Ozcan et al, 2009). Leptin did also show the propensity to extend the anorectic effects of the gut hormone PYY3-36 (Unniappan and Kieffer 2008).

1.14.2 Insulin

Insulin is primarily produced within the β-cell of the islet of Langerhans as preproinsulin and subsequently cleaved by proteolytic enzymes to produce insulin. It has also been localised in the CNS (Schwartz et al 1992). The β-cell is able to sense elevated plasma glucose. Glucose enters the β-cell via glucose transporter-2 (GLUT2) leading to an increase in adenosine triphosphate, and an alteration in ATP/ADP ratio. This activates the ATP-dependant potassium channels (KATP channels) resulting in depolarization of the cell membrane and calcium entry into the cell. This leads to insulin vesicle exocytosis. Furthermore, amino acids, parasympathetic nerve stimulation and gut hormones can also stimulate insulin secretion from beta cells. Insulin is rapidly transported to the liver where it exerts its effects by binding to the insulin receptor to activate a cascade of signalling events which culminate in the phosphorylation and activation of glycogen synthase. This results in an increase in glycogen production and a lowering in plasma glucose. Plasma glucose is also influenced by insulin concomitantly inhibiting lipolysis, gluconeogenesis and activating protein synthesis. In the periphery, insulin also leads to an increase in the number of plasma membrane GLUT4 molecules (Pessin and Saltiel 2000). It has long been known that gut-derived factors can stimulate endocrine secretions from the islets of Langerhans following nutrient ingestion (Bayliss and Starling 1902). This endocrine effect is highlighted when oral glucose stimulated insulin secretion is compared to intravenous glucose infusion (Elrick et al 1964). This is termed the 'incretin effect'. The incretin effect accounts for between 50 and 70 % of total insulin secretion following an oral glucose administration. Two major incretins have been characterized glucose-dependant insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP- 1) (Drucker 2006).

Insulin was first implicated in the control of body weight in the 1970's (reviewed by Figlewicz DP 2008). Plasma insulin is proportional to body fat content. The saturable relationship between CNS and plasma insulin is consistent with a receptor-mediated transport process (reviewed by Schwartz MW et al 2000). Central insulin levels in the cerebrospinal fluid are decreased in obesity, therefore chronic peripheral hyper-insulinaemia in obesity will lead to less adiposity signaling in the CNS (reviewed by Figlewicz DP 2008). It is also known that functional insulin receptors are expressed in the homeostatic and reward pathways including the hypothalamus, hippocampus and amygdala (reviewed by Figlewicz DP 2008). The central administration of leptin and insulin leads to a reduction in food intake and body weight (reviewed by Figlewicz DP 2008). As with leptin, insulin serves as a humoral signal in a negative-feedback loop linking feeding behaviour to adiposity (reviewed by Figlewicz DP 2008). Insulin

mediates its central effects through the activation of key hypothalamic nuclei to regulate energy balance. Central insulin administration is able to modify behaviors that reflect acute and learned reward evaluation. High-fat diet can lead to impairment in centrally administered insulin's ability to maintain body weight (reviewed by Figlewicz DP 2008). Further direct administration of insulin to the reward pathway is able to reverse feeding of palatable foods (reviewed by Figlewicz DP 2008).

Some point to the difficulties in separating out the central effects of insulin and leptin (Michael W. Schwartz et al 2000). They point out that as insulin promotes both fat storage and leptin synthesis it is difficult to tease out their independent influences. Further, weight gain cannot occur when insulin deficiency is present, even if food is consumed in large amounts as this leads to loss of calories through renal excretion (Schwartz M W et al 2000). However, the fact that leptin deficiency causes severe obesity, with hyperphagia that persists despite high insulin levels has led some to argue that insulin does not play as important part as leptin (Schwartz M W et al 2000). Also obesity is not induced by insulin deficiency. A recent study sought to clarify this issue by conducting a study in diabetic hyperphagia. This study selectively replenished leptin (but not insulin) to non-diabetic levels in an animal model of uncontrolled, insulin-deficient diabetes. The finding that this intervention prevented diabetic hyperphagia has added further weight to the conclusion that leptin deficiency, but not insulin, is required for hyperphagia (Schwartz M W et al 2000). Therefore current evidence points to leptin having a critical role in obesity. However both leptin and insulin participate in the CNS control of energy homeostasis (Schwartz MW et al 2000).

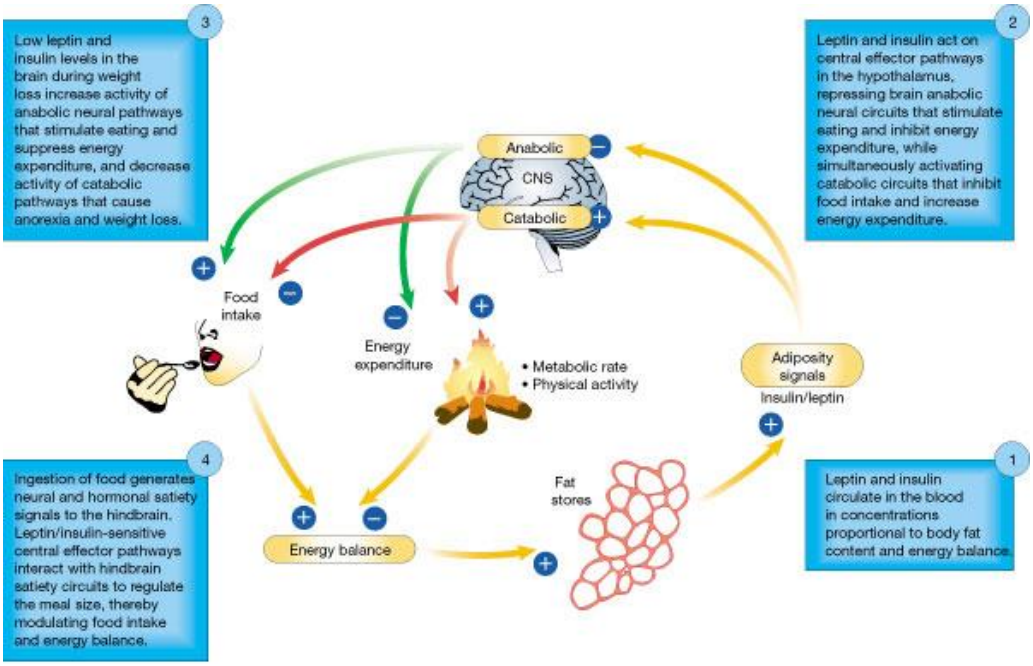


Figure 7; the central role of leptin and insulin in energy balance is displayed in this schematic diagram. Leptin and insulin are secreted in proportion to body fat stores. They act on the homeostatic (hypothalamus and brainstem) and reward pathways to modulate energy balance. A reduction in adiposity leads to compensatory changes in anabolic and catabolic pathways. This in turn maintains adiposity. This diagram was reproduced from a recent publication by Schwartz and colleagues (Schwartz MW et al 2000).

1.14.3 Ghrelin

Ghrelin is a 28 amino acid peptide formed by cleavage of precursor, pre-proghrelin. It is released from X/A-like cells of the gastric oxyntic glands. Lower levels of ghrelin are also expressed in the small intestine and hypothalamus (Kojima et al 1999). The biologically active acyl-ghrelin has post-translational modification on the third serine amino acid with a medium chain fatty acid molecule, typically octanoate (Kojima et al 1999). Gastric O-acyl transferase (GOAT) enzyme is responsible for this acylation (Yang et al 2008). Ghrelin is degraded by butyrylcholinesterase and lysophospholipase (De Vriese et al 2004). Acyl-ghrelin is extremely labile in the circulation and is readily degraded by endogenous non-specific enzymes including esterases (e.g. butyrylcholinesterase) and phospholipases (e.g. lysophospholipase 1) resulting in the production of des-acyl ghrelin, the likely inactive yet more abundant form (Hosoda et al 2000). Ghrelin stimulates food intake, and is thought to be a putative meal initiator (Cummings et al 2001, Callahan HS et al 2004). It has been shown to stimulate short

term food intake in animal and human studies, and even in obese individuals (Cummings D E 2006). Ghrelin is released in a pulsatile manner. Fasting leads to an increase in plasma ghrelin levels, in anticipation of meals and then decline with feeding. Further, the timing of plasma ghrelin peaks can be altered by modifying eating habits (Drazen et al 2006).The diurnal variation in ghrelin secretion is related to meal times and sleep (Figure 8) (Shiiya T et al 2002). Plasma ghrelin levels decline in proportion to calorie ingestion` (Callahan HS et al 2004), and correlates to the inter-meal interval (Blom WA et al 2009), but is not altered by ingestion of water illustrating that gastric distension does not regulate the post-prandial decrease in circulating ghrelin (Tschop et al 2000). Plasma ghrelin levels peak immediately before meals and fall to a nadir about an hour after feeding (Callahan HS et al 2004) (Figure 8). Circulating plasma ghrelin reflects recent food intake, thus circulating ghrelin acts as a short term signal of energy stores or energy gains, to hypothalamic neurons that govern food intake (Callahan HS et al 2004, Palmiter RD 2007, Lenard NR and Berthoud HR 2008). The elevation in pre-prandial ghrelin concentrations strongly correlate with subjective hunger scores (Cummings et al 2004).

The raised ghrelin levels seen during the first hours of sleep are thought to promote growth hormone secretion and contribute to the promotion of slow wave sleep (Dzaja et al 2004). Interestingly, lack of sleep has been associated with increased ghrelin, decreased leptin and a higher BMI (Taheri et al 2004). Only acyl-ghrelin binds to GHS-R1a (growth hormone secretagogue receptor) and cross the blood–brain barrier (Banks et al 2002). GHS-R1a is present in many tissues (Hosoda et al 2006); further, the appetite-stimulating effects of ghrelin are thought to be mediated, in part by neurons within the arcuate nucleus of the hypothalamus (Nakazato et al 2001 and Tamura et al 2002). Tamura and colleagues also demonstrated total abolition of ghrelin's effect on food intake by ablating the arcuate nucleus; however the brainstem and vagus nerve are also important in mediating ghrelin-induced food intake (Williams et al 2003 and le Roux et al 2005a).

Ghrelin is the only gut hormone known to increase food intake, and weight-gain over a week (Tschop et al 2000). Intravenous infusion of ghrelin increased calorie intake at a buffet meal by 28% in healthy volunteers (Wren et al 2001), Ghrelin secretion is dysregulated in obese individuals (Tschöp M et al 2001, Cummings DE et al 2002). Diet-induced weight loss leads to a compensatory rise in ghrelin (Tschöp M et al 2001, Cummings DE et al 2002). This rise in ghrelin is thought to contribute to the rebound weight gain by stimulating appetite (Shiiya T et al 2002, Cummings DE et al 2002). Conversely anorexic individuals have abnormally high ghrelin concentrations which

decline following weight gain (Otto et al 2001). High ghrelin level precedes the development of obesity in Prader-Willi syndrome (Cummings et al 2002a). Furthermore, genetic abnormalities in the *ghrelin* gene and its receptor have also been identified and are associated with both risk and protection from obesity respectively (Hinney et al 2002, Korbonits et al 2002, Ukkola et al 2002, and Wang et al 2004). Circulating plasma ghrelin concentration is negatively correlated with bodyweight in both rodents (Moesgaard et al 2004, Qi et al 2008) and humans (Cummings et al 2002b, Haqq et al 2003, Shiiya et al 2002, Tschop et al 2001). Plasma ghrelin has been inversely correlated with BMI; obese individuals have low circulating ghrelin levels; they do not show the typical ghrelin spikes throughout the day coincident with meal times and ghrelin levels do not fall rapidly in response to a meal (Shiiya et al 2002, le Roux et al 2005b), suggesting a role for ghrelin in the long-term regulation of body weight (Tschop et al 2001). The lower ghrelin in obesity was thought to suggest greater sensitivity, therefore despite low endogenous levels, blocking its activity could still lead to an anorectic effect. The reduction in 24-hour ghrelin levels concomitant with a profound reduction in hunger after bariatric by-pass surgery is supportive of this (Cummings et al 2002 and le Roux et al 2007), and the converse seen after diet-induced weight-loss is thought to be responsible for the increased appetite that promotes weight regain (Cummings et al 2002). Blocking ghrelin-induced food intake in individuals on a diet may help to sustain weight-loss.

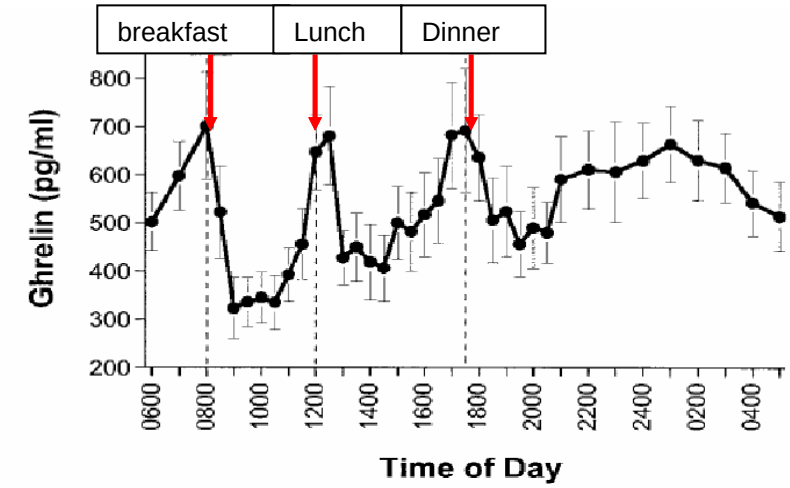


Figure-8, Temporal profile of ghrelin through the day (Cummings D E et al 2001). Circulating levels of ghrelin peak prior to a meal and reach a nadir within an hour.

Ghrelin's influence on appetite is thought to be mediated through the bloodstream and not through the vagal afferents, after a careful analysis of results from rats with selective vagal de-afferentation (Arnold et al 2006, Lenard and Berthoud 2008). The

majority of studies suggest that ghrelin regulates appetite via central mechanisms (Nakazato et al 2001). Only the acylated form is able to cross the blood brain barrier and bind to the specific ghrelin receptor, GHSR1a (Banks et al 2002). Peripheral injection of ghrelin alters *c-fos* expression in the NTS (Lawrence et al 2002), and in the ARC. The chemical ablation of ARC neurons abolishes the orexigenic effects of peripheral ghrelin administration (Tamura et al 2002). A recent functional imaging study has also implicated ghrelin in brain activity in reward centres such as the OFC, ventral tegmental area (VTA), insular and amygdala (Malik et al 2008). In addition, ghrelin administration has been shown to reduce metabolic rate and increase fat deposition (Kawakawa et al 2001, Tschop et al 2000). Ghrelin activates dopamine neurons in the reward pathway and stimulates food intake when locally administered (Jerlhag et al 2007 and Balleine 2007, Palmiter RD 2007), and ghrelin receptor blockade in the reward pathway blunted feeding following fasting (Jerlhag et al 2007), suggesting that the orexigenic actions of ghrelin are at least in part mediated by the dopaminergic reward pathway. Intravenous ghrelin administration increased neural response to images of food in the above areas (Malik et al 2008). Further, activity in these areas correlated with hunger. Thus ghrelin may promote food consumption by enhancing hedonic responses to food cues (Malik et al 2008). The exact interaction between these regions of the brain to bring about ghrelin-induced feeding is still unclear. An integrated complex neuro-humoral pathway is thought to mediate feeding behavior (Lenard and Berthoud 2008). The high ghrelin and low leptin seen in the fasting state will sensitize the dopaminergic reward pathway to “go for” the food represented and retrieved from memory. Once food is consumed, the drop in ghrelin and the rise in leptin, PYY3-36, GLP-1, and amylin mediate meal termination, and satiety maintained for a certain time beyond the end of nutrient absorption by PYY3-36 and GLP-1. The cycle repeats itself when glucose availability diminishes, until eventually, fat oxidation returns. Ghrelin also appears to play a role in glucose homeostasis. Several studies have demonstrated an inverse relationship between fasting ghrelin and fasting insulin levels (Purnell et al 2003). Additionally, insulin resistance and T2DM are associated with reduced fasting total ghrelin levels, (Poykko et al 2003) a correlation that has even been shown to exist independently of bodyweight (McLaughlin et al 2004). It appears that intravenous administration of ghrelin improves glucose disposal *in vivo* through peripheral insulin sensitization (Heijboer et al 2006), but curiously has been shown to inhibit insulin secretion both *in vivo* and *in vitro* (Dezaki et al 2008, Reimer et al 2003). Ghrelin increases gastric motility and gastric emptying and intestinal transit (Masuda et al 2000). The role of ghrelin in glucose homeostasis is unclear but may provide therapeutic potential for T2DM in the future.

Several strategies targeting the ghrelin axis have been explored for the treatment of obesity. Firstly, ghrelin receptor (GHSR1a) antagonism reduced food intake and bodyweight in obese rodents (Asakawa et al, 2003a, Beck et al 2004). However, as yet these findings have not been replicated in man (Halem et al 2004). Secondly, ghrelin specific RNA-spiegelmers (stable oligonucleotides which bind acyl-ghrelin) are being developed and have been shown to reduce food intake and bodyweight in rodents (Helmling et al 2004, Kobelt et al 2006 and Shearman et al 2006). However the efficacy of these compounds in humans is yet to be realised. Thirdly, an anti-ghrelin vaccine has been trialed with promising results in animals but no effect on obese humans despite strong antibody responses (Cytos Biotechnology 2006, Vizcarra et al 2007, Zorrilla et al 2006). Finally, the recent discovery of GOAT presents a new treatment target through inhibition of acylation and hence activation of ghrelin. A recent study suggests inhibition of GOAT is possible by administration of octanoylated pentapeptides *in vitro* (Yang et al 2008b).

1.14.4 Peptide tyrosine tyrosine

PYY has a tyrosine residue (amino acid abbreviation, Y) at each terminus of the 36 amino acid polypeptide, and was first isolated from porcine intestine in 1980. It is primarily synthesized and secreted from the entero-endocrine L-cells in the distal GI tract (Tatemoto and Mutt 1980). It has also been identified in the pancreas and brainstem (Adrian et al 1985a). Nutrient content and neural reflexes are thought to govern secretion (Anini et al 2002, Herrmann et al 1995a, and Herrmann et al 1995b). PYY, neuropeptide Y (NPY) and pancreatic polypeptide (PP) are members of the pancreatic polypeptide-fold family of peptides, and share a common structure consisting of a hairpin-fold motif which mediates receptor binding (Fuhlendorff et al 1990b). The NPY family of peptides bind to the NPY Y-subtype of G-protein coupled receptors with varied affinity. PYY1-36 is synthesized by the L-cells throughout distal gut. It undergoes cleavage of tyrosine and proline from the N-terminus of PYY1-36 to produce PYY3-36 (Grandt D et al 1992); the major circulating form of PYY in the fed and fasted state (Batterham et al 2006 and Korner J et al 2006). Several studies have demonstrated that DPP-IV is responsible for this cleavage (Grandt et al 1993, Mentlein et al 1993, Unniappan et al 2006), The process of elimination of PYY3-36 is unknown at present. Circulating levels of PYY begin to rise within 15 minutes of a meal, continue to increase over the next 2 hours, and reach peak levels at 1-2 hours after the meal, and remains elevated for several hours (Adrian et al 1985). This temporal profile with a sustained elevation of PYY post-meal ingestion suggest PYY is a satiety factor, in other words it is involved in delaying the next meal rather than acting as a meal terminator.

Circulating concentrations of PYY reflect calorie intake as well as macronutrient composition (Adrian et al 1985, Batterham et al 2006, Essah et al 2007, Helou et al 2008).

Exogenous administration of PYY3-36 leads to a dose-dependent reduction in food intake (Batterham et al 2002). PYY null animals exhibit hyperphagia resulting in significantly increased bodyweight and a marked elevation in body fat percentage (Batterham et al 2006). The initial controversy surrounding the anorexigenic functions of PYY3-36 (Tschop et al 2004), has been resolved following the replication of the original findings in rodents (Challis et al 2003, Cox and Randich 2004, Halatchev et al 2004, Martin et al 2004, Pittner et al 2004), primates (Koegler et al 2005) and humans (Degen et al 2005, le Roux C W et al 2006, Sloth et al 2007). Furthermore, chronic administration of PYY3-36 to PYY null animals led to a reversal of the obese phenotype (Batterham et al 2006). PYY over expression protects against diet-induced obesity, and genetic obesity (Boey et al 2008). In humans, peripheral administration of PYY3-36 reduces appetite and food intake in both lean and obese subjects (Batterham et al 2003a). PYY1-36 also reduces food intake but in an order of magnitude less potent than PYY3-36 (Chelikani et al 2004, DeCarr et al 2007). It is thought that the inhibitory effects of PYY1-36 on food intake occur following truncation of the peptide *in vivo* (Chelikani et al 2004, DeCarr et al 2007). These findings demonstrate that PYY plays a critical role in the regulation of food intake. The role of the vagus nerve in mediating the central effects of PYY is ambiguous. It is known that vagotomy abolishes the anorexigenic effects of PYY3-36 (Abbott et al 2005a, Koda et al 2005). However, others have reported the opposite (Halatchev and Cone 2005). At the cellular level anorectic effects of PYY3-36 are thought to be mediated by the Y_2-receptor, found throughout the CNS and vagal afferents (Batterham et al 2002, Abbott et al 2005). Peripheral administration of PYY3-36 up-regulates c-*fos* expression (a marker of neuronal activation) in hypothalamic feeding centres including the arcuate nucleus (Batterham et al 2002, Challis et al 2003). The administration of PYY3-36 directly into the ARC causes a dose-dependent decrease in food intake. This is ablated in PYY Y2 receptor (Y2R) knock-outs, and in pharmacological antagonism of the Y2R, suggesting that Y2R mediates these effects (Abbott et al 2005b, Batterham et al 2002). Radio-labelled PYY accumulates in the median eminence and area postrema suggesting PYY does also influence brainstem circuits (Dumont et al 2007). Recent evidence points to PYY3-36 activating non-homeostatic areas. In this functional magnetic resonance imaging study (fMRI), PYY3-36 was infused to mimic fed-state concentrations. This led to significant modulation of brain activity in areas involved in reward processing, such as: the orbital frontal cortex (OFC), ventral tegmental area (VTA), insula and globus

pallidus (Batterham et al 2007). Furthermore, post-prandial PYY3-36, and concomitant changes in neural activity in the OFC predicted subsequent feeding behaviour. This has led some to propose that the regulation of appetite has switched from homeostatic to hedonic control. The subjective hunger scores strongly correlate with changes in plasma PYY concentrations both in a post-prandial setting (Guo et al 2006, Stoeckel et al 2007) and during infusion of PYY (Batterham et al 2007).

Animals exposed to a high-fat diet have reduced PYY levels (le Roux et al 2006, Rahardjo et al 2007, and Yang et al 2005). In humans, bodyweight is negatively correlated with plasma PYY levels in adults (Alvarez Bartolome et al 2002, Batterham et al 2003a, Guo et al 2006, le Roux et al 2006 and Sodowski et al 2007). However not all studies concur (Kim et al 2005, Korner et al 2006, Pfluger et al 2007). Obese individuals have an attenuated post-prandial PYY secretion (Batterham et al 2003a, le Roux et al 2006). The converse is also true in patients with anorexia, where an elevated fasting and post-prandial PYY is seen (Misra et al 2006, Nakahara et al 2007). Stress can increase plasma PYY3-36 levels, and must be controlled for when measuring PYY in animals and humans (Chandarana et al 2009).

In contrast to leptin, obese individuals retain sensitivity to PYY3-36, raising the possibility of therapeutic potential (Batterham et al 2003a). Therefore pharmacological therapies aimed at the modulation of the PYY-Y2R axis are under development. Trials on modified PYY molecules that are potent Y2R agonists have demonstrated reduced feeding and a reduction in bodyweight (Balasubramaniam et al 2007, Ortiz et al 2007). PYY and glucagon-like peptide-1 (GLP-1) are co-secreted by entero-endocrine cells in the lower small intestine and colon. PYY3-36 combined with GLP-1 or GLP-1R agonist synergistically reduces food intake in humans and rodents (Neary et al 2005, Talsania et al 2005). Trials on the PYY3-36 nasal spray were terminated despite encouraging reduction in food intake and weight loss due to a lack of efficacy above current available treatments (MDRNA, 2008).

1.14.5 Glucagon like peptide-1

GLP-1 is made by the post-translational modification of pre-proglucagon precursor polypeptide. The tissue-specific activities of pro-hormone convertases 1 and 2 direct the differential cleavage of pre-proglucagon. These convertase enzymes are expressed in the α-cells of the pancreas, L-cells of the intestine and also within the CNS. The pattern of enzyme expression determines hormone synthesis. GLP-1 is secreted primarily from entero-endocrine L-cells in two forms: GLP-1(1-37) and GLP-1(1-36) amide and cleaved at the N-terminus to form the biologically active fragments GLP-1(7-

37) and GLP-1(7-36) amide (Mojsov et al 1986). GLP-1(7-36amide) is thought to be present in greater concentration but both isoforms are equipotent (Orskov et al 1994). GLP-1 is rapidly degraded by the ubiquitously expressed aminopeptidase dipeptidyl-peptidase IV (DPP-IV). DPP-IV is expressed in endothelial and epithelial tissues. The enzyme selectively cleaves N-terminal dipeptides (Lambeir et al 2003), and in the case of GLP-1 leading to inactive metabolites GLP-1(9-37) or GLP-1(9-36amide) (Deacon et al 1995). A significant amount of GLP-1 is degraded to the inactive metabolite before leaving the gut (Hansen et al 1999), with further degradation in the liver (Deacon 2005). Therefore it is thought that only a small proportion of intact active GLP-1 reaches the systemic circulation. Therefore DPP-IV inhibitors should be added to blood samples to prevent degradation of active GLP-1 after collection. Luminal nutrients are thought to be the main stimulus for GLP-1 release. Plasma GLP-1 is noted to rise 10 min after a meal and peak at 30 min after a meal. Plasma GLP-1 levels remain elevated for several hours (Orskov et al 1996, Vilsboll et al 2003). Quickly absorbed proteins lead to a greater GLP-1 response (Herrmann et al 1995). A quicker luminal passage has been linked to GLP-1 secretion. Entero-endocrine L-cells are located throughout the GI tract and the presence of nutrients in the proximal GI tract also stimulates GLP-1 release (Roberge and Brubaker 1991, Eissele et al 1992). The processes underlying GLP-1 synthesis and secretion from the L-cell are not unknown. Vagotomy abolishes nutrient-stimulated GLP-1 from the proximal GI tract suggesting a neural control mechanism directing GLP-1 secretion (Rocca and Brubaker 1999). GLP-1 has a short half-life (~ 2 min) and therefore peripheral and vagal GLP-1 receptors may play a substantial role in mediating the anorectic effects of GLP-1. It is possible that GLP-1 activates receptors before entering the local capillary network. GLP-1 evokes action potentials in vagal afferent neurons (Nakagawa et al 2004 and Kakei et al 2002). Lesions on the neuronal projections linking the brainstem to the hypothalamus lead to a decline in the anorectic effects of GLP-1, implicating these in the relaying of anorectic signals to the hypothalamus (Abbott et al 2005).

GLP-1 activates the GLP-1 receptor (GLP-1R), a G-protein coupled receptor widely expressed in pancreatic islets, brain, heart, kidney and throughout the GI tract (Bullock et al 1996). GLP-1 may act directly on gastric GLP-1 receptors to delay gastric emptying (Young et al 1996 and Naslund et al 1999, Nauck et al 1997). Centrally, the afferent nodose ganglion is known to be activated and in turn sending impulses to the NTS and hypothalamus (Holst and Deacon 2005). Peripherally and centrally administered GLP-1 lead to a reduction in food intake (Tang-Christensen et al 1996 and Turton et al 1996). Global deletion of the GLP-1R led to impaired glucose tolerance (Scrocchi et al 1996). The activation of GLP-1R in the hepatic portal vein augments the insulin response through a neural reflex (Balkan and Li 2000). It is thought that active

GLP-1 at lower concentrations activates neural pathways, and at higher concentrations activates islet and CNS receptors. Intra-cerebroventricular administration of GLP-1 leads to c-*fos* expression in the hypothalamus and inhibited feeding (Turton et al 1996). Peripheral injection of GLP-1 led to c-*fos* expression in the brainstem, also an important site of action (Baggio et al 2004). Further, central GLP-1 receptor blockade, doubled food intake in satiated rats (Turton et al 1996).

Exogenous GLP-1 directly stimulates insulin secretion from isolated islets in vitro (Fridolf and Ahren 1991). Furthermore, studies on isolated islets have displayed that GLP-1 potentially inhibits apoptosis and promotes cell proliferation (Drucker 2003). GLP-1 acts as an incretin, enhancing glucose-stimulated insulin release. The meal associated increase in GLP-1 is responsible for the glucose-dependant insulinotropic effect (Holst et al 1987, Kreymann et al 1987). Conversely, blockade of the GLP-1R by GLP-1R antagonist exendin 9-39, significantly impairs glucose tolerance (Kreymann et al 1987, Tseng et al 1999, Wang et al 1995). GLP-1 stimulates insulin gene transcription and biosynthesis (Drucker et al 1987), and improves α-cell glucose sensing leading to inhibition of glucagon secretion from α-cells (Byrne et al 1998, Orskov et al 1988).

The exact mechanisms underlying GLP-1 central effects remain unclear (Tang-Christensen et al 1996). Intra-cerebro ventricular (ICV) and PVN administration of active GLP-1 in rodents leads to a decrease in food intake, and chronic administration leads to a reduction in bodyweight (Meeran et al 1999, Turton et al 1996, Verdich et al 2001a). Interestingly deletion of GLP-1R does not lead to obesity reflecting the redundancy in this signalling pathway (Scrocchi et al 1996). Studies in humans have demonstrated that obesity is associated with reduced fasting and post prandial total GLP-1 secretion (Holst et al 1983, Ranganath et al 1996, Verdich et al 2001b). However, not all studies are in agreement (Laferrere et al 2007) and one study has even reported hyper-secretion in obese individuals (Fukase et al 1993). Furthermore, when weight-matched patients with T2DM, are compared to their obese counterparts, the incretin effect is severely reduced in T2DM patients, and thought to contribute to the pathogenesis of the disease (Nauck et al 1986). Patients with T2DM display a dose dependent response to exogenous GLP-1 (Kjems et al 2003). Glucose-induced insulin secretion can be normalized with infusion of GLP-1 in subjects with T2DM (M A Nauck et al 1993). A six week infusion of GLP-1 in patients with T2DM led to a significant improvement in HbA1c and an associated weight loss (Zander et al 2002). A naturally occurring GLP-1R agonist was isolated from the saliva of the Gila monster (*Heloderma suspectum*) and led to the drug exenatide being developed (Byetta; Eli Lilly, IN, USA). Exenatide has a half-life 30 times longer than GLP-1 (60 - 90 minutes) (Buse et al

2004). It is used in the treatment of T2DM. However, exenatide can lead to nausea; this together with the sub-cutaneous route of administration can lead to poor compliance. An acylated, albumin-bound long-acting GLP-1 analogue named Liraglutide (Novo Nordisk, Denmark) has now been developed. Liraglutide shows 97% similarity to native human GLP-1. It has a half-life of up to 14 hours (Agerso et al 2002). An alternative approach to extend the half life of GLP-1 is inhibition of DPP-IV. Vildagliptin and Sitagliptin are examples of DPP-IV inhibitors that are currently in use for the treatment of T2DM. The therapeutic effects of DPP-IV inhibitors are similar to incretin mimetics, although weight loss is not a common feature with this class of drugs (Aaboe et al 2008).

1.14.6 Amylin

Amylin is a 37 amino acid peptide produced by the β-cells in the islets of Langerhans. It is cleaved from a precursor and amidated at the C-terminal. It is co-released with insulin in a molar ratio (100:1, insulin: amylin). This molar ratio can be perturbed in T2DM or obesity (Butler et al 1990, Moore and Cooper 1991). The meal-stimulated amylin profile mirrors that of insulin (Butler et al 1990), Amylin is degraded by the insulin-degrading enzyme (Shen et al 2006). Amylin binds to the calcitonin receptor. Tissue specificity is conferred by receptor activity modifying proteins (RAMPs) binding to the receptor (chen et al 1997). The central anorectic effects of amylin appear to be mediated through the nucleus tractus solitarius (NTS) and area postrema in the brainstem (Riediger et al 2001), and the hypothalamus (Clline et al 2008). Vagotomy does not abolish the anorectic effects of amylin (Morley and Flood 1991). Amylin inhibits gastric emptying (Young et al 1996) and glucagon secretion (Silvestre et al 2001). The latter makes amylin a T2DM drug candidate. Peripheral amylin administration led to a reduction in food intake in normal fed and food-deprived mice. This reduction was independent of diabetes status (Morley and Flood 1991). Centrally administered amylin reduced 24-hour food intake by up to 30% in a week (Rushing et al 2000). The temporal profile of amylin, and its ability to reduce food intake, support a role for amylin in satiety. Obese subjects exhibit fasting hyper-amylinaemia, conversely obese individuals with impaired glucose tolerance or diabetes exhibit lower fasting levels. However, both diabetic and non-diabetic obese subjects demonstrate a post-prandial increase in circulating amylin levels. This temporal profile is thought to be mediated by hyperglycaemia and cortisol (Hartter et al 1991 and Thomaseth et al 1997). It is thought that an increased ratio of amylin/ insulin expression may act as a marker for beta cell dysfunction (Weng HB et al 2008). Hyperglycaemia is thought to lead to the hyper secretion of amylin relative to insulin, and increase the amylin /insulin ratio in insulin-resistant rats (Leahy JL et al 1998). GLP-1 (7–36) stimulates the

expression and secretion of amylin, whilst also increasing insulin protein expression in GK rats treated with GLP-1. Amylin has multiple physiologic effects on glucose homeostasis (Karlsson E 1999, Nyholm B et al 1999), including making GLP-I a less effective stimulus for insulin secretion (reviewed by Cluck MW et al 2005). It has been proposed that the amylin/ insulin ratio may be a better measure than the absolute amylin mRNA level (Weng HB et al 2008). Whilst the promoter elements and transcription factors that regulate rat and human insulin gene expression have been described, amylin gene expression is not well characterized (reviewed by Cluck MW et al 2005). The amylin promoter does contain elements similar to those present in insulin genes. Therefore a mechanism for parallel gene expression may exist (reviewed by Cluck MW et al 2005). It is thought that separate transcription factors regulate independent transcription of amylin and insulin (reviewed by Cluck MW et al 2005). Several transcription factors such as HNF-1 are now implicated in the selective expression of the amylin gene (reviewed by Cluck MW et al 2005). Under normal physiological conditions amylin and insulin are regulated in concert, but in pathological states such as diabetes and obesity their regulation may diverge (reviewed by Cluck MW et al 2005). Second messengers utilised by GIP/ GLP-1, calcium and fatty acyl molecules can differentially regulate amylin, insulin secretion, and gene expression (reviewed by Cluck MW et al 2005). Amylin and insulin mRNA content does increase in parallel following glucose challenge (Mulder H et al 1996). Supra-physiologic levels of exogenous amylin inhibit glucose-induced insulin secretion in humans, (Bretherton-Watt D et al 1992). Insulin secretion is inhibited by amylin both in vitro and in vivo, (Gebre-Medhin S et al 1998, Wang ZL et al 1993 and reviewed by Cluck MW et al 2005). Also recent studies have highlighted a role for amylin therapy in obesity (Ravussin E et al 2009, Smith SR et al 2008).

1.15 Treatments for obesity

1.15.1 Lifestyle Intervention

Pharmacotherapy and lifestyle intervention programs to modify diet, feeding behavior and encourage exercise, are widely used in various combinations to treat obesity (Guntram and Morton 2008). These approaches to managing obesity achieve long term weight loss in only a small minority of highly motivated individuals (Mark 2008). Clinically significant weight loss is generally very modest and transient, particularly in patients with severe obesity (Guntram and Morton 2008). Poor compliance and high rates of relapse are thought to be driven by the homeostatic mechanisms that exist to maintain bodyweight (Heymsfield et al 2003, Leibel et al 1995, Tsai and Wadden 2005). However, when patients with a BMI >40 kg/m^2 were willing to complete an intensive behavioral program they did achieve a remarkable weight loss of 35% of

initial weight after 40 weeks (Anderson et al 2006*),* but The long-term maintenance of this weight loss was difficult for most (Anderson et al 2006*)*.

1.15.2 Pharmacological treatments

Orlistat, the only pharmacological weight loss therapy available in Europe for the treatment of obesity has limited efficacy and is associated with undesirable side effects. It is a bacterial enzyme that blocks pancreatic lipase. This leads to a reduction in triglyceride digestion (reviewed by Bray GA and Tartaglia LA 2000). Orlistat blocked digestion of 30% of triglyceride when taken as 120 mg three times daily. Orlistat therapy leads to about 9–10% weight loss in a year (reviewed by Bray GA and Tartaglia LA 2000). However, diabetics lost less, reaching 6% after one year. It is thought that orlistat may increase postprandial GLP-1 levels by an increase in intestinal fat content, thereby enhancing the insulin secretory response to the meal and improve weight loss (Tanner et al 2004). Clinically orlistat leads to a modest weight loss in patients who tolerate it, and can only be prescribed for short periods of time (Rosenbaum et al 1997). The side effects of Orlistat therapy include oily spotting, faecal urgency and increased defecation. These side effects decline over time (reviewed by Bray GA and Tartaglia LA 2000). A study of 80 adults with mild to moderate obesity (BMI 30–35 kg/m^2) compared lifestyle intervention with pharmacotherapy (very-low-calorie diet, orlistat, and lifestyle change) to surgical intervention (gastric banding). Surgical treatment was significantly more effective than nonsurgical therapy in reducing weight, resolving the metabolic syndrome, and improving quality of life during a 24-month treatment program (O'Brien et al 2006*)*. At 2 years, the surgical group had greater weight loss- 21.6% of initial weight, whereas the nonsurgical group had only a 5.5% of initial weight loss. The XENDOS study demonstrated that orlistat accompanied with lifestyle changes was able to significantly reduce the incidence T2DM over 4 years through weight loss when compared to lifestyle changes alone (Torgerson JS et al 2004). This was through halting progression from impaired glucose tolerance. (Torgerson JS et al 2004) The comparator group also lost a meaningful amount of weight over the 4 years. A relative risk reduction of 37.3% was noted with Orlistat. The mean weight loss after 4 years was significantly greater with Orlistat (Torgerson JS et al 2004). Orlistat 120mg is FDA-approved for use in adults and adolescents age 12–16y. The increase in undigested stool triglycerides may cause considerable gastrointestinal adverse effects (reviewed by Yanovski SZ and Yanovski JA 2014).

Recent reviews on new drugs to treat obesity point to clinically meaningful weight loss with Orlistat, lorcaserin, and phentermine/topiramate-ER when combined with lifestyle intervention over a year (reviewed by Yanovski SZ and Yanovski JA 2014). The

comparison to placebo has ranged from ~3% for orlistat and lorcaserin to 9% for phentermine/topiramate-ER at one year. However, it is noted that the risks may out weigh the benefits for those that do not lose weight, and in some cases pose a cardiovascular risk in susceptible individuals (reviewed by Yanovski SZ and Yanovski JA 2014). The teratogenic risk and regular pregnancy tests whilst on phentermine/topiramate-ER must be weighed against the weight loss benefits (reviewed by Yanovski SZ and Yanovski JA 2014). The use of antidepressants is common in the obese population and the interaction with Locaserin may lead to serotonin syndrome (reviewed by Yanovski SZ and Yanovski JA 2014). Phentermine is low cost and has many years of clinical experience, but long-term effects on cardiovascular outcomes are unknown (reviewed by Yanovski SZ and Yanovski JA 2014).

A naltrexone-SR and bupropion combination drug is currently undergoing late-phase safety trials to assess cardiovascular risk (reviewed by Yanovski SZ and Yanovski JA 2014). Trials point to 5-10% weight loss at 1 year, varying with intensity of lifestyle intervention (reviewed by Yanovski SZ and Yanovski JA 2014).
phentermine/ topiramate-ER at the top dose led to a mean loss of 10.9% at a year against 1.6% with placebo (reviewed by Yanovski SZ and Yanovski JA 2014). However, 31% of participants withdrew. This is an area of considerable concern (reviewed by Yanovski SZ and Yanovski JA 2014). Women with childbearing potential should have a negative pregnancy test prior to starting phentermine/topiramate-ER and monthly thereafter (reviewed by Yanovski SZ and Yanovski JA 2014).

Lorcaserin is a selective serotonin 2C receptor agonist. It was approved in 2012 following two large randomized, placebo-controlled trials in nondiabetic patients (reviewed by Yanovski SZ and Yanovski JA 2014). There was 3.2% weight loss in comparison to placebo. The average weight loss of 5.6 kg was noted over 2 years (reviewed by Yanovski SZ and Yanovski JA 2014). Diabetics treated with lorcaserin had lower body weight and improved glycated hemoglobin concentrations. The FDA has requested a post-approval trial to assess long-term valvulopathy and hypertension (reviewed by Yanovski SZ and Yanovski JA 2014).

Further novel treatments options are keenly anticipated (Neary and Batterham, 2009). Others are less optimistic (Ledford H 2010). A recent review of drugs considered for approval by the food and drug administration (FDA) authority paints a bleak picture (Ledford H 2010). It is argued that appetite regulatory pathways overlap with mood and other important cerebral functions leading to long periods of monitoring on therapy, in

turn leading to an increase in development costs. They site this as a cause for the difficulty in developing new therapies. The most recent drug to be recommended by the FDA is a combination of an anti-depressant and neurotransmitter. It is thought to reduce hunger by its effects on the hypothalamic neurons (Ledford H 2010). They also propose that GLP-1 agonists are the next set of drugs to be considered for obesity therapy (Ledford H 2010). GLP-1 an injectable incretin used to treat type 2 diabetes is also known to produce weight loss. A meta-analysis points to 3% weight loss at 6 to 12 months, further studies in non-diabetic obese highlights 3.5 to 5.8 kg weight loss at 6 to 12 months (reviewed by Yanovski SZ and Yanovski JA 2014). liraglutide and exenatide are currently undergoing trials for treatment of obesity. Initial results point to 6.2% weight loss at 1 year when compared to placebo, with both groups undergoing effective lifestyle advice (reviewed by Yanovski SZ and Yanovski JA 2014). Those on GLP-1 were more likely to maintain weight loss and to lose ≥5%-10% (reviewed by Yanovski SZ and Yanovski JA 2014).

1.15.3 Bariatric surgery

Bariatric medicine deals with the causes, prevention and treatment of overweight and obesity. Bariatric surgery is the only effective treatment that randomized controlled trials have shown to produce effective long term weight loss (Buchwald H et al 2004, Colquitt et al 2009, and Sjöström L et al 2007). A systematic review has concluded that surgery is superior to conventional treatment in reducing weight. However, the review failed to show the superiority of one surgical method over another (Colquitt et al 2005*).* In recent decades the use of bariatric surgery to treat obesity has evolved significantly. Current National Institute for Clinical Excellence (NICE) guidelines advise consideration for bariatric surgery where non-surgical therapies have failed in individuals with a BMI > 40 kg/m2 or with BMI 35-40 kg/m2 with other significant disease that could be improved by weight loss (NICE, 2006). All non-surgical methods should have failed to achieve or maintain clinically beneficial weight loss for at least six months. Surgery is appropriate only if the patient will receive specialist management, is fit for general anaesthesia, and is committed to long term follow-up-(NICE 2006). Surgery can be considered as a first line option in patients with a body mass index >50. Recent evidence from the United States and Australia also shows the benefit of bariatric surgery in patients with lower body mass indices (30 to 35) (Yermilov I et al 2009).

1.16 Classification of bariatric surgery

Surgical procedures are categorized by presumed mechanisms of action. Procedures are called malabsorptive, restrictive or a combination of the two. The first mal-

absorptive surgical bypass to report weight loss was done in dogs (Kremen et al 1954). The distal small intestine was connected proximally with the jejunum, resulting in profound weight loss due to malabsorption of lipids- jejunoileal bypass (JIB) (Kremen et al 1954). Jejuno-ileal bypass (JIB), duodenal–jejunal bypass (DJB) and biliopancreatic diversion (BPD) are all classed as mal-absorptive procedures. These procedures are associated with significant mal-absorption and nutritional deficits. This limits their use in clinical practice (Organ et al 1984). Restrictive procedures work by limiting stomach volume, and restrict food entry into the stomach. They include laparoscopic adjustable gastric band (LAGB). In LAGB an inflatable synthetic band device is placed below the gastro-oesophageal junction, and the degree of restriction is adjusted by injecting saline through a subcutaneous port to inflate a balloon. Vertical gastric banding (VBG) leads to restriction by combining vertical stapling of the stomach with banding. In sleeve gastrectomy (SG), a ‘‘sleeve’’ of stomach is created by stapling and removing a large part of the stomach. SG was originally described as a first-stage procedure in a staged process for patients deemed high risk for invasive surgical intervention. SG was followed by either bilio-pancreatic diversion-duodenal switch (BPDDS) or Roux-en-Y gastric bypass (RYGB) in high risk patients with a BMI >60 kg/m^2. More recently, SG has been undertaken as a stand-alone bariatric procedure as an alternative to RYGB (Bohdjalian A et al 2010). SG is no longer considered a restrictive procedure. Early results suggest that SG results in comparable weight loss and resolution of co-morbidities, but longer term outcome studies are awaited. Hybrid operations include RYGB and BPD-DS. RYGB is the most commonly performed bariatric operation and is considered the ‘‘gold standard’’ treatment for severe obesity (Buchwald et al 2004, Sjostrom et al 2007). In RYGB a small stomach is created, and connected to the Roux limb of small bowel. Bowel continuity is restored by an entero–entero anastomosis, between the biliary limb and alimentary limbs (Cummings DE et al 2004). Ingested nutrients bypass most of the stomach, duodenum and the proximal jejunum (Buchwald et al 2004). Biliary and pancreatic secretions mix with the nutrients at the site of the entero–entero anastomosis. RYGB combines mal-absorptive and restrictive elements and is more effective and successful (Sjostrom et al, 2007).

Approximately 80% of bariatric surgery patients are women In the U.S. (Santry et al 2005) and in various European countries (Favretti et al 2007*)*, as well as in New Zealand (White et al 2005*).* Women and patients from more socially deprived areas were more likely than men, and patients from more affluent areas to have bariatric surgery in NHS hospitals (Burns et al 2010). Between April 2000 and March 2008, 6953 adults had a primary elective bariatric procedure in the NHS. Of these, 3191 patients had gastric bypass, 3649 had a gastric banding procedure, and 113 patients

had a sleeve gastrectomy. There was a marked increase in the number of bariatric procedures carried out during the study period from 238 in 2000-1 to 2543 to 2007-8, and a substantial increase in the use of laparoscopy over time was also noted. In 2000, 28% (66/238) of bariatric procedures were done laparoscopically by 2007 74.5% (1894/2543) of procedures were laparoscopic (Burns et al 2010).

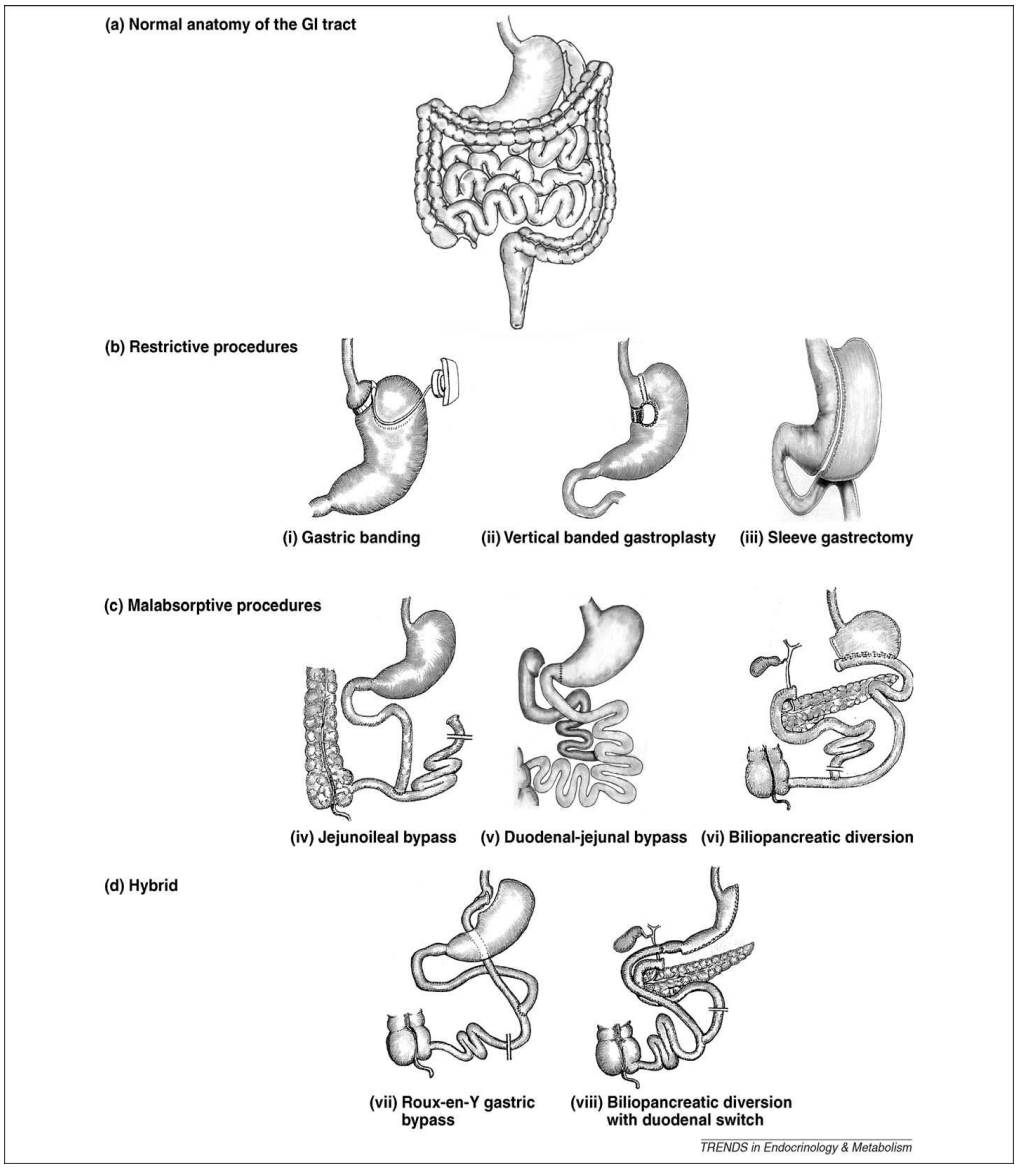


Figure-9; The GI tract anatomy (a), Restrictive procedures are shown in (b): (i) gastric banding (GB), an inflatable device is placed below the gastro-oesophageal junction, and the degree of restriction is adjusted by injecting saline through a subcutaneous port. (ii) Vertical gastric banding (VBG) a small pouch of stomach is created with a band and staples. This pouch opens into the GI tract at the lower end (iii) Sleeve gastrectomy (SG), a "sleeve" of stomach is created by stapling and removing a large part of the stomach. (c) Malabsorptive procedures (iv) Jejunoileal bypass (JIB), the jejunum and ileum are excluded from contact with food. (v) Duodenal–jejunal bypass (DJB), duodenum and jejunum are bypassed. It is an experimental procedure in humans. (vi) Biliopancreatic diversion (BPD), a section of the stomach is resected, and the smaller stomach connected to the distal gut, and the nutrients flow directly into the ileum. (d) Hybrid procedures aim to combine restriction with malabsorption. (vii) Roux-en-Y gastric bypass (RYGB) is the most widely performed procedure; the divided stomach is connected to the divided parts and the small bowel. The small bowel is divided into two and rearranged into a Y-configuration. Nutrients pass from the small stomach pouch to the jejunum via a "Roux limb". The biliary limb and the alimentary limb are connected by an entero–entero anastomosis. The bile and pancreatic secretions enter the common channel and mix with nutrients at the entero–entero anastomosis. (viii) Biliopancreatic diversion with duodenal switch (BPD-DS), A large part of the stomach is resected to create a "sleeve" of stomach, and a lengthy part of the small bowel is rerouted and re-arranged into a Y configuration. Food flows from the stomach into the shorter bowel loop, called the digestive loop and then to the common channel. Bile and pancreatic enzymes empty through the longer bowel loop, the biliopancreatic loop, and in to the common channel (Reproduced from Karra E et al 2009).

Surgical outcome is evaluated by change in weight or body mass index, or percentage loss of excess body weight and by resolution or improvement in co-morbidities. Excess body weight is calculated by subtracting the ideal weight of a patient, assuming a body mass index of 25, from his or her actual weight (Biagini and Karam 2008*).* A systematic review of bariatric surgery published in 2006 examined 43 studies, the mean percentage excess weight loss for Roux-en-Y gastric bypass was 67% at one and two years. The excess weight loss for Roux-en-Y gastric bypass at 10 years was 52% (O'Brien et al 2006*).* The Swedish Obese Subjects (SOS) study, in which 2,010 overweight patients wishing surgery were matched with 2,037 obese patients not desiring surgery, is an important long-term prospective outcome study. There was a greater percentage of initial weight loss after bariatric surgery (gastric bypass 32%, vertical banded gastroplasty 25%, and gastric banding 20%) than with conventional

treatment (2%) (Sjostrom et al 2007). There was also a significant reduction in the adjusted hazard ratio for death (29%) after an average follow-up of 10.9 years. Surgery is more effective at ensuring weight loss and controlling co-morbidities than medical treatment (Sjostrom et al 2007). It reverses, ameliorates, or eliminates major cardiovascular risk factors, including diabetes, hypertension, and lipid abnormalities (Sjöström et al 2007). Another long term (11 years follow up) study of 228 gastric bypass patients with a significant proportion (36.8%) of extremely obese patients (BMI >50 kg/m^2), showed that the extremely obese patients lost weight more rapidly, but also went on to gain weight more rapidly. In the morbidly obese, the BMI before surgery was 44.3 kg/m^2; the nadir was 26.4 kg/m^2, and occurred 1.9 years after surgery but increased again to 31.0 after 11.4 years after surgery. In the extreme obese, the initial BMI of 56.2 kg/m^2 decreased to 31.4 kg/m^2 at 2.2 years after surgery, but increased to 38.3 kg/m^2 at 11.6 years after surgery (Christou et al 2006). Patients who regain large amounts of weight say they are eating almost as much as before the operation (Christou et al 2006). The study by Pories and colleagues showed a remarkable stability of postoperative weight for up to 14 years after gastric bypass. This study on 608 patients showed a 58% excess weight loss after 5 years, and 55% at 10 years (Pories et al 1995). Even though there are varying degrees of evidence for different surgeries, there is a clear preponderance of evidence for all weight loss surgeries to be vastly superior to traditional weight loss therapies in promoting weight reduction.

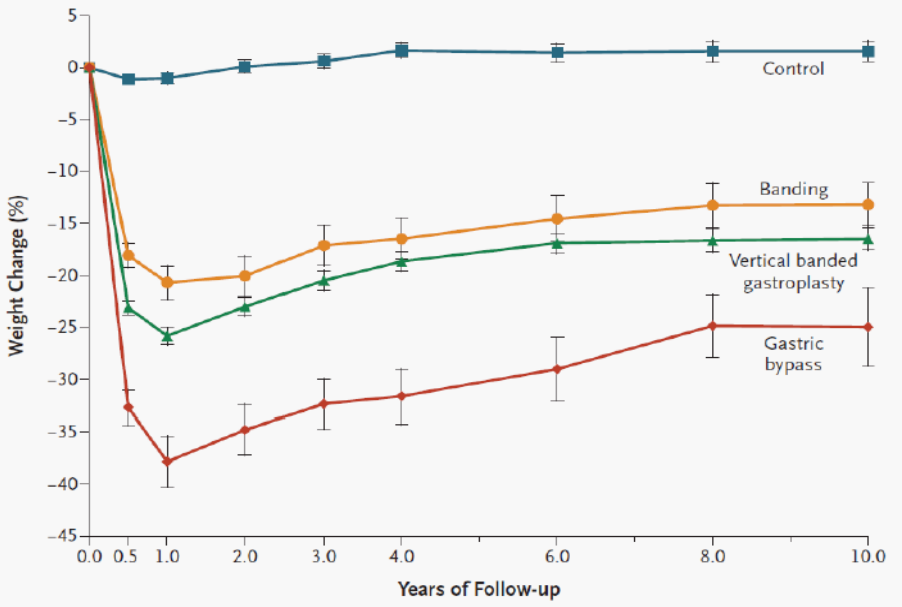


Figure 10; Mean change in bodyweight following bariatric surgery procedures: This graph outlines the results from the SOS study demonstrating mean percentage change in bodyweight over 10 years following bariatric surgery procedures; gastric banding (n = 156), vertical-banded gastroplasty (n = 451) and gastric bypass (n = 34). The control

group (n = 627) consisted of matched obese individuals receiving non-surgical treatment for obesity, adapted from (Sjostrom et al 2004).

1.17 Mechanisms mediating weight loss after surgery

Clinical observation that patients who underwent partial gastrectomy for peptic ulcer disease remained underweight, led Mason and Ito to undertake the first gastric bypass (Mason and Ito 1967). It was initially thought that reducing the stomach volume would lead to early satiety and smaller meals (Halmi et al 1981). However, the compensatory homeostatic mechanisms that maintain bodyweight would be expected to limit weight loss and increase meal frequency. In fact to the contrary patients report a reduction in appetite, reduction in energy-dense foods and fewer meals (Halmi et al 1985). The mechanisms mediating the beneficial effects of bariatric surgery are still not elucidated. Studies in patients having bypass surgery indicated that malabsorption was not the major cause of weight loss after intestinal bypass (Sclafani et al 1978). The changes associated with malabsorptive states, such as reduced circulating levels of albumin and proalbumin and increased faecal fat excretion are not observed following RYGB (MacLean et al 2001). Furthermore, despite a significantly higher malabsorptive component in JIB, there is comparable weight loss after RYGB and JIB (Griffen et al 1977). It was noted that after JIB, food intake and preference for sweets was reduced and feeding patterns normalized (Sclafani et al 1981). This was reproduced in obese rats undergoing JIB. A reduction in caloric intake and decreased palatable food consumption was noted (Sclafani et al 1978). Several lines of evidence now suggest malabsorption does not play a significant role in the weight loss post-RYGBP (Kenler et al 1990). The changes in bodyweight are consequential to a reduction in appetite (Hafner et al 1991, Halmi et al 1981, Borg et al 2006, Korner et al 2005). A number of recent studies have demonstrated that changes in the concentrations of gut hormones may underlie the observed effects of bariatric surgery (Korner J et al 2005, Le Roux et al 2006, de Fatima Haueisen Sander Diniz et al 2006, Karamanakos SN et al 2008, Peterli R et al 2009, Neary and Batterham, 2009, Karra et al 2010, De Paula et al 2010, Basso N et al 2011, Chambers AP et al 2011, Yousseif A and Emmanuel J et al 2013). Though changes in gut hormones favour weight loss, correlation between changes in gut hormone secretion and weight loss has not yet been shown in humans. The pace of resolution of T2DM does differ between days (RYGB) to months to years (GB) (Pories1995, Cummings and Flum 2008, Dixon et al 2008). The rapid resolution of T2DM cannot be explained by weight loss alone. This effect is also able to overcome the up regulation of counter-regulatory stress hormones- cortisol and catecholamine (McAlister et al 2003) seen after surgery. There is increasing evidence that alterations in circulating gut hormone concentrations engendered by surgery play a key role in

mediating both the altered eating behaviour and improved glucose homeostasis (reviewed by Karra E et al 2010).

1.17.1 The hindgut hypothesis

It is thought that the re-routing of food through an anatomically altered and/or shorter gastrointestinal tract leads to an increased delivery of incompletely digested nutrients to the ileum and colon. This leads to over stimulation of the specialised enteroendocrine L cells. The greater nutrient exposure of the L-cells leads to an exaggerated GLP-1 and PYY release (reviewed by Karra and Batterham 2010). GLP-1 also exerts gluco-regulatory properties. Surgical procedures that increase nutrient delivery to the distal gut such as BPD, JIB and RYGB result in rapid resolution of T2DM (Buchwald et al 2004). Further, compelling evidence from ileal transposition (IT) studies in rodents, where interposition of an ileal segment, with intact neural and vascular supply, to the proximal intestine mount an exaggerated nutrient-stimulated PYY, GLP-1 and enteroglucagon responses and exhibit reduced food intake and body weight (Koopmans et al 1984, Strader et al 2005, Patriti et al 2005). In IT the total gut length remains unchanged but the exposure of the transposed ileum to undigested nutrients is enhanced, leading to improved insulin sensitivity and overall glucose homeostasis (Koopmans et al 1984, Strader et al 2005, Patriti et al 2005, Wang et al 2008). These changes are seen in the absence of any restrictive or mal-absorptive surgery, and some argue leave no doubt that the hindgut plays a major role in mediating the weight loss and anti-diabetic effects of bariatric surgery (Karra E et al 2010).

1.17.2 The foregut exclusion hypothesis

An alternative explanation for the improvements in weight and glucose homeostasis was proposed by Hickey and colleagues (Hickey et al 1998). They proposed that the exclusion of an inhibitory factor from the foregut may mediate the rapid improvement in diabetes (Hickey et al 1998). Studies of exclusion of the foregut by doudeno-jejunal bypass (DJB) (a stomach-sparing variation of RYGB with a comparable extent of foregut exclusion, where food is diverted from the pyloric area to the jejunum, bypassing the duodenum and the proximal jejunum) led to no alteration in food intake or body weight in Goto–Kakizaki (GK) rats. However, there was an improvement in glucose homeostasis and resolution of T2DM (Rubino and Marescaux 2004). They also undertook gastro-jejunostomy (GJ), in GK rats. GJ creates a shortcut for ingested nutrients between the stomach and jejunum. Nutrients can either empty directly into the jejunum or follow the physiological pyloric–duodenal route. This procedure failed to

improve glycaemic control in these rats. They proposed that the foregut produces an unidentified factor with anti-incretin properties in the diabetic state, and foregut exclusion prevents the release of this molecule resulting in improved glucose homeostasis (Rubino and Marescaux J 2004, Rubino et al 2006). Foregut exclusion improved T2DM independently of weight loss (Rubino and Marescaux J 2004). GK rats are a spontaneous non-obese model of T2DM. Results from another duodenal exclusion procedure, duodenal–jejunal sleeve (insertion of a plastic endoluminal sleeve into the duodenum that extends into the jejunum) that results in early impressive improvements in glucose homeostasis (Aguirre et al 2008, Rodriguez-Grunert et al 2008), is also proposed as an example for the foregut anti-incretin molecule. However, these procedures increase the exposure of the hindgut L-cells to nutrients also resulting in an exaggerated hind gut response. Further, DJB disrupts the pylorus and thus loss of control over gastric emptying rate and nutrient delivery to the duodenum. The entry of food into the duodenum usually stimulates duodenal osmoreceptors and results in pyloric contraction and a slowing down in gastric emptying (Mason 2005). Procedures that eliminate the pyloric muscle control on gastric emptying result in accelerated gastric emptying, stimulation of intestinal peristalsis and rapid nutrient delivery to the hindgut and an exaggerated hind gut response (Mason 2005). Some have argued that Rubino and colleagues findings resulted from pyloric disruption (Mason 2005). The endoluminal duodenal–jejunal sleeve insertion is thought to prevent nutrient digestion and absorption in the proximal gut, and abolishes the duodenal control of pyloric contraction and therefore again resulting in enhanced hindgut nutrient exposure (Mason 2005). A recent study to compare IT and duodenal-jejunal exclusion (DJE) in GK rats reported comparable weight loss, glucose tolerance and rise in GLP-1 in both groups post-operatively. Interestingly exendin 9-39, a GLP-1 receptor antagonist did reverse the improvement in glucose homeostasis seen after DJE. These findings are thought to indicate that the postoperative improvement in glucose homeostasis is mediated by enhanced GLP-1 signaling rather than from absence of a presumed foregut anti-incretin molecule (Kindel et al 2009, reviewed by Karra et al 2010).

1.18 Mortality rate and complications after bariatric surgery

Author	Patients (n)	Peri-operative mortality	Complications (%)	Early(<30days) complications	late (>30 days) complications	Re-operation rate
Biertho et al	456	2	NR	19	37	8
Boza et al	91	0	NR	12	33	16
Christou and Efthimiou	886	3	15.2	74	27	49
Jan et al	492	1	32	73	115	82
Kim et al	232	0	5.6	5.2%	0.43%	NR
Lakdawala et al	50-SG 50-RYGBP	0 0	2 3	NR NR	NR NR	1 1
Lee et al	25	0	32	NR	NR	4
Weber et al	103	1	NR	21	14	11
Wong et al	30 –SG 7-RYGBP	0 0	3 4	NR NR	NR NR	0 2

Figure 11-A table to summarize mortality and morbidity after bariatric surgery (Franco JVA et al 2011). In the study by Biertho et al early was defined as <1 week and late as >1 week. Patients requiring therapeutic endoscopic interventions were included in the re-operation rate.

Bariatric surgery patients are at risk of developing nutrient deficiencies following surgery, as risk of vomiting compounds, decreased food intake and food intolerance (reviewed by Sawaya RA et al 2012). This is further complicated by the reduction in gastric secretions and reduction in small bowel surface area for absorption. Therefore it is necessary to routinely screen for metabolic bone disease and deficiencies of iron,

thiamine, B_{12}, calcium, folate, and Vitamin A and D (reviewed by Sawaya RA et al 2012). As most patient undergoing bariatric surgery are women of childbearing age folic acid and iron levels are particularly important (reviewed by Sawaya RA et al 2012). Monitoring every three months in the first year after surgery, every six months in the second year, and every 6–12 months in the third year is recommended (reviewed by Sawaya RA et al 2012). It is widely recommended that all patients undergo selective micronutrient measurements before and after bariatric surgery, and be guided by the type of surgical procedure even in the absence of vomiting or diarrhea (reviewed by Sawaya RA et al 2012). The supplementation regimen varies according to the procedure, following RGYBP multivitamin–mineral preparation with iron, vitamin B12, and calcium with vitamin D is common (reviewed by Sawaya RA et al 2012).

Iron deficiency is thought to be prevalent following bariatric surgery, and is thought to be multi-factorial; reduced iron intake with reduced meat intake, reduced gastric capacity, and reduced hydrochloric acid production leading to reduced conversion to Fe^{2+} ion, also limiting the release of iron from structural proteins, and reduction in its affinity to specific co-transporters (reviewed by Sawaya RA et al 2012). Following RYGBP there is reduced exposure to the duodenum and proximal jejunum, primary sites of iron absorption. The presence of peri-operative iron deficiency anemia further compounds this problem (reviewed by Sawaya RA et al 2012). The serum iron along with the total iron binding capacity is the preferred testing method (reviewed by Sawaya RA et al 2012). In some cases, despite appropriate supplementation and addition of Vitamin C, intravenous doses of iron gluconate are required (reviewed by Sawaya RA et al 2012).

Recent studies point to vitamin D deficiency in up to 60% of obese patients (reviewed by Sawaya RA et al 2012). This lipid soluble vitamin is essential to optimize bone mineralization and maintain calcium homeostasis (reviewed by Sawaya RA et al 2012). Prolonged deficiency can lead to osteopenia, osteoporosis, and hypocalcemia (reviewed by Sawaya RA et al 2012). It is thought that adipose tissue is able to take-up and clear 1, 25-dihydroxycholecalferol (reviewed by Sawaya RA et al 2012). Multiple prospective case series report 50% deficiency and 25% hypocalcaemia after RYGB, with the risk of developing metabolic bone disease, and thus lifelong prophylaxis with oral calcium and vitamin D supplementation is strongly recommended, calcium citrate is significantly better absorbed than its carbonated form, and supra-physiological doses are recommended (reviewed by Sawaya RA et al 2012).

Vitamin B_{12} and folate deficiency can lead to macrocytic anemia (reviewed by Sawaya RA et al 2012). Further, long-standing B_{12} deficiency can lead to irreversible neurologic sequelae (reviewed by Sawaya RA et al 2012). The reduction in intrinsic factor after bariatric surgery leads to vitamin B_{12} deficiency, leading to about one third developing vitamin B_{12} deficiency after RYGBP (reviewed by Sawaya RA et al 2012). Folate deficiency occurs commonly after RYGBP, due to by-passing the proximal small bowel where most absorption occurs (reviewed by Sawaya RA et al 2012). Red cell folate and homocysteine levels are measured (reviewed by Sawaya RA et al 2012). The reduction in Fat malabsorption can lead to vitamin A deficiency and night blindness or ocular xerosis, Vitamin K deficiency may lead to clotting abnormalities and chondrogenesis during fetal development, and Vitamin E deficiency with reduced antioxidant levels (reviewed by Sawaya RA et al 2012).

The numerous post-prandial symptoms including “dumping syndrome” do pose a difficulty in characterizing these set of symptoms (reviewed by Foster-Schubert KE 2011). Dumping syndrome occurs due to food reaching the small intestine rapidly after surgery with the altered anatomy, with ensuing abdominal symptoms of pain, bloating, and diarrhea, associated with vasomotor symptoms of flushing, hypotension, and tachycardia (reviewed by Foster-Schubert KE 2011). Hypoglycemia occurs late at 1-3 hours after meals, and typically responsive to dietary modification encompassing frequent small, low carbohydrate meals (reviewed by Foster-Schubert KE 2011). Medical therapy encompassing: alpha-glycosidase inhibitors (acarbose); somatostatin analogs to reduce gastric emptying and inhibit gastrointestinal hormone release, and diazoxide to inhibit calcium-induced insulin release have all been tried (reviewed by Foster-Schubert KE 2011). Difficult cases rarely require reversal of surgical intervention or enteral feeding (reviewed by Foster-Schubert KE 2011). The post-gastric bypass hypoglycemia has required more invasive intervention with majority requiring partial pancreatic resection at 2 years (reviewed by Foster-Schubert KE 2011). Thus establishing symptoms of hypoglycemia confirming low plasma glucose with symptoms and relief of symptoms with the correction and concomitant measurement of C-peptide together with a negative sulfonylurea screen (Whipple's triad) is useful in order to avoid un-necessary invasive intervention (reviewed by Foster-Schubert KE 2011). The timing of these measurements has led some to utilize the oral glucose tolerance test (OGTT), to induce symptoms and signs. Historically OGTT was used to identify reactive hypoglycemia. This test was found to be positive in at least 10% of normal people, Continuous glucose monitoring may help document low glucose episodes in free-living conditions (reviewed by Foster-Schubert KE 2011).

It is not yet clear as to the aetiology of severe, hyperinsulinaemic hypoglycemia following RYGBP (reviewed by Foster-Schubert KE 2011). Some propose an extreme altered physiology following surgery, bringing metabolic benefit. Others propose an underlying genetic predisposition unmasked by surgery. A better understanding of this will enable us to prevent this complication, through careful selection (reviewed by Foster-Schubert KE 2011). A range of severity in post-gastric bypass patients is reported, with mild “dumping syndrome” cases at one end and hyperinsulinemic hypoglycemia associated with neuro-glycopenic symptomatology on the other (reviewed by Foster-Schubert KE 2011). The former managed with dietary manipulation and the latter requiring invasive intervention as outlined above. There was no notion of pre-operative hypo-glycaemia in these patients, nor did diabetes state play a part (reviewed by Foster-Schubert KE 2011). These symptoms were also absent in patients undergoing a restrictive procedure, and pointing to the exclusion of the foregut and faster nutrient delivery playing a part (reviewed by Foster-Schubert KE 2011). The absolute risk remains low 0.2% post RYGBP, compared to 0.04% of the general population (reviewed by Foster-Schubert KE 2011). Beta cell mediated and non-beta cell mediated factors have been proposed; hypertrophic islets with obesity, however onset is late after surgery, and typically years later. A median time of 2.7 years from surgery has been reported; also the lack of incidence with restrictive procedures goes against this. The difficulty obtaining pancreatic specimens in appropriately matched patients precludes the use of islet morphology in post-gastric bypass hypoglycemia (reviewed by Foster-Schubert KE 2011). Interestingly the severity of nesidioblastosis does not seem to correlate with duration following RYGBP (reviewed by Foster-Schubert KE 2011). GLP-1 is a possible candidate as an exaggerated response is seen after RYGBP, and with RYGBP patients with neuro-glycopenic symptoms had higher GLP-1 matched for duration post-surgery without hypoglycemia, and non-surgical weight-matched controls (reviewed by Foster-Schubert KE 2011). GLP-1 does correlate to insulin secretion. The post-gastric bypass hypoglycemic patients also had higher insulin and C-peptide levels (reviewed by Foster-Schubert KE 2011). It is not yet clear if GLP-1 plays a causative role or is simply a marker. The lower ghrelin with its insulin counter-regulatory mechanisms has also been proposed to play a part (reviewed by Foster-Schubert KE 2011). The opponents site no difference in these peptide hormones whilst others site case reports leading to amelioration by feeding through the bypassed gut, and suggesting the altered nutrient flow with gastric bypass being the only difference causing the exaggerated incretin response and hence hypoglycemia. Other as yet unknown factors could play a role; anatomic changes could still contribute, but seem unlikely given that reversal of anatomy does not ameliorate the symptoms (Foster-Schubert KE 2011).

1.19 Gut and islet hormone alterations after RYGBP and SG

An accurate assessment of appetite scores and gut hormone levels are dependent on study design and experimental protocol in human studies on obesity surgery. The heterogeneity in study protocols and procedures has led to difficulties in making substantial comparison between studies. Though changes in gut hormones favour weight loss, correlation between changes in gut hormone secretion and weight loss has not yet been shown in humans, but has been shown in rats after RYGBP (Shin AC et al, 2010). This discrepancy may be related to study design and sample processing. Several studies have looked at gut hormone changes after surgery (Cummings D E et al 2002, Langer et al 2005, Korner J et al 2006, Whitson BA et al 2007, Karamanakos SN et al 2008, De Paula et al 2009, Y Wang et al 2009, Li F et al 2009, Peterli R et al, 2009, Bose M et al 2010, Abbatini F et al 2010, Bohdjalian A et al 2010, Basso N et al 2011, Chambers AP et al 2011). However, not all have measured the active forms of the circulating hormone (Cummings D E et al 2002, Langer et al 2005, Korner J et al 2006, Whiston BA et al 2007, Karamanakos SN et al 2008, De Paula et al 2009, Y Wang et al 2009, Li F et al 2009, Bose M et al 2010, Abbatini F et al 2010, Bohdjalian A et al 2010, Basso N et al 2011, Chambers AP et al 2011). Further, samples were not collected in to tubes containing protease inhibitors to ensure no degradation of these peptides occur prior to analysis (Cummings De et al 2002, Korner J et al 2005, Langer FB et al 2005, Korner J et al 2006, Whitson BA 2007, Wang Y et al 2009, Li F et al 2009, Lopez PP et al 2009, Bohdjalian A et al 2010, N Basso et al 2011). This has led some authors to admit poor collection practice and being unable to detect consistent changes in hormone profiles (Buchwald et al 2007). Some studies that have looked at PYY 3-36 and active GLP-1 but have failed to add DPP4 inhibitor to the samples (DePaula AL et al 2009, Korner J et al, 2005, Korner J et al 2006, Whitson BA et al 2007, Karamanakos et al 2008, Li F et al 2009, Valderas JP et al 2010). Other studies have shown a low initial hind gut response that increased with the passage of time after SG (Peterli R et al 2009), perhaps suggesting a lack of standardisation in study protocol. In the case of acyl-ghrelin no study to date has collected blood samples with HCL and protease inhibitors to measure this active octanoylated form prior to degradation, as per manufacturer's instructions on assay protocols. The meal stimulated acyl-ghrelin is not significantly altered after RYGBP in rats (Shin AC et al 2010). There have been no studies to investigate meal stimulated PYY3-36 secretion after SG. Several studies looked at post operative changes several months to years after surgery (Korner J et al, 2005, Korner J et al 2006, Y Wang et al 2009, Karamanakos et al 2008, Bohdjalian A et al 2010), perhaps missing early physiological changes. Others have compared post-surgical changes in gut hormones against

control groups (Cummings De et al 2002, Korner J et al, 2005Lopez PP 2009, Whitson BA 2007, Oliván B et al 2009, Bose M et al 2010, Valderas JP et al 2010), and not to their pre-operative state, making it difficult to draw conclusions on an individual's physiological changes and corresponding correlations to anthropometry. Comparison of matched cohorts can lead to natural inter-individual variation masking true change in an individual. Further, it is not possible to make comparisons across temporal profiles of individual's and correlate this to outcome measures.

No study to date has found a correlation between changes in active gut hormones after RYGBP/ SG and changes in perception of hunger, satiety or prospective food consumption. This may be because those studies that have looked at visual analogue scores (VAS) utilised only two time points per visit to assay change (Korner J et al, 2005, Korner J et al 2006, Karamanakos et al 2008) and when they did employ several time points to measure VAS, suitable inhibitors to prevent degradation of the hormones were not added (Buchwald et al 2007, Karamanakos SN et al 2008, DePaula AL et al 2009, Valderas JP et al 2010). Also, whilst several studies have examined the change in insulin, glucose, insulin resistance (HOMA IR) post RYGBP (Korner J et al 2005, Whiston BA et al 2007, De Paula AL et al 2009, Oliván et al 2009, Bose M et al 2010, Abbatini F et al 2010, Chambers AP et al 2011, Basso N et al 2011), none have so far examined insulin: amylin ratio nor explored the relationship between acyl-ghrelin and insulin resistance measured by the HOMA IR model, in morbidly obese individuals undergoing RYGBP and SG. A significant increase in meal stimulated amylin secretion is seen on rats undergoing RYGBP (Shin AC et al 2010). However, no correlation between increased amylin secretion and weight loss is seen (Shin AC et al 2010). There have been no studies on meal-stimulated active amylin secretion after SG. Sleeve gastrectomy (SG) has gained prominence as a sole operation for morbid obesity (Bohdjalian A et al 2010). The gut hormone changes that follow this procedure have also been extensively investigated recently (Karamanakos SN et al 2008, Peterli R et al 2009, De Paula et al 2009, 2010, Basso N et al 2011, Chambers AP et al 2011, Valderas JP et al 2010, Papailiou J et al 2010). Gut hormone changes that occur after SG are thought to be similar to that seen after RYGBP, favouring weight loss and early satiety (Karamanakos SN et al 2008, Peterli R et al 2009, De Paula et al 2009, 2010, Basso N et al 2011, Chambers AP et al 2011, Valderas JP et al 2010, Papailiou J et al 2010). No study has yet documented paradoxical changes in gut hormones in patients who fail to respond to SG, perhaps if failure could be predicted at an early stage and second stage procedure instituted on time; this would enable long term weight loss, in those patients.

Prospective (Sjostrom et al 2004) and retrospective studies (Rosenthal et al 2008) have shown resolution of T2DM after bariatric surgery. The mechanism underlying this has been attributed to weight loss (Rosenthal et al 2008, Karamanakos SN, et al 2008), improved incretin response (Peterli R et al 2009, Li F et al 2009, Dezaki K et al 2008, Chambers A P et al 2011) and improvement in insulin resistance independent of weight loss (Peterli R et al 2009, De Paula et al 2009). A significant reduction in serum glucose, insulin and HOMA IR was seen at two weeks after SG but not in the control cholecystectomy group. This remained unchanged at 1 and 2 months after surgery despite further significant weight loss (Rizzello M et al 2010), and confirms no role for pre-operative interventions.

1.19.1 Insulin

A study to compare the effects of SG, RYGBP on glucose homeostasis in morbidly obese T2DM patients, that evaluated patients at 3 years after surgery to examine the role of weight loss in the resolution of T2DM, found similar resolution rates of 81.2% and 80.9% after RYGBP and SG (Abbatini et al 2010). Insulin resistance was restored to normal values in all the patients, including those patients with persisting T2DM (Abbatini et al 2010). RYGBP and SG led to a significant improvement in fasting plasma glucose at 3 months after the surgery. The resolution rate did not alter at 3 years, however longer follow up has led to halving in resolution of T2DM (Sjostrom et al 2007). The authors conclude that RYGBP and SG resulted in comparable resolution and improvement in glucose homeostasis after surgery (Abbatini et al 2010). In both groups resolution occurred at 3 months, and was unchanged at 12 months, despite further significant weight loss, between these time points in the two groups, suggesting a more direct effect after RYGBP and SG, probably mediated by endocrine mechanisms leading to an improvement in glycaemia independent of weight loss (Abbatini et al 2010). Interestingly, there was no statistically significant difference in weight loss between cured and T2DM patients, in any of the groups. However there was a statistically significant difference in the change in BMI between the cured and type-2 patients after RYGBP. This was not the case after SG (Abbatini et al 2010). In a randomised prospective parallel group study conducted by Peterli and colleagues, the fasting insulin concentrations and HOMA indices were significantly reduced a week after surgery before any significant weight loss had occurred (Peterli R et al 2009, Peterli R et al 2012, Jacobsen SH et al 2012).

Several studies have also explored the role of incretins post RYGBP and SG (Korner J et al 2005, Korner J et al 2006, Karamanakos SN et al 2008, De Paula et al 2009, Y

Wang et al 2009, Li F et al 2009, Peterli R et al, 2009, Bose M et al 2010, Abbatini F et al 2010, Basso N et al 2011, Chambers AP et al 2011). However some of these studies have been marred by the lack of standardization, and lack of suitable inhibitors being added to prevent degradation of the active hormone moiety (Korner J et al 2005, Buchwald et al 2007, Karamanakos SN, et al 2008, DePaula et al 2009). This again has led some authors to admit poor collection practice and being unable to detect consistent changes in hormone profiles (Buchwald et al 2007). The pronounced GLP-1 response seen after RYGBP is thought to promote insulin secretion in this group (Peterli R et al 2009, Li F et al 2009 and Dezaki K et al 2008). It is thought that the lack of such a pronounced GLP-1 response after SG may be compensated for by the decrease in ghrelin seen after SG and this is thought to lead to improved insulin sensitivity after SG (Peterli R et al 2009, Li F et al 2009 Papailiou J et al 2010, and Peterli R et al 2012).

Sleeve gastrectomy (SG) has gained prominence as a sole operation for morbid obesity (Bohdjalian A et al) and T2DM (DePaula AL et al 2009, Karamanakos SN et al 2008, Peterli R et al 2008, BassoN et al 2011, Chambers A P et al 2011). Studies on patients undergoing SG to explore the role of incretins in patients with a lower BMI and more advanced diabetes (De Paula et al 2009), found SG in combination with proximal ileal inter-position led to an exaggerated incretin response, restoration of the first phase insulin secretion and resolution of T2DM in two thirds of patients. However, the incretin response was probably under-estimated due to the lack suitable inhibitors to prevent degradation (De Paula et al 2009). The gut hormone changes that follow this procedure have also been extensively investigated recently (DePaula A L et al 2009, Karamanakos SN et al 2008, Peterli R et al 2008, Valderas JP et al 2010, Papailiou J et al 2010, BassoN et al 2011, Chambers A P et al 2011), and are thought to be similar to that seen post RYGBP. Faster gastric emptying (Braghetto I et al 2009) and small bowel transit time (Shah S et al 2010) post-surgery is thought to lead to quick delivery of nutrients to the hindgut and in-turn evoke a hind gut incretin hormone response not dissimilar to that seen following RYGBP (Peterli R et al 2009), leading to an improvement in insulin secretion (DePaula AL et al 2009).

1.19.2 Ghrelin

There has been much debate on the importance of ghrelin after LSG (Langer FB et al 2005, Frezza E E et al 2008). Several studies have assessed the impact of bariatric surgery on circulating ghrelin measuring both total (acyl- and desacyl-ghrelin) or acyl-ghrelin in the fasted and/ or meal-stimulated state (le Roux et al 2006, Korner et al

2005, Karamanakos et al 2008, C. Holdstock et al 2003, M. Sundbom et al 2007, Leonetti et al 2003, Wang and Liu, 2009). Some groups have reported significantly increased circulating ghrelin levels post-RYGB (Holdstock et al 2003, Sundbom et al 2007), whilst others showed no difference in circulating ghrelin levels after RYGB (le Roux et al 2006, J. Korner et al 2005, S.N. Karamanakos et al 2008, F. Leonetti et al 2003). Total ghrelin is known to be elevated after diet induced weight loss (Oliván B et al 2009) and it was initially thought that a decrease in total ghrelin after SG may explain the superior weight loss and maintenance of weight loss after LSG (Langer FB et al 2005). However, a recent meta-analysis of several studies was unable to reach a conclusion (Frezza E E et al 2008). A recent review points out that patients actively undergoing weight loss exhibit increased circulating ghrelin concentrations while this is not the case in weight stable patients (reviewed by Karra E et al 2010). These findings indicate that overall energy balance might be a more important determinant for postsurgical circulating ghrelin levels and explain the discrepancy between studies (M. Faraj et al 2003). To date no study has measured acyl-ghrelin, the active octanoylated form collected under standardised conditions to prevent degradation as recommended by the assay. The meal stimulated acyl-ghrelin is not significantly altered after RYGBP in rats (Shin AC et al 2010). The significant decline in acyl-ghrelin after SG is thought to be due to the complete removal of the gastric fundus, the segment of the stomach, thought to produce the vast majority of acyl-ghrelin (Langer F B et al 2005). The vagus nerve is also thought to play a part in this response (Papailiou J et al 2010). The suppression of ghrelin secretion seen after RYGBP is thought to be secondary to a permanent deprivation of nutrient stimulation to oxyntic gland cells responsible for the production and release of acyl-ghrelin (Papailiou J et al 2010). In support of this, recent evidence also points to prolonged fasting leading to a decline in acyl-ghrelin in the plasma (Papillou J et al 2010). Others have suggested that the absence of food from the stomach and duodenum after bypass surgery could lead to suppression of ghrelin by override inhibition, and this favours weight loss after RYGBP surgery (Cummings et al 2002). It is not yet clear if the reduced production of acyl-ghrelin seen after SG is temporary that may be reversed over time, through post-surgical gastric hyperplasia or other gastro-intestinal sites such as the duodenum taking over acyl-ghrelin production. Alternatively the central orexigenic effect of ghrelin may be restored by adaptations at the central sites of ghrelin action (Papailiou J et al 2010). Conversely ghrelin is increased in weight-matched subjects who achieved similar weight loss with dieting and therefore lead to compensatory food intake (Cummings et al 2002, Korner J et al 2005). Studies in patients undergoing SG report a significant and sustained decrease in fasting and meal-stimulated ghrelin concentrations immediately after surgery (Karamanakos et al 2008, Wang and Liu 2009, Peterli R et al 2012). Therefore it is

plausible that reduced ghrelin post surgery plays a role in mediating weight loss and improved glucose homeostasis after bariatric surgery.

The many effects of acyl-ghrelin linked to insulin resistance (eg suppression of the insulin-sensitizing hormone adiponectin, blocking of hepatic insulin signalling and inhibition of insulin secretion, increase in growth hormone cortisol, and epinephrine secretion) may be the reason why decreased acyl-ghrelin secretion after SG may help restore insulin sensitivity (Peterli R et al 2009). This has led some to speculate that the weight independent resolution of T2DM and improvement in glucose homeostasis seen after bariatric surgery may in part be mediated by acyl- ghrelin (Papailiou J et al 2010). The decline in acyl-ghrelin is thought to facilitate maximal capacity in the islets enabling the islets to respond adequately to the hyperglycaemia and meet the increased demand associated with obesity (Papailiou J et al 2010). Ghrelin is also known to decrease insulin secretion in vitro and in vivo ((Dezaki et al, 2008; Reimer et al, 2003). In the SG group where acyl-ghrelin is significantly reduced after surgery, the improvement in glucose homeostasis will lead to improvements in Type-2 DM (Peterli R et al 2012).

1.19.3 Peptide tyrosine tyrosine

A study to compare surgical intervention against medical treatment for obesity was able to achieve similar weight loss after RYGBP, SG, and medical treatment. However favourable PYY change was only seen after RYGBP and SG. The meal stimulated total PYY AUC did increase significantly after RYGB and SG, the magnitude of increase was significantly higher in the RYGBP group compared to the SG group and lean controls (Valderas JP et al 2010). Two studies reported comparable increases in fasting and meal-stimulated circulating PYY levels following RYGB and SG (Peterli et al 2009, S.N. Karamanakos et al 2008). A recent publication highlighted a similar pronounced hind-gut response after SG to that seen after RYGBP (Peterli R et al 2009). The one study to investigate PYY3-36 in patients undergoing RYGBP studied PYY3-36 response to a 3 hour glucose tolerance test (GTT) (Oliván B et al 2009). It is known that the meal stimulated total PYY response following SG is similar to that seen after RYGBP (Korner J et al 2005, Karamanakos SN et al 2008, Peterli R et al 2012). Fasted and meal stimulated PYY levels were noted to be elevated for up to twenty years after bypass surgery in humans (Naslund et al 1997). The numbers of enteroendocrine cells containing PYY are significantly increased at 30 years after by-pass surgery (Ockander et al 2003). This increase was in comparison to obese and normal weight controls, suggesting JIB increases PYY-containing cells (Ockander et al 2003). PYY levels are increased after JIB associated with a decrease in food intake, and PYY-antisera

increased food intake, indicating a role for PYY in mediating reduced food intake after bariatric surgery (le Roux et al 2006). Subsequent studies have since consistently reported increases in fasting and/or meal-stimulated PYY levels after RYGB (Korner J et al 2005, Morinigo et al 2006 and Korner J et al 2006, Peterli R et al 2009, Peterli R et al 2012, Jacobsen SH et al 2012, Yousseif A and Emmanuel J et al 2013). Three studies reported comparable increases in fasting and meal-stimulated circulating PYY levels following RYGB and SG (Peterli et al 2009, S.N. Karamanakos et al 2008, and Peterli R et al 2012). In summary, there appears to be a clear trend towards increased PYY levels after bariatric surgery. Overstimulation of the hindgut by increased nutrient exposure is the likely explanation for this exaggerated response. Recent evidence points to a more direct effect of PYY3-36 on insulin sensitivity (van den Hoek et al 2007), PYY3-36 is known to be co-secreted with GLP-1 by intestinal L cells in response to food intake, the role of PYY3-36 on insulin sensitivity independent of food intake is not confirmed (Papailiou J et al 2010).

1.19.4 GLP-1

The vast majority of studies have shown increases in basal and/or nutrient-stimulated GLP-1 levels after RYGBP (le Roux C W et al 2006, Korner J et al 2005, Morinigo R et al 2006 and Korner J et al 2007, Peterli R et al 2009, Peterli R et al 2012, Jacobsen SH et al 2012). Some recent studies have examined the role of active GLP-1 in patients undergoing RYGBP and SG (Peterli R et al 2009 and DePaula AL et al 2009, Basso N et al 2011, Chambers A P et al 2011, Peterli R et al 2012, Yousseif A and Emmanuel J et al 2013) though one study did not add DPP4 inhibitor to the samples (DePaula AL et al 2009), and a second had much lower plasma levels (Peterli R et al 2009), suggesting differences in collection protocols. Another recent study in rodents undergoing RYGBP found a significant post-prandial increase in GLP-1 that led to significantly higher post prandial plasma insulin, higher than lean controls, and significantly improved insulin resistance. The 20 minute peaks of GLP-1 and insulin did correlate after RYGBP. Further, there were significantly lower post-prandial plasma glucose levels reaching that of lean controls (Shin AC et al 2010). The study by Peterli and colleagues points to similar changes in GLP-1 after both RYGBP and SG (Peterli R et al 2009, Peterli R et al 2012). Although SG entails no mal-absorptive component, accelerated gastric emptying is thought to mediate this. However, a progressive decrease in gastro-esophageal reflux following SG is noted and may be indicative of increasing gastric compliance with time. These adaptive changes post-SG could potentially alter gastric emptying and consequently GLP-1 levels (J. Himpens et al 2006). A significant and similar decline in fasting glucose, insulin and HOMA-IR was seen in two groups after the loss of 10 kg when diet therapy and RYGBP were compared. However, a significant

increase in GTT stimulated GLP-1 occurred only after RYGBP but not after diet induced weight loss (Oliván et al 2009). This suggests that RYGB per se rather than weight reduction mediated the increased in GLP-1 (Laferrere B et al 2008, Ahren B et al 2003). A very recent publication compared GLP-1 (7-36) after RYGB and SG using a model of high fat diet-induced obese hyper-insulinaemic rats (Chambers et al 2011-b). The two bariatric surgery procedures led to a comparable improvement in glucose tolerance and GLP-1(7–36) secretion (Chambers AP et al 2011-b). The authors demonstrate that these improvements in glucose homeostasis are weight independent and GLP-1 (7-36) dependent (Chambers AP et al 2011- a). However, these studies were undertaken several months after the procedures. Basso and colleagues also recently demonstrated an improvement in glycaemia after SG mediated by restoration of the first phase of insulin secretion in diabetic obese patients mediated by GLP-1 (Basso N et al 2011).

1.19.5 Amylin

It is thought that amylin synthesis and secretion may be under the influence of GLP-1 (Ahrén B et al 1997), and amylin in turn may mediate some of the biological actions of GLP-1 (Asmar M et al 2010). A significant increase in meal-stimulated amylin secretion is seen in rats undergoing RYGBP (Shin AC et al 2010), though contrary to others who reported a decrease in amylin after RYGBP (Mousumi Bose et al 2010). Bose and colleagues found a non-significant decline in amylin after RYGBP. However, a decline in amylin in the diet induced weight loss control group suggests that sample collection and processing may have played a part in this un-expected result (Mousumi Bose et al 2010). Others have found a significant increase in amylin when SG is combined with an ileal interposition on to the proximal duodenum and proximal jejunum (DePaula AL et al 2009). However, no correlation between increased amylin secretion and weight loss is seen (Shin AC et al 2010). There have been no studies on meal stimulated active amylin secretion after SG.

1.20 Improvement in mortality after bariatric surgery

A recent longitudinal study found that the overall mortality of obese patients was 0.68% in the surgically treated group (vertical banded gastroplasty and Roux-en-Y gastric bypass) and 6.17% in obese patients who had not had bariatric surgery (Christou et al 2004). The Swedish Obese Subjects Study also found fewer deaths related to myocardial infarction and cancer in the surgically treated group (Sjostrom et al 2007), and Adams et al found a 40% reduction in mortality in surgically treated obese patients (Adams et al 2007). Even when the risks of death associated with surgery are

taken into account, the patients who have surgery are more likely to be alive a year after surgery than the patients who choose conservative treatment (Christou et al 2004).

1.21 Resolution of co-morbidities after bariatric surgery

In addition to the significant weight loss associated with bariatric surgery, vast improvement in hyperlipidaemia, hypertension, obstructive sleep apnoea and T2DM has been reported (Buchwald et al 2009, Sjostrom et al 2004) and surgery has been directly linked with a reduction in mortality from diabetes related illness, cardiovascular diseases and cancer (Adams et al 2007). The SOS study also showed that surgical intervention leads to considerable improvements in obesity related co-morbidities and heath related quality of life when compared to patients treated with diet alone (Sjostrom et al 2004). At two years, the incidence of hypertension is 3% in surgical patients, and 10% in those receiving conventional treatment (10%) (Sjostrom et al 2004). Bariatric surgery is associated with weight loss and improvements in glucose homeostasis (Buchwald et al 2009). A long-term controlled bariatric surgical intervention study on obesity showed that surgery resolved or markedly improved diabetes, reduced myocardial infarction by 43%, and provided a 31% reduction in overall mortality. Interestingly, the benefit in the reduction of myocardial infarction and overall mortality was almost exclusively seen in diabetic patients. The patients were matched by age, sex, BMI, and co-morbidities and both groups were followed for 15 years (Sjostrom et al 2006). Another meta-analysis of 22,094 patients where the mean age was 47 years, and mean BMI 46, showed diabetes resolution in 76.8% of patients and improvement in 86.0%. Further, other co-morbidities were also significantly improved after surgery. This included hyperlipidemia, which improved in ≥70% of patients, and hypertension which resolved in 61.7% and improved in 78.5%. Obstructive sleep apnea was resolved in 85.7% of patients (Buchwald et al 2004). A smaller long-term follow-up study of 342 severely obese patients who underwent gastric bypass in New Zealand also confirms excellent long-term outcomes. The excess weight loss after 1, 5, and 10 years were 89%, 70%, and 75% respectively. In addition, hypertension was resolved in 62% of individuals, and a further 25% had improved blood pressure. T2DM was resolved in 85%, and a further 10% showed improvement (White et al 2005). The weight loss and improvement in co-morbidities seen after surgery are maintained in the longer term (White et al 2005).

1.22 Bariatric surgery leads to an improvement in glucose homeostasis

Morbid obesity is known to be associated with insulin resistance and insulin hyper-secretion by the pancreatic β cells. However, β-cell function, β-cell glucose sensitivity, and potentiation are preserved *(*Camastra et al 2007). Diabetes is characterized by a progressive loss of β-cell function, independent of insulin resistance *(*Ferrannini et al 2005*)*. Bariatric surgery leads to a 2-3 fold improvement in insulin sensitivity. This is seen early after surgery before any substantial weight loss has occurred. The absolute insulin secretion decreases significantly after bariatric surgery (Kopp et al 2003, Wickremesekera et al 2005 Camastra et al 2005). The mechanisms underlying these dramatic effects on insulin sensitivity and β-cell function are yet to be elucidated. However, several mechanisms have been proposed *(*Cummings et al 2007). Among them, caloric restriction and changes in gut hormone release have received the most attention. Studies suggest that gastric bypass surgery results in an effective cure of T2DM in up to 79 % of cases (Buchwald et al, 2009). Hence surgery is now cautiously being considered as a treatment for T2DM in individuals with BMI's lower than the ranges prescribed by the current healthcare guidelines (Cummings and Flum 2008). A systematic review and meta-analysis of the resolution of T2DM after bariatric surgery also found a 78% total remission rate, and an improvement in 87% (Buchwald et al 2009). The relative effectiveness in resolving T2DM for Roux-en-Y gastric bypass was 80.3% (Buchwald et al 2009).

Others have reported much lower rates of remission at 5 years; complete and partial remission rates were 24% and 26%, and 16% remained unchanged (Brethauer SA et al 2013). Shorter duration of T2DM and higher long-term weight loss predicted long-term remission (Brethauer SA et al 2013). Recurrence was associated with longer duration of T2DM and weight regain (Brethauer SA et al 2013). Thirty-four percent of all patients had improvement in long-term diabetes control (Brethauer SA et al 2013). Overall, patients were taking fewer numbers of diabetic medications and requiring insulin therapy (Brethauer SA et al 2013). Patients with T2DM of 5 years or less had a high 76% long-term remission rate, compared with a 21% in those with longer duration (Brethauer SA et al 2013). There is generally agreement that RYGBP achieve higher rates of remission than procedures involving restriction of the gastric fundus and longer duration of T2DM have lower remission rates (Brethauer SA et al 2013).

A recent study to assess long term remission of diabetes after RYGBP noted 62% remission of diabetes maintained at 6years (Adams TD et al 2012). The mean total weight loss in the surgical group changed from 35% to 28% along with diabetes remission from 75% to 62% (Adams TD et al 2012).The pooled T2DM remission in four

studies with only T2DM patients highlighted a 22 times higher remission with bariatric surgery when compared to conservative approaches. However, Different definitions for diabetes remission were used. A conservative estimate puts remission at five times after bariatric surgery (Gloy et al 2013).

1.23 The economic costs of obesity can be counteracted by obesity surgery

A recent study attempted to quantify the effect of bariatric surgery on direct medical costs (Cremieux et al 2008). This study assessed the time required for third-party payers to recover the initial investment associated with bariatric surgery (the return on investment). Each bariatric surgery patient was matched to controls on multiple baseline characteristics, including age, sex, total pre-surgery medical costs, and co-morbid conditions, the control group of morbidly obese patients never underwent bariatric surgery (Cremieux et al 2008). Total surgery costs are fully recovered after 53 months. Costs of open surgery performed between 1999 and 2002 are fully recovered after 77 months and, as expected, costs of open surgery performed between 2003 and 2005 are recovered after 49 months. The costs associated with laparoscopic surgery are fully recovered after 25 months. These returns on investment result from reductions in prescription drug costs, physician visit costs, and hospital costs (including emergency department visits and inpatient and outpatient visits). The reduced costs are associated with multiple diagnosis categories, including diabetes mellitus, coronary artery disease, hypertension, and sleep apnoea (Cremieux et al 2008). This study demonstrates significant cost savings start accruing after 25 months for patients undergoing laparoscopic bariatric surgery. The study also shows that, whilst bariatric surgical costs took more than 6 years to be fully recovered in 2002, this was reduced to just over 2 years in 2005 for laparoscopic bariatric surgery. Examination of the cost per quality adjusted life year (QALY) for bariatric surgery has shown that bariatric surgery in European countries is also more cost effective than conventional treatment (Ackroyd et al 2006). Further a study of patients in employment found that significantly more worked after surgery than before (76% v 58%). Further, significantly more patients worked longer hours than they did before (35.8 h v 30.1 h), and fewer patients claimed welfare benefits (Hawkins et al 2007). Suggesting that the economic benefits to the individual and economy are further enhanced.

1.24 Our hypothesis and rationale for the study

Recent studies have compared weight loss and resolution of T2DM after RYGBP and SG (Karamanakos SN et al 2008, Peterli R et al 2009, and Valderas JP et al 2010, Chambers AP et al 2011, Kehagias I et al 2011, Woelnerhanssen B et al 2011, Peterli

R et al 2012, Ramon JM et al 2012). The mechanisms mediating weight loss and amelioration of T2DM after these procedures are yet to be fully elucidated. We conducted a prospective parallel group study to examine changes in fasting and meal stimulated gut hormones and subjective measures of hunger, fullness and prospective food consumption simultaneously after RYGBP and SG in a tertiary care hospital setting. Exclusion criteria were: male, smokers, positive hepatitis B or C status. Patients were studied at three time points; week before surgery, 6 and 12 weeks post surgery, to coincide with routine surgical follow up. Each Individual was compared against themselves. The hormones studied in this thesis are insulin, leptin, acyl-ghrelin, glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY) and amylin. We hypothesized a similar hind gut hormone response after RYGBP and SG. However, we proposed that acyl-ghrelin and amylin would be differentially altered following these procedures. We also hypothesized that the change in active plasma gut hormones may mediate the loss of weight through changes in appetite and satiety, and that the improvement in glucose and insulin resistance may also be mediated through active plasma gut hormones.

Chapter 2
Methods

2.1 Suppliers

Laboratory plastics, pipette tips, solvents and reagents were purchased from Sigma Aldrich (Sigma Aldrich, Dorset, UK), Invitrogen (Invitrogen, Paisley, UK) or VWR (VWR, Lutterworth, UK), if otherwise, it is indicated in the text. All clinical supplies were obtained through the NHS Supply Chain (NHS Supply Chain, Alfreton, UK). Purified and deionised water from an Elga water purification system was used in the experiments conducted (Elga; Veolia, Wycombe, UK).

2.2 Bariatric study

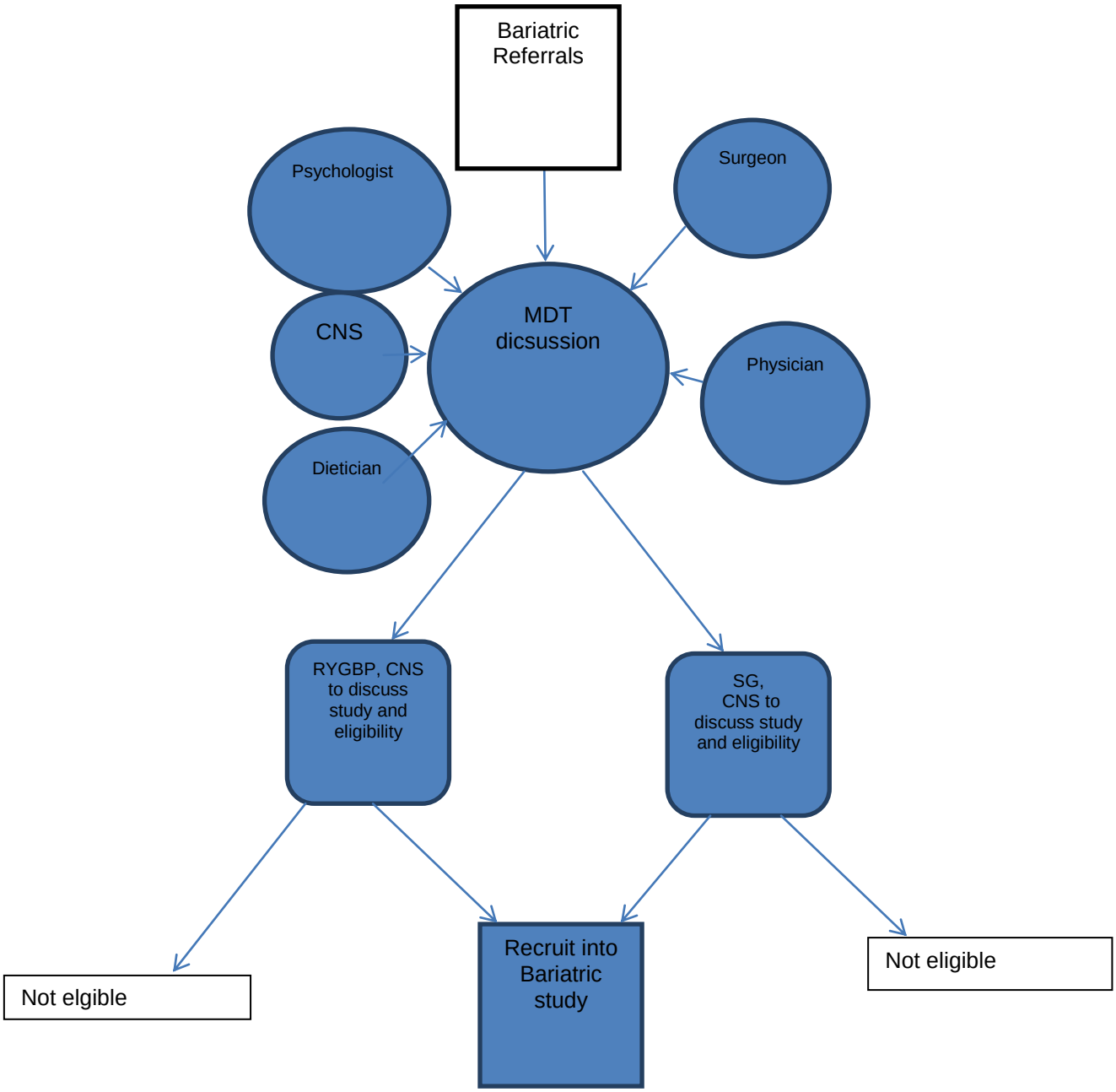
We conducted a prospective parallel group study on patients undergoing RYGBP and SG to measure changes in fasted and meal-stimulated active gut peptide hormones PYY3-36, acyl-ghrelin, GLP-1, active amylin and subjective measures of appetite simultaneously in a tertiary care setting, in order to study the mechanisms mediating weight loss after RYGBP and SG. Exclusion criteria were: male, smokers, positive hepatitis B or C status. Patients were studied at three time points; week before surgery, 6 and 12 weeks post surgery, to coincide with routine surgical follow up. Each individual was compared against themselves.

2.2.1 Ethics

Ethical approval was obtained from the University College London Hospital research ethics committee. The study was carried out in accordance with the principles of the Declaration of Helsinki. Patients attended a preliminary screening session where they received both oral and written information about the study. An informed consent form was completed on the first day of the study. Data was collected stored in accordance with the Data Protection Act 1998.

2.2.2 Subject recruitment

Patients undergoing Roux-en-Y gastric by-pass and sleeve gastrectomy surgery
who met the inclusion criteria (females aged 18-65 with a BMI of 40-55 Kg/m2) and exclusion criteria (male, smokers, positive hepatitis B or C status) were invited to participate in the study.



A consort flow diagram to illustrate patient assignment to surgical procedure through an multi-disciplinary team (MDT) approach. CNS- clinical nurse specialist.

The patient is seen by the psychologist, dietician, bariatric surgeon and bariatric physician. The best choice of procedure was discussed based on clinical criteria, by the multidisciplinary team. The patient was reviewed by the clinical nurse specialist and details of the study discussed with the aid of a leaflet. If the patient agreed to participate, then the patient was reviewed by the clinical research fellow (Julian

Emmanuel). The clinical assessment formed the basis of choice, and as such could have introduced a selection bias in the patients recruited. Every patient meeting inclusion and not conforming to the exclusion criteria was given the opportunity to take part in the research study.

2.2.3 Subject standardisation and acclimatization

Patients followed a standardization and acclimatization protocol (Chandarana K et al 2009). Patients were asked to refrain from alcohol on the day before the clinical study. They consumed a standard meal between 7PM and 8PM on the night before the study. They were then asked to fast with water only till 9AM on the study morning. Patients attended the clinical research centre at 9AM. Patients were cannulated and then rested for an hour to accommodate the stress of cannulation.

2.2.4 Blood collection

On arrival at the clinical facilities, a 20-gauge cannula (BD, Oxford, UK) was placed in the patients forearm vein by aseptic technique. A three-way tap (BD, Oxford, UK) was attached to the cannula and secured. The three-way tap was used for all subsequent blood collection. It was flushed with normal saline (BD, Oxford, UK) after each collection. Blood was collected into chilled syringes and immediately transferred to vaccutainers (BD, Oxford, UK) coated with ethylenediaminetetraacetic acid (EDTA). All blood samples were collected and processed according to the manufacturers' instructions of the commercially available assays that were subsequently used for quantification of hormones.

2.2.5 Standard meal

Patients were asked to consume a 250 ml liquid meal (Resource 2.0+fibre, Nestle, UK) over fifteen minutes at the end of the rest period. Baseline blood samples were taken prior to the meal. The nutritional composition of the meal is outlined in Figure- 13.

Constituent	Per 100 ml	Per meal
Energy (Kcal)	200	500
Protein (g)	9	22.5
Carbohydrate (g)	21.4	53.5
of which sugars (g)	6	15
Fat (g)	8.7	21.75
of which saturated fatty acid (g)	0.54	1.35
Mono-unsaturated fatty acid (g)	5.43	13.575
Poly-unsaturated fatty acid (g)	2.72	6.8
Fibre (g)	2.5	6.25

Figure-12; The nutrient composition of the liquid meal given to patients after an overnight fast.

2.2.6 Reagents added to blood to prevent degradation of active hormone.

Reagent	Location	Amount	Manufacturer
DPP-IV inhibitor	Syringe	10 µl/ml blood	Millipore, Watford, UK
Aprotinin (Trasylol)	Vaccutainer	5000 KIU/ml blood	Bayer, Newbury, UK
HCl (1 N)	Plasma Tube	50 µl/ml plasma	Sigma, Dorset, UK
4-(2-Aminoethyl)-benzenesulfonyl fluoridehydrochloride (AEBSF)	Plasma Tube	100 mg/ml	Fluka, Dorset, UK

Figure-13; The protease inhibitors and HCL added to blood samples or plasma to prevent degradation of the active hormone assayed.

2.2.7 Visual analogue score

Subjective appetite, satiety, prospective food consumption nausea and irritability were assessed through visual analogue scores (VAS). Each VAS was 100 mm in length with

text expressing the most positive and the negative ratings anchored at either end (Batterham et al 2007). VAS has been validated for the assessment of subjective hunger and satiety with a high degree of reproducibility (Flint A, 2000; Raben et al, 1995). Subjects were instructed to place a cross on a linear scale and complete each scale. Subjects were discouraged from looking at their previous scores. An example of the VAS sheet is displayed below (figure-15). VAS was measured with the aid of a 300mm ruler; and the measurement from the negative end was recorded. The actual value and the change from baseline (which was defined as score at t=0) calculated and recorded for each time point at each visit. The area under curve (AUC) for each individual visit was calculated utilizing the trapezoidal method. The change in AUC was used to analyze correlation to change in plasma hormones.

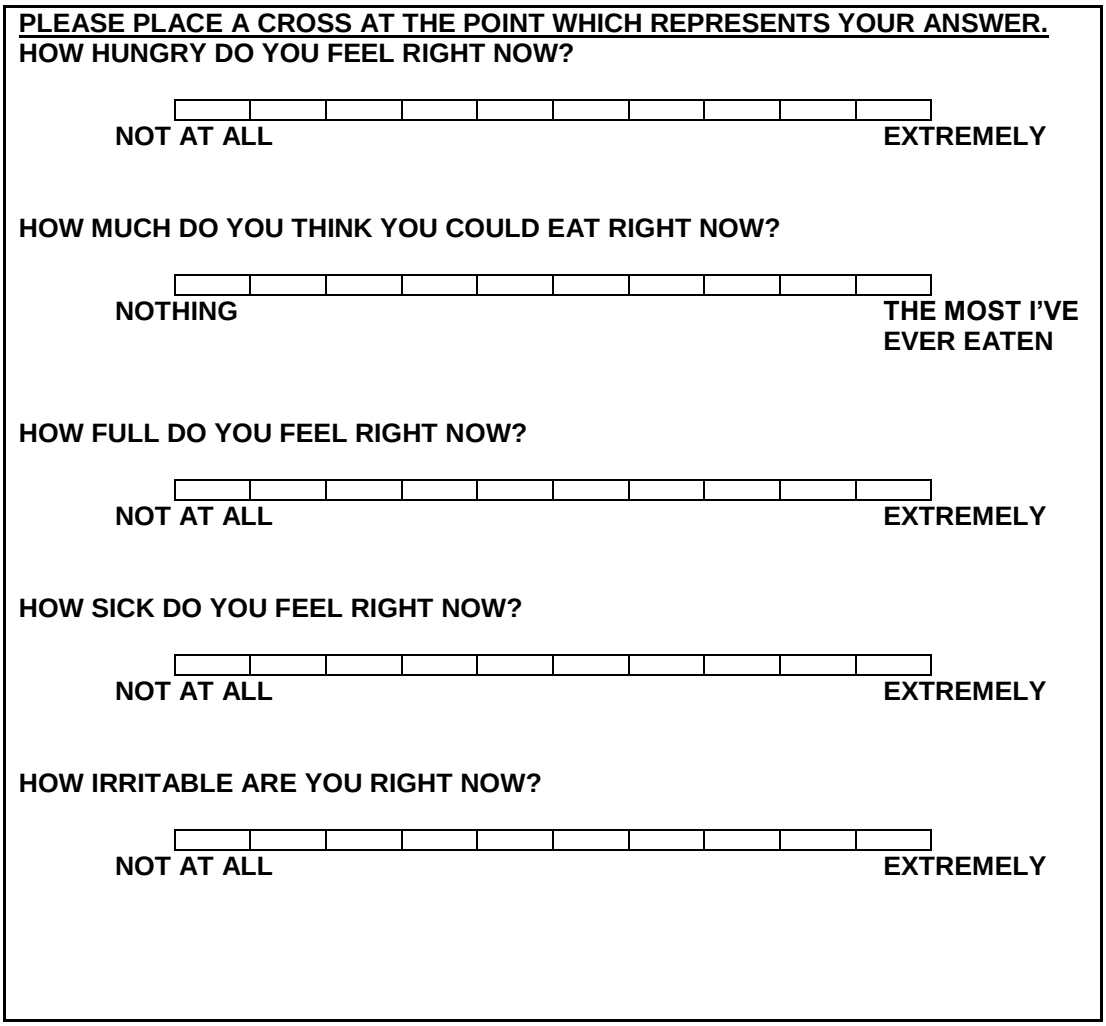


Figure-14; A sample VAS score sheet.

2.2.8 Hormone assays

All assays were performed according to the manufacturer's instructions.

Hormone	Assay	Sensitivity	Intra-assay variation	Inter assay variation	Catalogue number
Insulin	RIA	2 µU/ml	2.9	9.1	HI-14 HK
PYY3-36	RIA	20 pg/ml	4.4	8.9	PYY-67 HK
Acyl-ghrelin	RIA	7.8 pg/ml	4.0	9.4	GHRA-88 HK
Leptin	ELISA	0.5 ng/ml	3.3	N/A	EZHL-80 SK
Active GLP-1	ELISA	2 pM	5.8	2.3	EGLP-35 K
Amylin	ELISA	1 pM	4.4	3.6	EZHA-52 K

Figure-15; A list of all the assays used to measure plasma hormone levels in the study is listed. All assays were purchased from Millipore (Millipore, Watford, UK).

2.2.9 Radioimmunoassay (RIA)

RIAs were used to quantify hormones in plasma samples. The principle of a RIA is based on competitive binding between a fixed quantity of radioactively (Iodine-125, ^{125}I) labeled tracer antigen molecule of interest and a known concentration of standards of that antigen. This reaction utilises a constant volume of antibody with a fixed amount of binding sites, to the antigen molecule of interest. The amount of tracer bound to the antibody is proportionate to the concentration of the un-labelled antigen in solution. The binding of unlabeled antigen to antibody competitively inhibits tracer binding. The antibody-bound tracer antigen is precipitated from free tracer in solution. The radioactivity in the precipitate is measured with a gamma counter. The bound tracer antigen radio-activity is plotted against known standards, and this standard curve is utilized to measure samples with unknown concentration of antigen molecule of interest.

All RIA kits have similar protocols with hormone (antigen) specific reagents, antibodies and tracers. All reactions were carried out in duplicate. Each labelled polystyrene test tube (12 x 75 mm), apart from the total count tubes (TC) had the same reaction volume of 300 µl. The total count tubes had only the tracer added on the second day (100 µl). The non-specific binding tubes (NSB) consisting of assay buffer alone were utilized to assess background binding. The total binding tubes (Bo) with assay buffer (200 µl) and antibody (100µl) was utilized to assay the total binding capacity of the antibody. This ranges from 35-50% in each assay. Six serial dilutions of a known standard supplied by the manufacturer was undertaken to construct the standard curve. In all the other tubes, 100 µl assay buffer, 100 µl known standards (standard curve) or 100 µl of unknown samples and 100 µl antibody was added. This mixture was vortexed, and underwent incubation for 20-24 hours at 4 °C. The tracer was added on the second day, and again the reaction mixture vortexed, and incubated overnight for 20-24 hours at 4 °C. On the final day of the assay, 1 ml of precipitating reagent was added to all tubes except TC tubes. Tubes were vortexed and incubated for 20 minutes at 4 °C, and all tubes except the TC tubes were centrifuged at 10,000 rpm for 20 minutes at 4 °C. The supernatant was aspirated with the aid of a glass pipette and the radioactivity in the precipitate was quantified with a gamma emission counter (Packard Cobra, MN, USA). A flow chart of this procedure is presented in figure-17.

Tube Number	Description	Assay buffer (µl)	Sample (µl)	Antibody (µl)	Vortex, cover and incubate at 4 °C between 20 – 24 hours	I-125 labelled tracer (µl)	Vortex, cover and incubate at 4 °C between 20 – 24 hours	Precipitating reagent (ml)	Vortex, incubate for 20 minutes at 4 °C.	Centrifuge at 10,000 rpm for 20 minutes at 4 °C. Decant supernatant, count.
1, 2	TC	0	0	0		100		0		
3, 4	TC	0	0	0		100		1.0		
5, 6	NSB	300	0	0		100		1.0		
7, 8	NSB	300	0	0		100		1.0		
9, 10	Bo	200	0	100		100		1.0		
11, 12	Bo	200	0	100		100		1.0		
13, 14	Standard, X	100	100	100		100		1.0		
15,16	X/2	100	100	100		100		1.0		
17,18	X/4	100	100	100		100		1.0		
19, 20	X/8	100	100	100		100		1.0		
21,22	X/16	100	100	100		100		1.0		
23, 24	X/32	100	100	100		100		1.0		
25, 26	X/64	100	100	100		100		1.0		
27, 28	control	100	100	100		100		1.0		
29, 30	unknown	100	100	100		100		1.0		
31, n	unknown	100	100	100		100		1.0		

Figure-16; sample flow diagram for RIA

The average count from the duplicates was calculated. The average NSB value was subtracted from all the average counts. These values were used for subsequent calculations. The percentage binding for each sample was calculated by dividing the average counts by the total binding (Bo) count. The measured counts per minute of radiation are proportional to the amount of bound labeled tracer antigen antibody complex. A standard curve graph of known concentration versus percentage binding constructed, and the concentrations of unknown samples extrapolated from this curve. The assay was accepted if the quality controls measured with the assay were within the range provided by the manufacturer. Quality controls supplied by the manufacturer were run in duplicate. The quality control sample values from each assay were utilized to calculate inter-assay variability. Intra-assay variability was calculated from the mean variability in the standard curves.

2.2.10 Enzyme linked immunosorbant assay ELISA

ELISA's were used to quantify the concentrations of peptide hormones in plasma. The principle of an ELISA is based on capture of the antigen molecule of interest with an immobilised biotinylated antibody; this is followed by the attachment of an enzyme to this antigen-antibody complex and proportional conversion of a substrate to an end product that can be quantified with a spectrophotometer. The hormone (antigen) specific ELISA plate, reagents, and protocol varied between assays. All samples were done in duplicate. The following is an overview of the assay procedure. The 96-well plate coated with a pre-titred amount of specific monoclonal antibody to the hormone

molecule of interest was washed with 300 µl wash buffer to initiate the assay. This was decanted and residual buffer removed by tapping smartly on to absorbent paper. A specified amount of assay buffer was added to all wells apart from the standard curve wells. Matrix solution (consisting of charcoal-stripped plasma) was added to the standard curve and NSB wells. Then known standards, quality controls and unknown samples were added to the plate. This is followed by a constant volume of detection antibody to each well to allow formation of antibody-protein complexes with biotinylated polyclonal antibodies, the plate sealed and incubated for 2 hours at room temperature on an orbital microtitre plate shaker set at 600 rpm. The plastic film was removed, the contents of the wells decanted and residual solution removed as above, the plate was washed 3 times to remove unbound material, each wash followed by tapping the plate on to absorbent paper. Then a specified amount of enzyme solution was added to each well; the plate sealed again and incubated on the plate shaker for a further 30 minutes. At the end of the incubation period the contents of the wells were decanted and residual solution removed as above. The plate was washed 5 times to remove all unbound materials, each wash followed by tapping smartly onto paper towels. Finally, a specified amount of substrate solution was added to each well and the plate covered with foil to prevent degradation of the light sensitive substrate. This reaction was terminated after 15 minutes by the addition of an acidic buffer. The substrate concentration in each well was measured by a spectrophotometer, within 5 minutes. A sample flow diagram of assay procedure is attached below (figure-18).

The enzyme and substrate used for the leptin ELISA was horseradish peroxidase and 3,3’5,5’ tetramethylbenzidine (TMB). The end product of TMB degradation is a coloured product. The active GLP-1 and active amylin ELISA both utilize alkaline phosphatase as the enzyme and methyl umbelliferyl phosphate (MUP) as substrate. The MUP degradation product is fluorescent. The concentration of the end products in each well was measured spectrophotometrically through absorbance (leptin) or fluorescence (GLP-1 and amylin) on a plate reader. The results were analysed with Magellan software (Tecan, Reading, UK). As with RIA, an average of the duplicates was calculated. A standard curve constructed from known standards. The concentrations of the unknown samples were extrapolated from this curve. The assay was accepted if the manufacturer’s quality control fell within the manufacturer’s specified range.

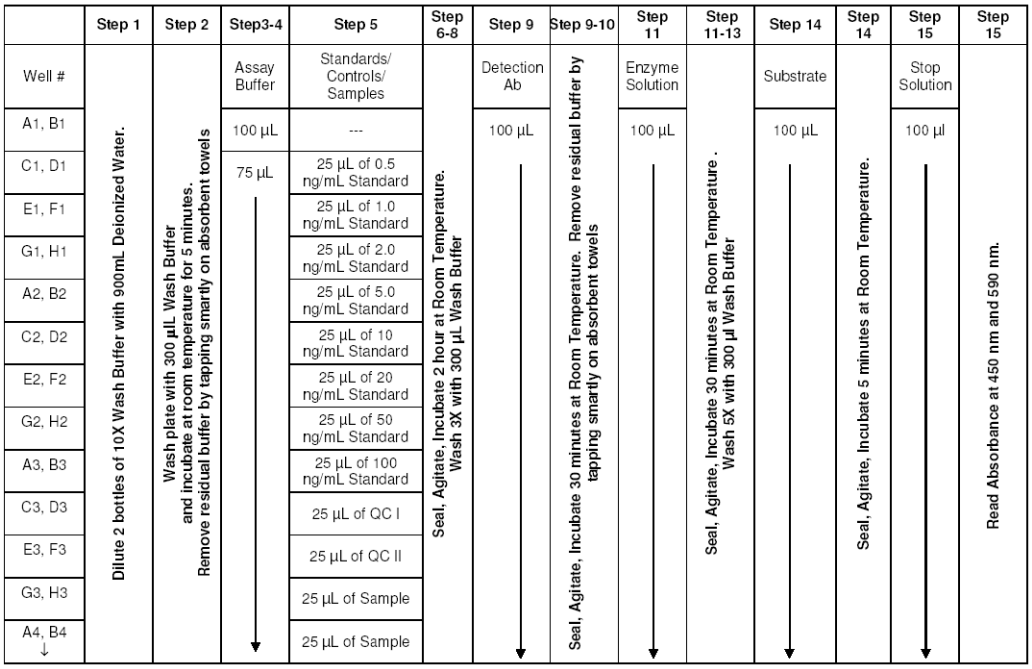


Figure-17 sample flow diagram for ELISA

2.2.11 Glucose assay

The glucose oxidase method was employed to assay plasma glucose concentrations in plasma samples. The glucose oxidase reaction with an auxiliary hydrogen peroxide indicator reaction which couples 4-aminoantipyrine to a coloured phenolic compound was used to determine glucose concentration. The amount of phenolic compound formed is proportional to the glucose concentration in solution. Six serial dilutions of a known glucose standard was incubated with glucose oxidase to construct a standard curve, As per manufacturer's instructions 3µl of plasma and 450 µl glucose oxidase was incubated at room temperature for 5 minutes in a translucent 96 well plate. The plate was then read on a spectrophotometer. The results were analysed with Magellan software (Tecan, Reading, UK). Assay sensitivity was 0.035 mmol/L, intra/ inter-assay variability: 2.59 and 2.63% respectively. All reagents for this assay were supplied by Thermo scientific, Auchtermuchty Fife, UK.

2.2.12 HOMA IR

An individual's insulin resistance at each visit was calculated using the fasting glucose and fasting insulin values from each visit and the HOMA calculator available on line at www.dtu.ox.ac.uk.

2.2.13 body composition analysis

Each patient's weight and anthropometry at each visit was measured with a body composition analyser (Inbody 720, Derwent Healthcare, UK). The body composition analyzer combines the use of multi frequency current with a direct segmental measurement to give a detailed printout. Height was measured with a stadiometer. BMI was calculated from the above values.

It is known that the electrical resistance of tissues is directly related to their fluid content. The fat-free mass is a good electrical conductor, whereas adipose tissue is an electrical insulator. Bio-impedence correlates to total body water. The conducting volume can be measured utilizing Ohm's law; volume of constant is proportional to the length squared divided by its resistance. Limitations include geometry of conductor. There is a linear relationship between height and fat free mass, provided the conductor measured is cylinder or has a constant section. This is not accurate as most machines utilise wrist to ankle, and consists of two long thin conductors (limbs) separated by a shorter and thicker one (trunk). The measured impedance of the trunk is dependent on body position and respiratory cycle. An assumption of homogeneous conductor is also made. It is also worth noting that the anatomic disposition of the injecting and sensing electrodes also play a part in determining accuracy. The relative error rates when compared DEXA is dependent on the segment measured (Martinsen OG and Grimnes S 2011)

2.2.14 Statistical analysis

All data was collected in Microsoft Excel and further analysis and preparation for graphical presentation was carried out in SPSS 11.0 (SPSS, IL, USA) and GraphPad Prism 4 (GraphPad Software, CA, USA). All data is presented as mean ± standard error of mean (SEM), otherwise indicated. The sample size for the two human studies are noted in each section by 'n ='. Area under curve was calculated using the trapezoid rule. Statistical analysis was performed using paired or unpaired two-tailed Students' t-test, repeated measures one way and two way analysis of variance (ANOVA) with Bonferroni post-hoc tests as described. The change in plasma hormone values from baseline is shown as Δ and displayed where appropriate. P values of * $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$ were reported as statistically significant.

This was a pilot study. However, previous studies from Batterham and colleagues (Batterham R et al 2003) did point us towards expected means, standard deviations (6 pmol/ L) and assay variability. In the application we suggested a sample size of 12 subjects per group which will have a 90% power to detect a difference in PYY levels of 5.85 pmol/L at the 5% confidence level. We supplemented this knowledge with initial data on our first 7 patients: Insulin; pre-op peak 94.4± 18.8 pM at 6 weeks 262.8± 96.4 and at 12 weeks 159.9± 54.4, PYY3-36; pre-op peak 38.3 ±3.6 and 82.2 ±8.5 at 6 weeks and 94.4± 18.8 at 12 weeks. We utilized this data to supplement our initial assertions. The inter-assay variation for PYY3-36 was 6.4% (Chandarana K et al 2009). The inter-assay variation for acyl-ghrelin was 4.5% (Chandarana et al 2009), and for active GLP-1 the inter-assay variability was 6.4% (Chandarana et al). We were able to recruit 17 patients in 2 years.

Chapter 3

Appetite and weight loss after RYGBP and SG correlate to gut hormones

3 The effect of RYGBP and SG on appetite, anthropometric indices and gut hormones

3.1 Introduction

The homeostatic mechanisms to maintain bodyweight limit the effectiveness of lifestyle interventions (Heymsfield et al, 2003; Leibel et al, 1995; Tsai and Wadden, 2005). Current National Institute for Clinical Excellence (NICE) guidelines advice bariatric surgery for individuals with a BMI > 40 kg/m^2 or BMI 35-40 kg/m^2 with other significant disease such as T2DM and OSA that could be improved with weight loss, and where non-surgical therapies have failed (NICE, 2006). Bariatric surgery is the most effective therapy for morbid obesity (Buchwald H et al 2004, SOS- Sjostrom et al 2007). A greater percentage excess weight loss and (29%) reduction in mortality is seen after an average follow-up of 10.9 years (Sjostrom et al 2007). RYGBP is the commonest bariatric surgical procedure performed (Buchwald et al 2009). SG has gained prominence as a sole operation for morbid obesity (Bohdjalian A et al 2010). SG represented 5.3% of all bariatric procedures performed worldwide in 2008 (Buchwald et al 2009). The change in gut hormones after RYGBP has been shown to correlate to weight loss in animal models (Shin AC et al 2010). Further, changes in bodyweight are thought to be a consequence of a reduction in appetite (Hafner et al 1991, Halmi et al 1981, Borg et al 2006, Korner et al 2005).

The equivalent outcome after RYGBP and SG has led to studies and reviews comparing RYGBP and SG with respect to weight loss and glucose homeostasis (karamanakos SN et al 2008, Peterli R et al 2009, Li F et al 2009, Juan P. Valderas et al 2010, F Abbatini et al 2010, Valderas JP et al 2010, Franco JVA et al 2011, Basso N et al, Chambers AP et al 2011, Peterli R et al 2012 and Ramon J M et al 2012, Yousseif A and Emmanuel J et al 2013). They highlight gut hormone changes that are not dissimilar despite quite different anatomical arrangements of the gastrointestinal tract after these two procedures. However, the mechanisms mediating weight loss after these procedures are still debated (Sharkey KA 2011, Kenny PJ et al 2011, De Silva A et al 2011, Gass M et al 2011, Karra et al 2010, Scott W and Batterham R 2011, Peterli R et al 2012, Ramon J M et al 2012, Yousseif A and Emmanuel J et al 2013).

The one study to compare PYY3-36 after RYGBP and SG found a similar response after both procedures; this response was attenuated at 3 months after surgery (Peterli R et al 2009). Valderas and colleagues confirmed improvement in appetite scores after surgery, further total PYY AUC did correlate to appetite (Valderas JP et al 2010). A cross sectional study found correlation between increase in total PYY and appetite after RYGBP (Pournaras DJ et al 2010). Most studies have measured total ghrelin following

RYGBP and SG (Langer et al 2005, Karamanakos et al 2008, Li F et al 2009, Wang Y et al 2009, Peterli R et al 2009, Bohdjalian A et al 2010, Lee WJ et al 2011, and Peterli R et al 2012). One has noted a decline in fasting total ghrelin after SG (Ramon J M 2012), the decline in total ghrelin is maintained for up to 5 years after SG (Bohdjalian A et al 2010). Other studies have shown no change or rise in ghrelin (reviewed by Papilou J et al 2010). No study to date has measured acyl-ghrelin after RYGBP and SG. Others have recently shown a paradoxical rise in acyl-ghrelin after RYGBP at 12 months after surgery (Barazzoni R et al 2013).

Recent studies have also examined the active GLP-1 response after RYGBP in humans (Bose M et al 2009, Peterli R et al 2009, Mela L et al 2011, and Peterli R al 2012) and animals (Shin AC et al 2010). Studies comparing RYGBP and SG have noted; significantly less pronounced response after SG (Peterli R et al 2009, and Peterli R et al 2012). The cross sectional study by Pournaras and colleagues found correlation between GLP-1 and appetite (Pournaras DJ et al 2010). The active GLP-1 response has also been noted to correspond to weight loss after surgery, when an arbitrary cut off was utilised (le Roux CW et al 2007). Somatostatin, an inhibitor of gut hormone release reversed appetite. Further, a recent study on patients having undergone RYGBP showed that infusions of PYY3-36 and long acting GLP-1 analogoues at pharmacological doses were able to cause further weight loss.

A significant increase in meal stimulated active amylin secretion is seen in rats undergoing RYGBP, but no correlation between active amylin secretion and weight loss is seen (Shin AC et al 2010). Two other studies (Bose M et al 2010, Jacobsen S H et al 2012) have examined the role of total amylin after RYGBP. There have been no studies on meal stimulated active amylin secretion after RYGBP and SG in humans.

The heterogeneity in study protocols and procedures has led to difficulties in making substantial comparison between studies. An accurate assessment of appetite scores and gut hormone levels are dependent on study design and experimental protocol in human studies on obesity surgery. Though changes in gut hormones favour weight loss, correlation between changes in individual gut hormones and weight loss has not yet been shown in humans. This may be related to study design and sample processing. Several studies have looked at gut hormone changes after surgery (Cummings De et al 2002, Langer et al 2005, Korner J et al 2006, le Roux et al 2006, le Roux et al 2007, Whitson BA et al 2007, Karamanakos SN et al 2008, De Paula et al 2009, Y Wang et al 2009, Li F et al 2009, Peterli R et al, 2009, Bose M et al 2010, Abbatini F et al 2010, Bohdjalian A et al 2010, Basso N et al 2011, Chambers AP et al

2011, Jacobsen S H et al 2012, Peterli R et al 2012). However, not all have measured the active forms of the circulating hormone under investigation (Cummings De et al 2002, Langer et al 2005, Korner J et al 2006, Whiston BA et al 2007, Karamanakos SN et al 2008, De Paula et al 2009, Wang Y et al 2009, Li F et al 2009, Bose M et al 2010, Abbatini F et al 2010, Bohdjalian A et al 2010, Basso N et al 2011, Chambers AP et al 2011, Peterli R et al 2012, Jacobsen S H et al 2012). Further samples were not collected into tubes containing protease inhibitors to ensure no degradation of these peptides occur prior to analysis (Cummings De et al 2002, Korner J et al 2005, Langer FB et al 2005, Korner J et al 2006, Whitson BA 2007, Wang Y et al 2009, Li F et al 2009, Lopez PP et al 2009, Bohdjalian A et al 2010, N Basso et al 2011). Some studies looked at post operative changes several months to years after surgery (Korner J et al, 2005, Korner J et al 2006, Y Wang et al 2009, Karamanakos et al 2008, Bohdjalian A et al 2010, Chambers A P et al 2012) missing early physiological changes. Others have utilized cohorts to compare post-surgical changes in gut hormones in patients against control groups (Cummings De et al 2002, Korner J et al, 2005Lopez PP 2009, Whitson BA 2007, Oliván B et al 2009, Bose M et al 2010, Valderas JP et al 2010), and not to their pre-operative state, making it difficult to draw conclusions on individual physiological changes and correlation to outcomes after surgery. Comparison of matched cohorts can also lead to natural inter-individual variation masking procedure related small changes in gut hormones in individuals. Further, it is not possible to make comparisons of temporal profiles across cohorts of individual's and correlate this to outcome measures. In the case of acyl-ghrelin no human study to date has collected blood samples with HCL and protease inhibitors to measure this active octanoylated form prior to degradation, as per manufacturer's instructions on assay protocols. The suppression of acylated ghrelin did correlate to weight loss after RYGBP in rats (Shin AC et al 2010). However, this study like others (Oliván B et al 2009, Langer FB 2005, Bohdjalian A et al 2010) only added protease inhibitors but not HCL to the collection tubes. Other studies measured total ghrelin without the addition of protease inhibitors or HCL (Lopez PP et al 2009, Korner J et al 2005, Whitson BA et al 2007, Karamanakos et al 2008, Bose M et al 2010, Wang Y 2009, Li F et al 2009, Peterli R et al 2012- only Aprotinin, Ramon J M et al 2012) or did use HCL without protease inhibitors (Korner J et al 2006). Further, some studies that have looked at PYY 3-36 and active GLP-1, failed to add DPP4 inhibitor to the samples (DePaula AL et al 2009, Korner J et al, 2005, Korner J et al 2006, le Roux CW et al 2006, Whitson BA et al 2007, le Roux CW et al 2007, Karamanakos et al 2008, Li F et al 2009, Valderas JP et al 2010, Umeda L M et al 2011). There have been no studies to investigate meal stimulated PYY3-36 secretion after SG in humans.

With respect to how gut hormones mediate weight loss; no study to date has found correlation between changes in active gut hormones after RYGBP and changes in appetite. This may be because only a few studies have looked at appetite scores alongside gut hormones (Korner J et al, 2005, Korner J et al 2006, Buchwald et al 2007, Karamanakos SN et al 2008, DePaula AL et al 2009, Valderas JP et al 2010**)**. Studies that have looked at visual analogue scores (VAS) utilised only two time points per visit (Korner J et al, 2005, Korner J et al 2006, Karamanakos et al 2008). Therefore correlation analysis between changes in gut hormones measured at several time points after a mixed meal and change in appetite and satiety measured at a single time point after a meal has not been feasible. Some studies did employ several time points to measure VAS (Buchwald et al 2007, Karamanakos SN et al 2008, DePaula AL et al 2009, and Valderas JP et al 2010).

We conducted a prospective parallel group study on patients undergoing RYGBP and SG to measure changes in fasted and meal-stimulated active gut peptide hormones PYY3-36, acyl-ghrelin, active GLP-1, active amylin and subjective measures of appetite simultaneously in a tertiary hospital setting. Patients were studied at three time points; week before surgery whilst mid-way through the liver reducing diet, and at 6 and 12 weeks post-surgery. This enabled us to standardise patients prior to the study day across three visits. Patients consumed a high calorie standardised mixed liquid meal following acclimatisation to the stress of canulation. This enabled us to study comparative post prandial response on all three visits, after SG and RYGBP, where volumes consumed over a 15 minute period were severely restricted. The calorie content of the mixed meal drinks offered to patients was in keeping with previous published studies by Batterham and colleagues (le Roux et al 2006). An estimated percentage of energy requirement mixed meal drink would have required higher volumes to be consumed soon after surgery, and thus longer to consume. This would have led to difficulties with standardisation across three visits and would have also skewed the timing of hormone sampling after the meal. The restrictive nature of these procedures limit volume consumed immediately after the procedure, and thus a large volume liquid meal post-operatively may adversely influence hormone measurements and analysis for comparison across three visits.

3.2 Results

3.2.1 Comparison of baseline anthropometry/ biochemistry/ gut hormone profile and VAS between RYGBP and SG groups

N=18 in all calculations apart from when noted	RYGBP mean± (95%CI)	SG mean ± (95%CI)
Age (years)	49.3 ± (45.6-53.0)	41.5 ± (34.0-49.0)
weight (kg)	125.7 ± (116.4-135.0)	127.5 ± (109.0-133.1)
fat mass (kg)	68.1 ± (62.0-74.2)	66.0 ± (54.9-69.2)
BMI kg/m^2	47.6 ± (44.6-50.6)	46.7 ± (40.2-47.9)
Fasting glucose (mmol/L)	5.61 ± (4.1-6.5)	4.93 ± (4.3-5.3)
Hunger AUC (mm)	4018 ± (1271-6764)	4377 ± (2188-6566)
Fasting hunger (mm)	34.7 ± (12.4-57.2)	48.5 ± (37.5-59.5)
Satiety AUC (mm)	7614 ± (4302-10925)	6829 ± (3977-9680)
Fasting satiety (mm)	23.4 ± (10.4-36.5)	22.3 ± (9.6-34.9)
Prospective food consumption AUC (mm)	4498 ± (2015-6980)	5372 ± (3569-7175)

N=18 in all calculations apart from when noted	RYGBP mean ± (95%CI)	SG mean ± (95%CI)
Fasting prospective food consumption (mm)	**30.6 ± (11.9-49.4)**	**45.8 ± (34.0-57.5)**
HOMA IR	**1.5 ± (0.85-2.1)**	**3.1 ± (0.75-2.27)**
PYY 3-36 AUC pM	**6364 ± (5443-7284)**	**6083 ± (2485-9680)**
Fasting PYY3-36 pM	**19.4 ± (11.7-27.1)**	**21.9 ± (2.4-41.4)**
Acyl- ghrelin AUC pM	**7606 ± (5520-9692)**	**7023 ± (5791-8254)**
Fasting acyl-ghrelin pM	**53.0 ± (32.8-73.2)**	**44.7 ± (34.6-54.9)**
Insulin AUC pM	**83743 ± (43478-118041)**	**111133 ± (27190-195077)**
Fasting insulin pM	**76.7 ± (47.0-106.5)**	**180.2 ± (15.4-345.1)**
Amylin AUC pM (n=17)	**2123 ± (582-3665)**	**2317 ± (1394-3241)**
Fasting amylin pM (n=17)	**6.5 ± (0.8-12.3)**	**6.9 ± (3.6-10.2)**
GLP-1 AUC pM (n=17)	**1339 ± (819-1859)**	**931 ± (836-1026)**
Fasting GLP-1 pM (n=17)	**4.4 ± (2.9-5.9)**	**4.2 ± (4.0-4.4)**
Leptin pM	**4596 ± (3754-5438)**	**4278 ± (3491-5065)**

Figure-18: A comparison of baseline anthropometric characteristics; baseline AUC and fasting values of measured VAS; calculated HOMA IR (calculator available on line at www.dtu.ox.ac.uk); fasting and calculated baseline AUC of all the plasma hormone temporal profiles and fasting plasma leptin in the two groups of patients. The SG group was significantly younger.

The SG group was significantly younger, and tended to have a lower BMI than the RYGBP group (Figure-19). Other published comparative studies have also had similar differences. The patients in the SG group were younger in the study by Karamanakos and colleagues (Karamanakos et al 2008). This difference in baseline BMI was within the inclusion criteria at recruitment, and within the criteria for referral for bariatric surgery. Two patients in the SG group had a BMI below 40. This was due to peri-operative weight loss. In the study by Valderas and colleagues some patients did also start with a BMI below expected due to the peri-operative diet (Valderas JP et al 2010).

All patients followed a low calorie diet for two weeks prior to surgery. There was no difference in any other baseline characteristics (weight, fat mass, glucose, HOMA IR, meal-stimulated PYY3-36/ acyl-ghrelin/ insulin/ active amylin/ active GLP-1/ leptin, subjective hunger/ fullness/prospective food consumption) between the two groups.

3.2.2 Equivalent excess weight and BMI loss in both RYGBP and SG groups

At 6 weeks post-surgery we found that both groups had similar percentage excess weight loss (%EWL) after RYGBP (23 ± 2%) and SG (26 ± 4%) p=0.46 (see table). At 12 weeks post-surgery we also found similar percentage excess weight loss (%EWL) after RYGBP (34 ± 3%) and SG (37 ± 5%) p= 0.66. There is also equivalent BMI loss at 6 weeks and 12 weeks after both procedures. Despite starting with a lower BMI, the SG group lost similar BMI points (4.5 ± 0.45) to the RYGBP group (4.9 ± 0.28, p=0.43) at 6 weeks, and at 12 weeks SG after surgery (6.6 ± 0.62) RYGBP (7.4 ± 0.40, p=0.28). This is in keeping with the published literature (Karamanakos et al 2008, Peterli R et al 2009, Valderas JP et al 2010 and De Gordejuela AG et al 2011)

	RYGBP	SG	t-test p value
% EWL at 6week	23 ± 2%	26 ± 4%	0.46
Change in BMI at 6wk (kg/m^2)	4.9 ± 0.28	4.5 ± 0.45	0.43
% EWL at 12 weeks	34 ± 3%	37 ± 5%	0.66
Change in BMI at 12 wk (kg/m^2)	7.4 ± 0.40	6.6 ± 0.62	0.28

Figure-19; a comparison of excess weight and BMI loss after RYGBP and SG, an equivalent excess weight loss and BMI loss at 6 and 12 weeks after both surgical procedures.

3.2.3 Visual analogue scores for hunger, satiety and prospective food consumption.

3.2.3.1 Hunger VAS in the RYGBP group

Subject Number	Pre-op fasting (mm)	Pre-op AUC (mm)	6 wk fasting (mm)	6 Wk AUC (mm)	12 wk fasting (mm)	12 wk AUC (mm)
2	44	10853	5	975	6	1358
5	56	3833	5	697.5	5	922.5
6	56	7268	4	1005	5	1020
7	16	4215	25	2985	16	1358
8	84	6218	15	877.5	92	1448
9	0	30	2	713	3	900
11	5	802.5	6	862.5	3	855
12	46	2070	25	1208	14	1463
14	6	870	4	1020	5	900

Figure-20; Fasting and total AUC hunger before and after RYGBP

3.2.3.2 Hunger VAS in the SG group

Subject Number	Pre-op fasting (mm)	Pre-op AUC (mm)	6-wk fasting (mm)	6 wk AUC (mm)	12 wk fasting (mm)	12 wk AUC (mm)
1	24	7763	94	1500	54	3278
4	55	3750	14	2235	96	2715
10	44	1380	75	7193	65	1380
13	47	4770	6	967.5	6	2130
15	44	1650	43	1410	97	3398
17	45	3105	6	1313	26	967.5
18	65	4005	26	1725	14	3315
19	64	8595	14	3533	75	4283

Figure-21; Fasting and total AUC hunger before and after SG

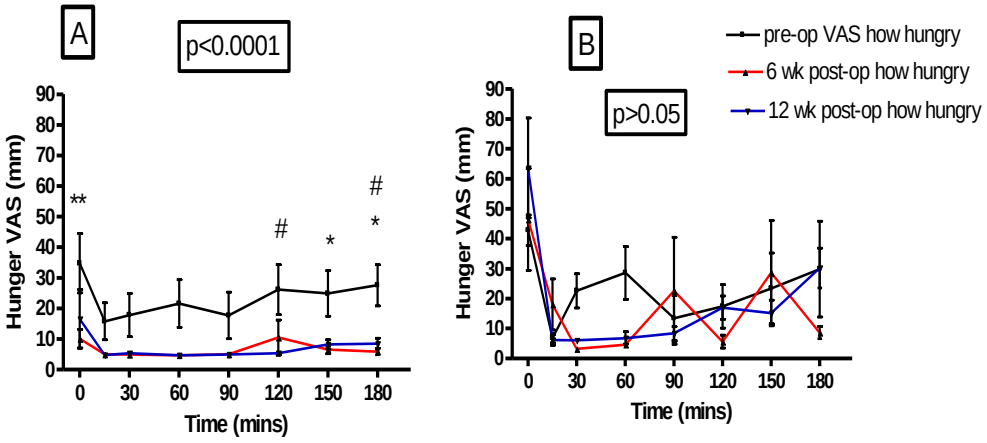


*Figure-22; Temporal changes in hunger VAS over the three visits in the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant (p<0.0001) decline in hunger- two way matched ANOVA, comparison of pre-operative against post-operative time points. This decline does not reach statistical significance in the SG group (p>0.05) - two way matched ANOVA, comparison of pre-operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows significant decline at fasting (t=0) and at t=150, t=180 at 6 weeks, and at t=120, t=180 at 12 weeks in the RYGBP group (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

3.2.3.3 Prospective food consumption in the RYGBP group

subjects	Pre- op fasting (mm)	Pre-op AUC (mm)	6-wk fasting (mm)	6 wk AUC (mm)	12 wk fasting (mm)	12 wk AUC (mm)
2	44	10635	35	2168	15	3300
5	6	4785	14	780	16	1103
6	46	6488	5	960	5	937.5
7	25	5303	14	1178	6	1268
8	74	6263	15	840	43	1170
9	0	795	3	705	5	975
11	4	907.5	43	1155	5	1665
12	33	4163	26	1035	24	1410
14	44	1140	15	1005	25	1305

Figure-23; Fasting and total AUC prospective food consumption VAS before and after RYGBP

3.2.3.4 Prospective food consumption in the SG group

subjects	Pre-op fasting (mm)	AUC (mm)	6-wk fasting (mm)	AUC (mm)	12 wk fasting (mm)	AUC (mm)
1	25	7238	87	1575	75	5550
4	64	6210	36	3165	95	2453
10	35	2655	55	1545	43	2738
13	64	7178	24	2325	25	3750
15	44	2153	34	1395	95	3075
17	44	5078	16	1838	36	1170
18	36	4530	14	1620	25	3488
19	54	7935	24	4208	54	4215

Figure-24; Fasting and total AUC prospective food consumption VAS before and after SG

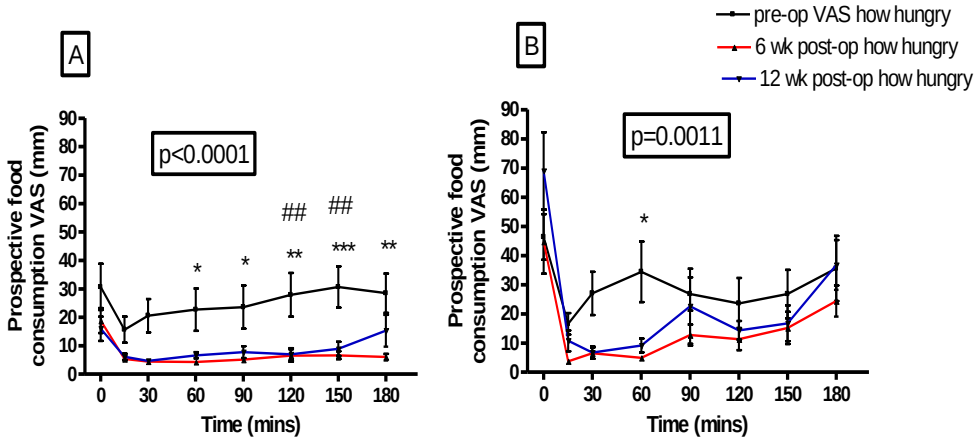


*Figure-25; The temporal changes in prospective food consumption VAS over the three visits for the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant (p<0.0001) decline in prospective food consumption- two way matched ANOVA, comparison of pre-operative against post-operative time points. This decline is also significant in the SG group (p=0.0011)- two way matched ANOVA, comparison of pre-operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows significant decline at t=60, t=90, t=120, t=150 and t=180 at 6 weeks, and at t=120, t=150 at 12 weeks after RYGBP. In the SG group significant decline is only noted at t=60 at 6 weeks after surgery, (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

3.2.3.5 Satiety VAS in the RYGBP group

subjects	Pre-op fasting (mm)	Pre-op AUC (mm)	6-wk fasting (mm)	6 wk AUC (mm)	12 wk fasting (mm)	12 wk AUC (mm)
2	15	4148	75	15473	44	4688
5	45	7673	84	17040	24	16305
6	8	5363	65	16560	75	16148
7	25	2918	44	15593	15	15098
8	5	7380	66	11655	4	12180
9	50	4553	62	11535	5	2783
11	35	7695	5	6030	4	5738
12	23	12405	26	15743	63	15818
14	5	16388	5	14753	6	15930

Figure-26; Fasting and total AUC satiety VAS before and after RYGBP

3.2.3.6 Satiety VAS in the SG group

Subjects	Pre-op fasting (mm)	AUC (mm)	6-wk fasting (mm)	AUC (mm)	12 wk fasting (mm)	AUC (mm)
1	36	6915	5	14715	5	11423
4	34	9600	6	12458	6	14753
10	15	8093	7	14858	15	14790
13	44	7283	48	14415	47	13485
15	26	2475	5	14828	5	12998
17	5	12690	77	16058	56	16688
18	16	3525	84	15248	65	13853
19	2	4050	45	11348	16	11220

Figure-27; Fasting and total AUC satiety VAS before and after SG

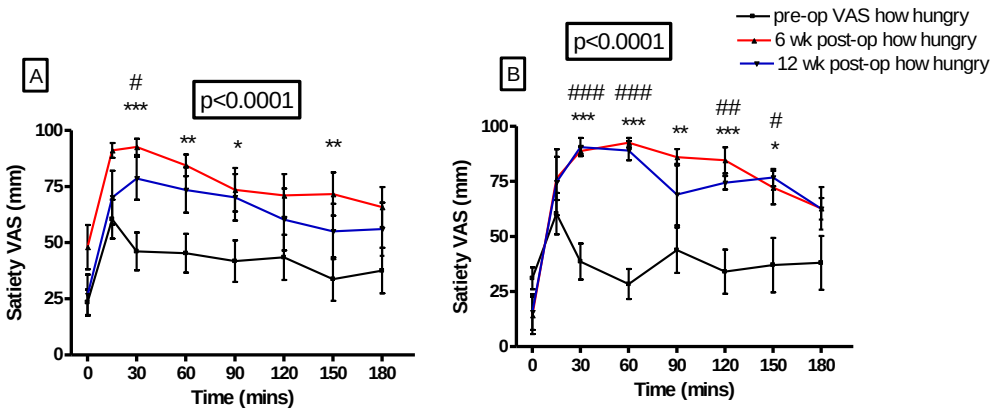


Figure-28; A comparison of changes in satiety VAS over the three visits for the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant

*(p<0.0001) increase in satiety- two way matched ANOVA, comparison of pre-operative against post-operative time points. This increase is also highly significant (p<0.0001) in the SG group- two way matched ANOVA, comparison of pre-operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows significant increase at t=30, 60, 90 and 150 at 6 weeks after RYGBP and at t=30 at 12weeks after surgery. This increase is significant at t=30, 6, 90, 120 and 150 at 6 weeks after SG, and at t=30, 60, 120 and 150 at 12 weeks after surgery (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

3.2.4 A differential change in subjective appetite and satiety after RYGBP and SG

There was a significant decline in the hunger score AUC from 4018 ± 1191mm at baseline pre-operative state to 1149 ± 236 mm (p=0.044) at 6 weeks after surgery and to 1136 ± 88 mm (p=0.036) at 12 weeks after surgery in the RYGBP group. There is no significant difference between 6 and 12 week AUC (p=0.95, paired t-test). In contrast no significant change in hunger AUC was observed following SG, which declines from 4377 ± 926 mm at baseline pre-operative state to 2485 ± 728 mm at 6 weeks (p=0.189) and then to 2683 ± 396 mm at 12 weeks (p=0.059). However, this change from baseline to 12 weeks does show a trend towards significance. As with the RYGBP group there is no significant (p=0.83, paired t-test) difference between 6 and 12 weeks. Comparison of RYGBP and SG groups hunger AUC at 6 weeks does show a trend towards significance (p=0.15). This difference is highly significant (P=0.0036) at 12 weeks. The hunger AUC decreased in both surgical groups but was statistically significant only in the RYGB group. The satiety AUC was found to be significantly augmented in the RYGB and SG groups.

In the RYGBP group baseline pre-operative prospective food consumption AUC significantly (p=0.009) declined from 4498 ± 1077 mm to 1092 ± 145 mm at 6 weeks, and then to1459 ± 242 mm (p=0.013) at 12 weeks. This increase between the 6 and 12 week time point is highly significant (p=0.0096). In the SG group there was also a significant (p=0.001) decline from 5372 ± 763 mm at baseline pre-operative state to 2209 ± 351 mm at 6 weeks and to 3305 ± 458 mm (p=0.018) at 12 weeks. This rise between 6 and 12 weeks does show a trend towards significance (p=0.086). Further, there is a significant difference between the RYGBP and SG groups at 6 weeks (p=0.033) and 12 weeks (p=0.0003), this latter difference is highly significant.

Following the mixed meal a more pronounced improvement in satiety was seen after surgery in the SG group. In the RYGBP group the pre-operative baseline satiety AUC

significantly (p=0.0093) increased from 7614 ± 1436 mm to 13820 ± 1172 mm at 6 weeks and to 11632 ± 1870 mm (p=0.057) at 12 weeks. The latter rise from baseline did show a trend towards significance. The increase between 6 and 12 weeks did also show a trend towards significance (p=0.171). The increase from baseline was more pronounced after SG. The baseline pre-operative AUC significantly (p=0.0005) increased from 6829 ± 1206 mm to 14241 ± 548 mm at 6 weeks and to 13651 ± 642 mm (p=0.0001) at 12 weeks. There was no significant (p=0.355) change between 6 and 12 weeks after SG. A comparison of RYGBP and SG groups does not show a significant difference at 6 weeks (p=0.759) and 12 weeks (p=0.291). Statistical analysis with two way matched ANOVA comparing pre-operative temporal profile to post–operative temporal profile at 6 weeks and 12 weeks after surgery does point to a differential response in hunger, satiety and prospective food consumption VAS between the RYGBP and SG groups. There is a more pronounced decline in hunger VAS after RYGBP (two way matched ANOVA, p<0.0001). In this group Bonferroni post tests analysis indicates significant decline at t=0 (p<0.01), t=150 (p<0.05) and t=180 (p<0.05) at 6 weeks, and at t=120 (p<0.05) and t=180 (p<0.05) at 12 weeks after surgery (figure-23). The same analysis comparing pre-operative temporal profile with 6 and 12 week post-operative temporal profiles points to no significant decline in hunger after SG (two way matched ANOVA, p>0.05). Bonferroni post tests also confirm no significant change at any time points. Interestingly, similar analysis with a two way matched ANOVA of the change in hunger VAS (delta hunger) from baseline (t=0) did not show a significant disparity between the procedures: after RYGBP (p=0.0159), and SG (p=0.0074). This analysis seems to point towards a more prominent decline after SG. Further, Bonferroni post-test analysis did not show any significant difference at any time point after both surgical procedures (figure-30)

3.2.5.1 Change in delta hunger

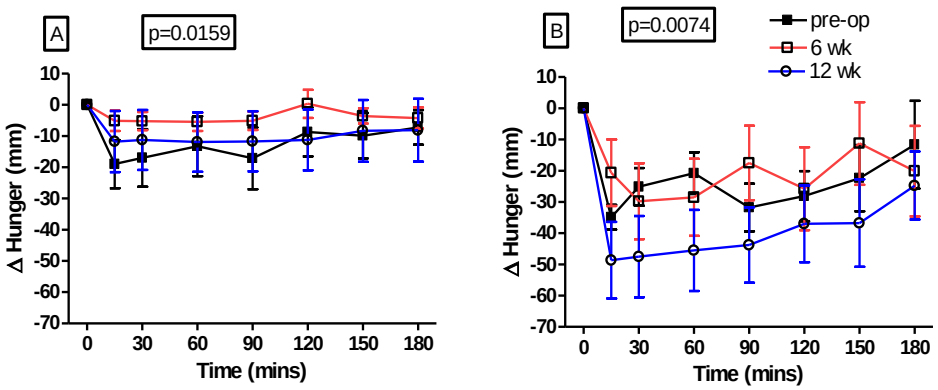


Figure-29; The temporal change in delta hunger VAS over three visits for the RYGBP (A) and SG (B) groups are shown. In the RYGBP group, surgery leads to a significant (p=0.0159) decline in delta hunger- two way matched ANOVA, comparison of pre-

*operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows no significant decline at any time point. This decline does also reach statistical significance (p=0.0074) in the SG group- two way matched ANOVA, comparison of pre-operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows no significant decline at any time point (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

The pronounced decline in hunger after RYGBP is also reflected in the analysis of prospective food consumption. In the RYGBP group surgery leads to a highly significant (p<0.0001) decline in pre-operative temporal profile (two way matched ANOVA). Bonferroni post-test analysis reveals significant decline at t=60 (p<0.05), t=90 (p<0.05), t=120 (p<0.01), t=150 (p<0.001), and t=180 (p<0.01) at 6 weeks, and at t=120 (p<0.01), t=150 (p<0.01) at 12 weeks after surgery. The prospective food consumption temporal profile does also decline significantly (p=0.0011) after SG, but is not as pronounced. Further, Bonferroni post-test analysis only shows significant decline at t=60 (p<0.05) at six weeks after surgery (figure-26). There is no significant decline at any time point at 12 weeks after surgery in the SG group. There was a significant decline in delta (change from baseline/ t=0) prospective food consumption after RYGBP (p=0.0505) and SG (p<0.0001). As with delta hunger there was a more pronounced decline in delta prospective food consumption after SG when compared to RYGBP. Again Bonferroni post-test analysis did not show any significant change at any time point after surgery in both groups (figure-31).

3.2.5.2 Change in delta prospective food consumption

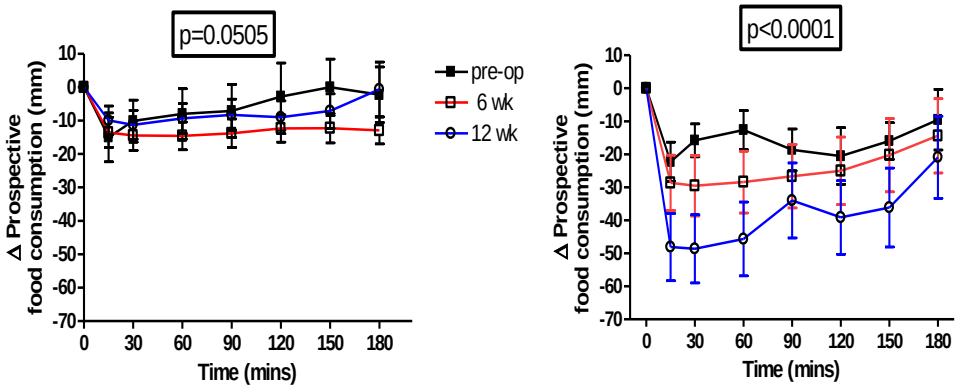


Figure-30; the change in delta prospective food consumption VAS over the three visits in the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant (p=0.0505) decline in prospective food consumption (two way matched ANOVA, comparison of pre-operative against post-operative time points). This decline is also highly significant in the SG group (p<0.0001) - two way matched ANOVA,

*comparison of pre-operative against post-operative time points. Post-hoc analysis with Bonferroni tests shows no significant decline at any time point at 6 week and 12 weeks after RYGBP and SG (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

There is a similar increase in satiety temporal profile after RYGBP and SG. Statistical analysis comparing pre-operative temporal profile with post-operative 6 week and 12 week temporal profiles shows a highly significant increase in both groups (two way matched ANOVA, p<0.0001 in both groups). However, Bonferroni post-test analysis does point to a more pronounced response after SG. After RYGBP at 6 weeks there is significant increase at t=30 (p<0.0001), t=60 (p<0.01), t=90 (p<0.05), t=150 (p<0.01), and after 12 weeks there is significant increase at t=30 (p<0.05) only. However, after SG there is a significant increase at 6 weeks at t=30 (p<0.001), t=60 (p<0.001), t=90 (p<0.01), t=120 (p<0.001), t=150 (p<0.05), and at t=30 (p<0.001), t=60 (p<0.001), t=120 (p<0.01) and t=150 (p<0.05) after 12 weeks (figure-29). The increase in delta satiety VAS (change from baseline/ t=0) was similar but more pronounced after SG. In the RYGBP group comparison of pre-operative time point with post operative time points point to a significant (p=0.0005) increase in delta satiety temporal profile (two way matched ANOVA). After SG there was a more pronounced significant (p<0.0001) increase in delta satiety after the mixed meal. Further, Bonferroni post test analysis did confirm a significant improvement in delta satiety at t=30 (p<0.01) at 6 weeks, and at t=30 (p<0.01), t=60 (p<0.01) at 12 weeks after SG but at no time points after RYGBP (figure-32).

3.2.5.3 Change in Delta satiety

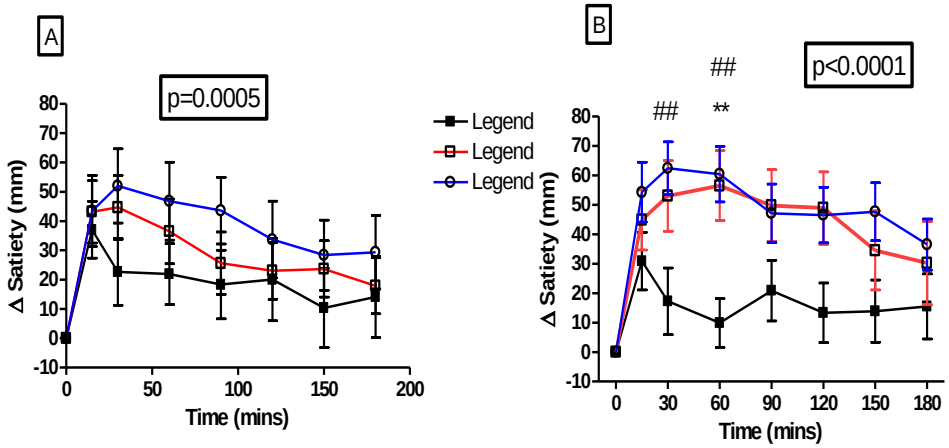


Figure-31; the temporal changes in delta satiety (change from t=0) VAS over the three visits in the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant (p=0.0005) increase in delta satiety (two way matched ANOVA,

*comparison of pre-operative against post-operative time points). This increase is also highly significant (p<0.0001) in the SG group (two way matched ANOVA, comparison of pre-operative against post-operative time points). Post-hoc analysis with Bonferroni tests shows no significant increase at any time point after RYGBP, and at t=60 (p<0.01) at 6 weeks and at t= 30 (p<0.01) and t=60 (p<0.01) at 12 weeks after SG (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 week).*

3.2.6 Comparison between RYGBP and SG plasma leptin

There is a similar reduction in plasma leptin after RYGBP and SG at 6 and 12 weeks after surgery (figure-33/ 34). The circulating fasting plasma leptin does decline in keeping with adiposity (figure-33). The leptin declined from 4596 ± 365 pM at baseline to 3266 ± 343 pM at 6 weeks (p=0.0032) and 3077 ± 346 pM at 12 weeks (p=0.0009) after RYGBP. There is no significant (p=0.43) difference between the 6 week and 12 week fasting leptin in the RYGBP group. Similarly, following SG a comparable reduction in fasting leptin was seen at 6 and 12 weeks post-surgery. After SG this declined from 4278 ± 333 pM at baseline to 2904 ± 356 pM at 6 weeks (p=0.0005) and 2575 ± 337 at 12 weeks (p=0.0003). There was no significant (p=0.12) decline between 6 and 12 weeks. There was no significant difference between RYGBP and SG groups at baseline (p=0.53), 6 weeks (p=0.48) and 12 weeks (p=0.32).

	fat mass	**leptin**	**fat mass**	**leptin**	**fat mass**	**leptin**
Subject	visit A	Visit A	Visit B	Visit B	Visit C	Visit C
1	63.5	4341.8	55.2	3492.2	49.8	2725.1
2	71.5	4059.7	64	3094.5	58.4	3282.7
4	74.2	4974.4	70.2	4186.9	66.6	4372.7
5	66.2	5283.4	59.8	4680.3	54.6	3679.4
6	74.4	5877.3	67.4	4914.7	63.6	4751.8
7	69.1	4821.3	59.8	2255.2	54.6	2962.5
8	59.3	5398.6	50	2327.2	40.6	1974.6
9	64.6	4728.4	57.6	3722.8	52.6	3610.3
10	66	5635.8	53.8	3470.8	47.7	2508.2
11	75.9	3881.6	64.9	2572.6	64.4	3379.2
12	77.9	5109.7	66.6	3581.4	60	2922
13	71.7	3375.8	63.2	1797.3	51.9	2055.7
14	54.1	2204	45.5	2245.3	38.8	1131.4
15	54.8	5384.2	46.8	3790	46.9	3475.9
17	49	3640.2	39.4	1321.8	33.8	1473.5
18	60.6	3515.9	52.6	2383.1	47.5	2239.5
19	56.7	3355.2	50.7	2791.4	47.6	1746

Figure-32; Patients fat mass (kg) and circulating fasting plasma leptin levels (pmol/L)

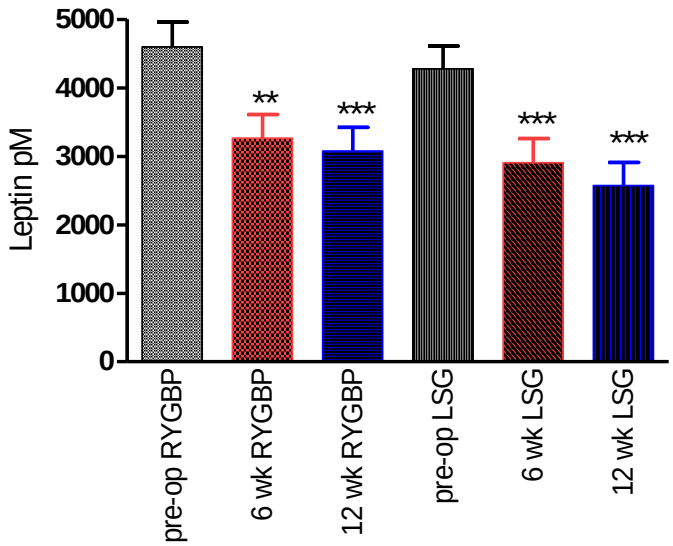


*Figure-33; Bar chart to display change in plasma leptin concentration after RYGBP and SG, when pre-operative levels compared to 6 and 12 weeks after surgery in the RYGBP and SG groups- one way ANOVA (** p<0.01, ***p<0.001).*

3.2.7 Weight, BMI, Fat mass and VFA correlate to leptin

In the RYGBP group weight (p<0.0001/ r=0.47), BMI (p<0.0001/ r=0.63), fat mass (p<0.000/ 1r=0.53) and visceral fat area (p<0.0001/ r=0.53) does correlate to circulating fasting leptin (figure-35). In SG group weight (p=0.004, r=0.32), BMI (p=0.0006/ r=0.42), fat mass (p=0.0007/ r=0.42) and visceral fat area (p=0.0024/ r=0.35) does also correlate circulating leptin (figure-36).

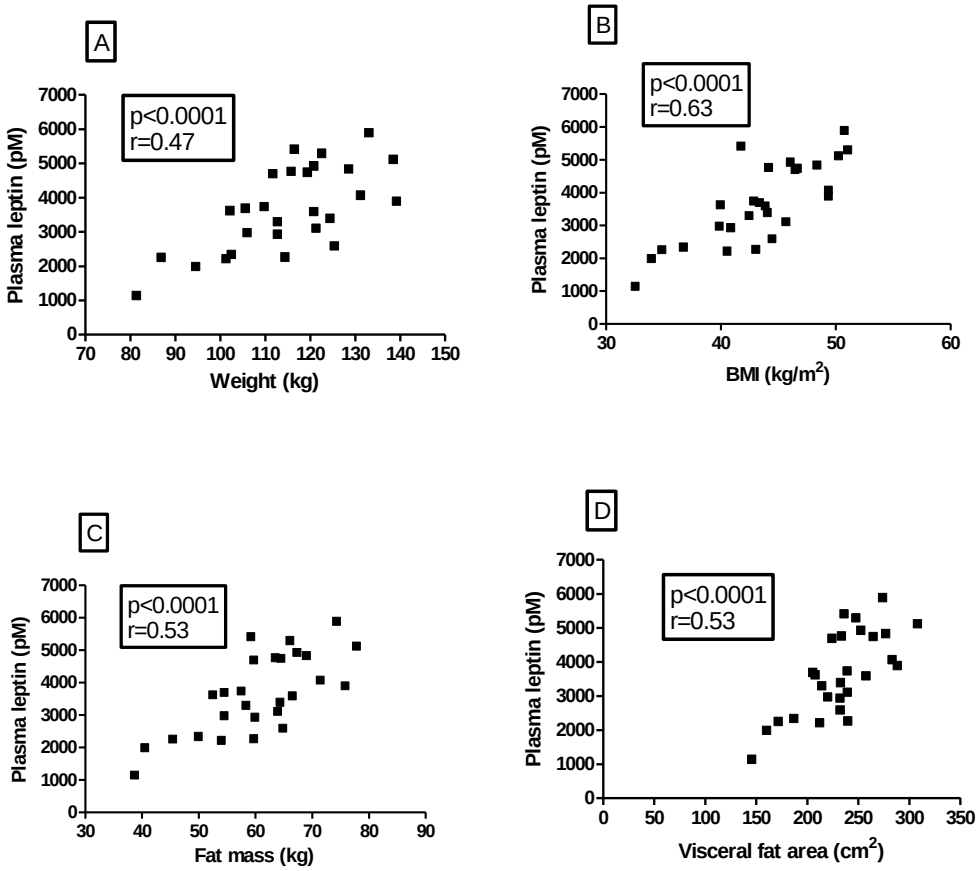


Figure-34; Plasma leptin does correlate to weight (A), BMI (B), fat mass (C) and VFA (D) in the RYGBP group.

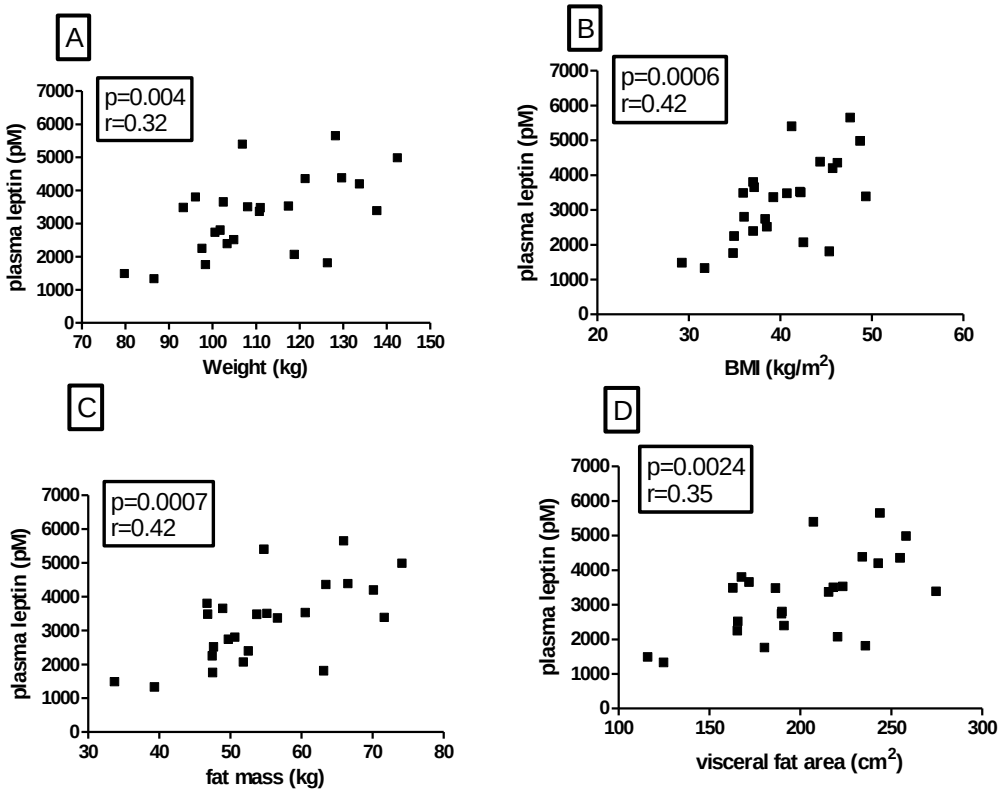


Figure-35; Plsma leptin does correlate to weight (A), BMI (B), fat mass (C) and VFA (D) in the SG group.

3.2.8 Comparison between RYGBP and SG plasma hormone profiles

	Pre-operation		6 weeks post-op		12 weeks post-op	
	Bypass	Sleeve	Bypass	Sleeve	Bypass	Sleeve
Leptin pM	4596±365	4278±333	3266±343 **	2904±356 ***	3077±346 ***	2575±337 ***
Fasting PYY3-36 pM	19.4±3.4	21.9±8.2	22.5±3.4	23.6±7.5	24.5±3.9 *	27.9±7.8
PYY3-36 AUC	6364±399	6083±1522	13780±1458 ***	10875±1868 ***	13186±976 ***	10372±1940 **
Fasting acyl-ghrelin pM	53.0±8.8	44.7±4.3	39.3±2.5	35.9±1.8 *P=0.096	53.5±6.6	33.6±2.1 *P=0.059, #
Acyl-ghrelin AUC	7606±905	7023±521	6878±342	6204±388 *P=0.056	7491±530	5738±252 *P=0.052, #
Fasting active GLP-1pM	4.4±0.6	4.2±0.1	5.6±1.2	4.2±0.1	5.1±1.0	4.2±0.1
Active GLP-1 AUC	1339±220 #(P=0.089) (1diabetic)	931±38	6095±1092 **, #	2804±414 **	6106±786 *** , ###	2254±307 **
Fasting amylin pM	6.5±2.4	6.9±1.4	11.0±6.4	6.5±1.3	11.5±7.3	6.0±1.0
Amylin AUC	2123±652	2317±390	3151±1490	2328±388	3032±1189	2524±610

*Figure-36; Summary of leptin, PYY3-36, acyl-ghrelin, active GLP-1 and amylin in the RYGBP and SG groups, mean ± SEM is shown, *p<0.05, ** p<0.01, ***p<0.001 when pre-operative values are compared with 6, 12 week post-operative values, and # p<0.05, ## p<0.01, ### p<0.001 for difference between RYGBP and SG groups.*

3.2.9 Change in PYY3-36 after RYGBP and SG

There is a rise in fasting circulating PYY3-36 levels from 19.4±3.4 pM at baseline to 22.5±3.4 pM at 6 weeks, and to 27.9±7.8 pM at 12 weeks after RYGBP. At 6 weeks post-RYGBP the increase in fasting PYY3-36 was not significant. However, by 12 weeks post-RYGBP this became significant (p=0.015). Fasting circulating PYY3-36 levels change from 21.9±8.2 pM at baseline to 23.6±7.5 pM at 6 weeks and to 27.9±7.8 pM at 12 weeks after SG. Following SG there was no significant effect on fasting PYY3-36 at either 6 or 12 weeks. Between groups comparisons of fasting PYY3-36

revealed no difference between groups at either 6 or 12 weeks post surgery. Analysis of temporal profiles by a two way matched ANOVA to compare pre-operative time point to post operative time points reveals the temporal profile of PYY3-36 is significantly ($p<0.001$- two way matched ANOVA) increased after RYGBP. Bonferroni post-hoc tests show significant increase at t=30, 60, 90, 120, 150 and 180 at 6 weeks, and at t=30, 60, 90, 120, 150 and 180 at 12 weeks after RYGBP (figure-38). The temporal profile of PYY3-36 is also significantly ($p<0.001$- two way matched ANOVA) increased after SG. Bonferroni post-hoc tests show significant increase at t=15, 30, 60, 90, 120, 150 and 180 at 6 weeks, and at t=15, 30, 60, 90, 120 and 150 at 12 weeks after SG (figure-38). The postprandial peak in PYY3-36 is significantly ($p=0.0006$) increased from 38.8±1.9 pM (t=180) at baseline to 93.9±10.0 pM (t=60) at 6 weeks, and significantly ($p=0.0006$) increased to 93.5±10.3 pM (t=60) at 12 weeks after RYGBP. After SG the postprandial peak is significantly ($p=0.0018$) increased from 39.3±9.2 pM (t=90) at baseline to 67.4±12.2 pM (t=60) at 6 weeks, and significantly ($p=0.0036$) increased to 70.0±11.6 pM (t=60) at 12 weeks after SG. The meal stimulated PYY3-36 AUC is significantly increased from 6364±399 at baseline, to 13780±1458 ($p=0.0007$) at 6 weeks and 13186±976 ($p=0.0001$) at 12 weeks after RYGBP. The meal stimulated PYY3-36 AUC is also significantly increased from 6083±1521 at baseline to 10875±1868 ($p=0.001$) at 6 weeks and 10372±1940 ($p=0.007$) at 12 weeks after SG. There is no significant difference between the RYGBP and SG PYY3-36 AUC at baseline, six week and 12 week time points (figure-38).

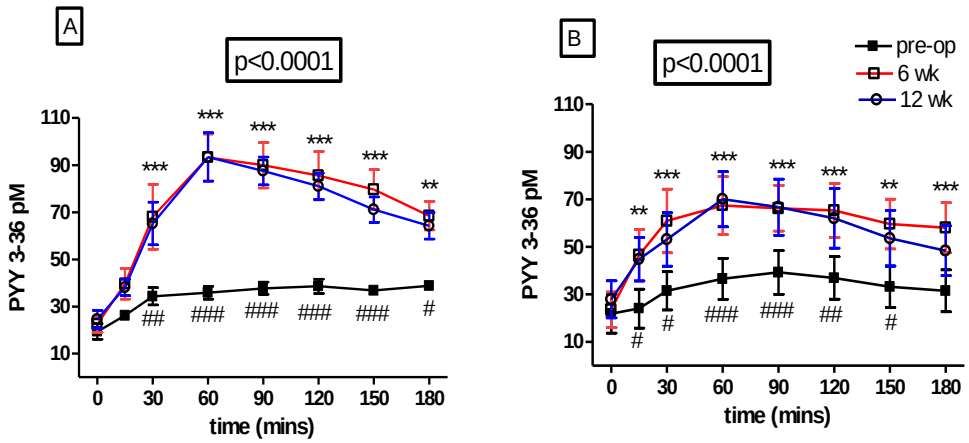


Figure-37; The temporal changes in meal stimulated circulating plasma PYY3-36 over the three visits in the RYGBP (A) and SG (B) groups are shown. In the RYGBP group surgery leads to a significant ($p<0.0001$) increase in PYY3-36 (two way matched ANOVA, comparison of pre-operative against post-operative time points). This increase is also highly significant ($p<0.0001$) in the SG group (two way matched ANOVA,

*comparison of pre-operative against post-operative time points). Post-hoc analysis with Bonferroni tests does show significant increase at t=30 (p<0.001), t= 60 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.001) and t=180 (p<0.01) at 6 weeks, and at t=30 (p<0.01), t=60 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.001) and t=180 (p<0.05) at 12 weeks after RYGBP. In the SG group Post-hoc analysis with Bonferroni tests point to significant increase at t=15 (p<0.01), t=30 (p<0.001), t= 60 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.01) and t=180 (p<0.001) at 6 weeks, and at t=15 (p<0.05), t=30 (p<0.05), t=60 (p<0.001), t=90 (p<0.001), t=120 (p<0.01) and t=150 (p<0.05) at 12 weeks after SG. (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks).*

3.2.10 Change in acyl-ghrelin after RYGBP and SG

Fasting acyl-ghrelin decreased from 53.0±8.8 pM at baseline to 39.3±2.5 pM at 6 weeks and rose again to 53.5±6.6 pM at 12 weeks after RYGBP. Fasting acyl-ghrelin was not significantly affected by RYGBP. There is a decrease in fasting acyl-ghrelin from 44.7± 4.3 pM at baseline to 35.9±1.8 pM at 6 weeks, and to 33.6±2.1 pM at 12 weeks after SG. These changes show a trend toward statistical significance at 6 weeks (P=0.096) and at 12 weeks (p=0.059) after SG. Further, the fasting plasma acyl-ghrelin in the SG group at 12 weeks after surgery is significantly lower than that seen in the RYGBP (p=0.0155) group. Analysis of the temporal profile of acyl-ghrelin with a matched two-way ANOVA, comparing pre-operative time point to post-operative time points shows a significant decrease after both RYGBP (p=0.015) and SG (p<0.001). The decline is more pronounced in the SG group. Further, Bonferroni post-hoc tests does not show significant decline at any time point in the RYGBP group. This contrasts with significant decline at t=15 (p<0.05) and t=30 (p<0.01) at 6 weeks, and t=0 (p<0.05), t=15 (p<0.01) and t=30 (p<0.01) at 12 weeks after SG (figure-39). The trough acyl-ghrelin level is altered from 39.5±4.5 pM (t=120) at pre-operative state to 35.9±1.3 pM (t=120) at 6 weeks (p=0.46), and 37.0±1.0 pM (t=60) at 12 weeks (p=0.56) after RYGBP. The trough acyl-ghrelin level is suppressed from 32.8±1.7 pM (t=120) at pre-operative state to 31.5±2.5 pM (t=30) at 6 weeks (p=0.46), and 28.1±2.7 pM (t=150) at 12 weeks (p=0.26) after SG. The acyl-ghrelin AUC declines from 7606±905 at baseline to 6878±342 at 6 weeks, and 7491±530 at 12 weeks after RYGBP. These changes do not reach statistical significance. The suppression in acyl-ghrelin AUC from 7023± 521 at baseline to 6204±389 at 6 weeks and 5738±252 at 12 weeks after SG does show a trend towards significance (p=0.056) at 6 weeks, and (p=0.052) 12 weeks. The difference in AUC between RYGBP and SG groups does also show a trend towards significance (p=0.012) at 12 weeks after surgery.

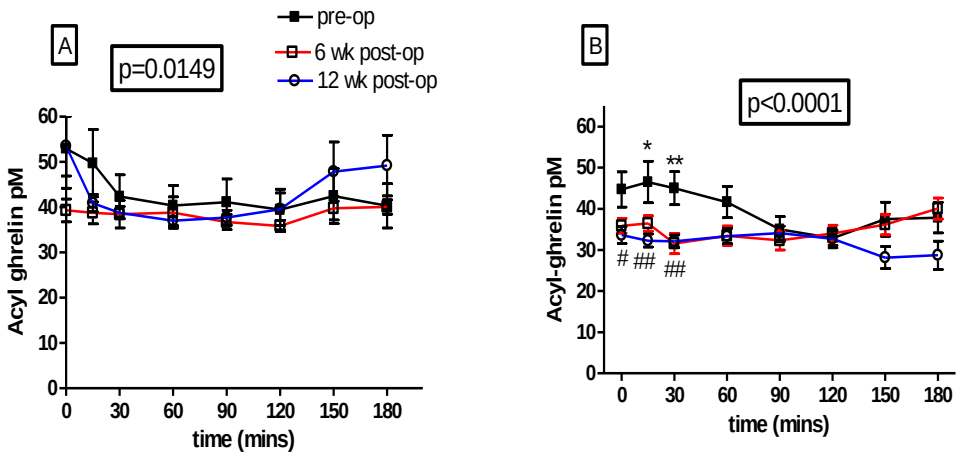


*Figure-38: The Comparison of meal stimulated plasma acyl-ghrelin response after RYGBP (A) and SG (B). Analysis of the plasma temporal profile of acyl-ghrelin after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points does show a significant decrease after both RYGBP (p=0.015) and SG (p<0.001). The decline is more pronounced in the SG group. Further, Bonferroni post-hoc analysis does not show significant decline at any time point in the RYGBP group. This contrasts with significant decline at t=15 (p<0.05) and t=30 (p<0.01) at 6 weeks, and t=0 (p<0.05), t=15 (p<0.01) and t=30 (p<0.01) at 12 weeks after SG (figure-39) (*p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, and # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks after surgery).*

3.2.11 Change in active GLP-1 after RYGBP and SG

There was a no increase in fasting active GLP-1 at 6 weeks and at 12 weeks after RYGBP and SG. In contrast, the temporal profile of meal stimulated active GLP-1 secretion was significantly altered after both RYGBP (p<0.0001) and SG (p<0.0001) (two way matched ANOVA, comparing pre-operative time point to post operative time points). Bonferroni post-hoc tests show significant increase at four time points at 6 weeks and at 12 weeks after RYGBP. In the SG group there was significant increase at three time points at 6 weeks, and two time points at 12 weeks (figure-40). There was a significant (p=0.0016) almost 8 fold increase in the peak active GLP-1 from 9.9±2.4 (t=30) pM at baseline to 76.1±13.5 pM (t=30) at 6 weeks, and (p=0.001) to 79.9±12.1 pM (t=30) at 12 weeks after RYGBP. After SG there was a fivefold significant (p=0.001) increase in peak active GLP-1 from 6.0±0.7 pM (t=60) at baseline to 29.7±4.2 pM (t=30) at 6 weeks, and (p=0.0091) to 27.2±5.4 pM (t=30) at 12 weeks. There was no significant difference between baseline active GLP-1 AUC in the RYGBP 1339±220 and SG 931±38 group, There was a significant (p<0.01) increase in meal stimulated

active GLP-1 AUC from 1339±220 at baseline to 6095±1092 at 6 weeks and 6106±786 ($p<0.001$) at 12 weeks after RYGBP. After SG there was also a significant ($p<0.01$) increase in active GLP-1 AUC from 931±38 to 2804±414 at 6 weeks and ($p<0.01$) 2254±306 at 12 weeks. Further, there was a significant difference in the GLP-1 AUC between the two groups at 6 ($p= 0.014$) and 12 ($p=0.0005$) weeks after surgery (figure-37).

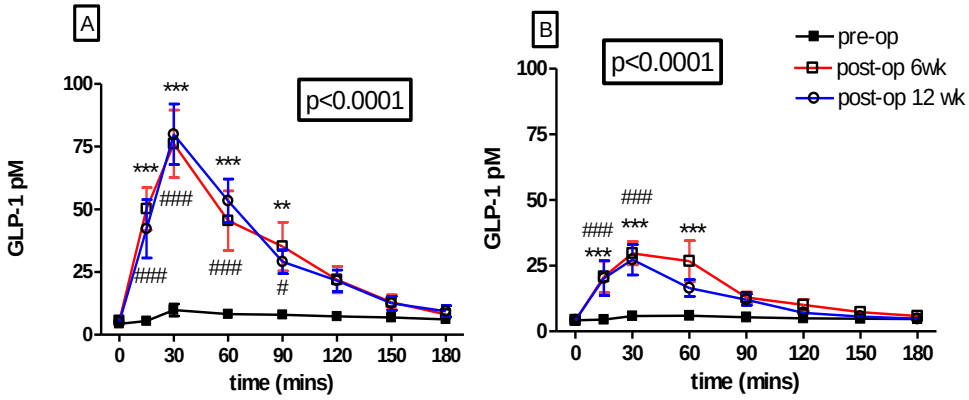


*Figure-39; Comparison of meal-stimulated GLP-1 response between RYGBP (A) and SG (B) groups. Analysis of the plasma temporal profile of GLP-1 after a mixed meal test utilizing a matched two-way ANOVA, comparing pre-operative time point to post-operative time points does show a significant increase after both RYGBP ($p<0.0001$) and SG ($p<0.0001$). Bonferroni post-hoc analysis does show significant increase at t=15 ($p<0.001$), 30 ($p<0.001$), 60 ($p<0.001$), and t=90 ($p<0.01$) at 6 weeks, and at t=15 ($p<0.001$), 30 ($p<0.001$), 60 ($p<0.001$) and at t=90 ($p<0.05$) at 12 weeks after RYGBP. This analysis in the SG group does also show significant increase at t=15 ($p<0.001$), 30 ($p<0.001$), 60 ($p<0.001$) at 6 weeks, and at t=15 ($p<0.001$), t=30 ($p<0.001$) at 12 weeks (figure-40). The symbols denote: *$p<0.05$, ** $p<0.01$, ***$p<0.001$ at 6 weeks, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ at 12 weeks*

3.2.12 Change in amylin after RYGBP and SG

There were no significant alterations in baseline fasting active amylin at 6 weeks and at 12 weeks after RYGBP and SG (figure-37). Analysis of the temporal profile of plasma active amylin following a mixed meal test, comparing pre-operative profile to post-operative time points (two way matched ANOVA) does show a significant ($p=0.005$) increase in active amylin secretion after RYGBP, but not after SG ($p=0.588$). Bonferroni post-hoc analysis did not show any significant increase at any time point in the RYGBP group at 6 and 12 weeks. There was no increase in the peak amylin after RYGBP and SG. SG does not lead to any significant change in the peak amylin level at 6weeks

(p=0.45), and 12 weeks (p=0.29). There was no significant effect of either surgical procedure on active amylin AUC (figure-41).

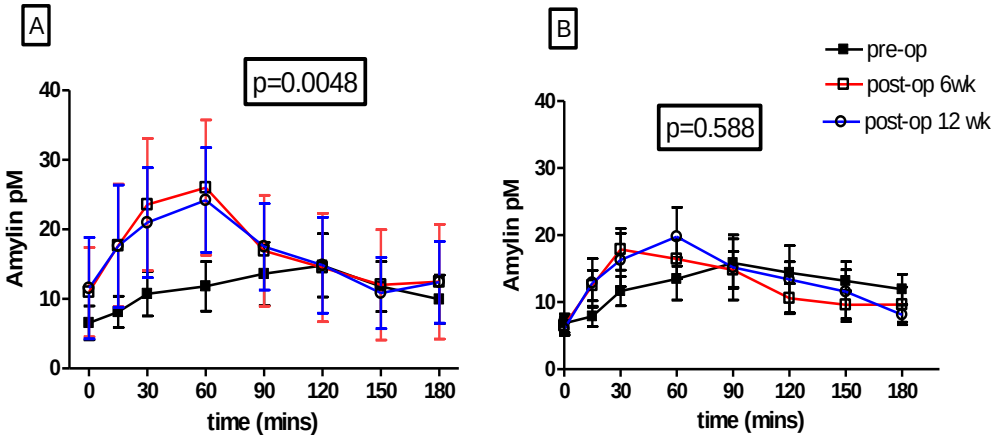


*Figure-40; The comparison of meal stimulated amylin response between RYGBP (A) and SG (B) groups. Analysis of the plasma temporal profile of amylin after a mixed meal test utilizing a matched two-way ANOVA, comparing pre-operative time point to post-operative time points does show a significant increase after RYGBP (p=0.0048), but not after SG (p=0.588). Bonferroni post-hoc analysis does not show significant increase at any time points after RYGBP. This analysis in the SG group does also not show any significant change. The symbols denote: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

3.2.13 Gut hormone changes are independent of weight loss

There was continued weight loss in the RYGBP and SG groups between 6 and 12 weeks after surgery (figure-42). However, there was no significant difference between 6 and 12 week PYY3-36, acyl-ghrelin, GLP-1, amylin and insulin in both groups.

	Ä 12-6 wk RYGBP	t-test, p value	Ä 12-6 wk SG	t-test, p value
%EWL	11 ± 1	*<0.0001	10 ± 2	*0.0003
BMI loss (Kg/m^2)	2.53 ± 0.3	*<0.0001	2.15 ± 0.33	*0.0003
AUC PYY3-36 pM	594 ± 1365	0.657	503 ± 999	0.63
AUC Acyl-ghrelin pM	613 ± 629	0.358	467 ± 386	0.266
AUC GLP-1 pM	189 ± 700	0.794	550 ± 397	0.209
AUC Amylin pM	105 ± 327	0.744	196 ± 387	0.629
AUC Insulin pM	18173 ± 16129	0.293	15132 ± 14246	0.323

Figure-41; A comparison of change in anthropometry and gut hormones after the two procedures between 6 and 12 weeks after surgery. There is a significant change in

percentage excess weight loss and BMI loss from 6 to 12 weeks in both groups. However, there is no significant change in AUC in any of the hormone indices through this same period.

3.2.14 Correlation analysis

We examined the relationship between change in individual hormones and change in appetite. This was undertaken in individual patients. The change in AUC from baseline to 6 weeks and 12 weeks was correlated to changes in appetite in the corresponding time periods. Correlation analysis between change in hormone indices, and change in hunger, prospective food consumption and satiety after RYGBP and SG was examined.

Statistical analysis is based on the assumption of independence of variables being studied, with no correlation in time or space. A study with repeated measures in individual subjects does therefore contain potential sources of non-independence. It is postulated that in repeated measures, unmeasured factors can produce correlations or temporal auto-correlation. Thus systematic bias can be introduced when we fail to account for this temporal non-independence and incorrectly inflate test statistics and increase the likelihood of false positives. This is termed type 1 errors in statistics. In our study the correlation between PYY3-36, acyl-ghrelin, active GLP-1, insulin/ amylin ratio and appetite and satiety and with excess weight loss may not be causally linked. It is possible that weight loss alone co-ordinated the change in both parameters. Others have argued a change in the gut anatomy and still others a change in calorie consumption may explain some of the changes seen following bariatric surgery. However, the comparison of the same individual across three visits and utilising the change in an individual to assay correlation attempts to mitigate this. Animal model studies with reproducible phenotype when active hormone supplementation overcomes the absence of the active hormone or the receptor would imply causation. Causation is difficult to establish in human studies.

3.2.14.1 VAS and hormone correlation analysis in the RYGBP group

In the RYGBP group; change in hunger and satiety did not correlate to PYY3-36, acyl-ghrelin, active GLP-1 and amylin. However, change in prospective food consumption was significantly and positively ($p= 0.014$. $r=0.23$) correlated to acyl-ghrelin and negatively to change in acyl-ghrelin from baseline ($p=0.039$, $r=0.17$) (figure-43). Further, PYY3-36 ($p=0.011$, $r=0.23$) and change in PYY3-36 from baseline ($p=0.008$,

r=0.25) and GLP-1 (p=0.036, r=0.19) did also negatively correlate to prospective food consumption in the RYGBP group (figure-43). Amylin did not correlate to satiety or prospective food consumption. At the 12 week time point the change in GLP-1 did correlate to the change in satiety. The very low r values may reflect the small sample size or a weak association. However the p values indicate significant correlation.

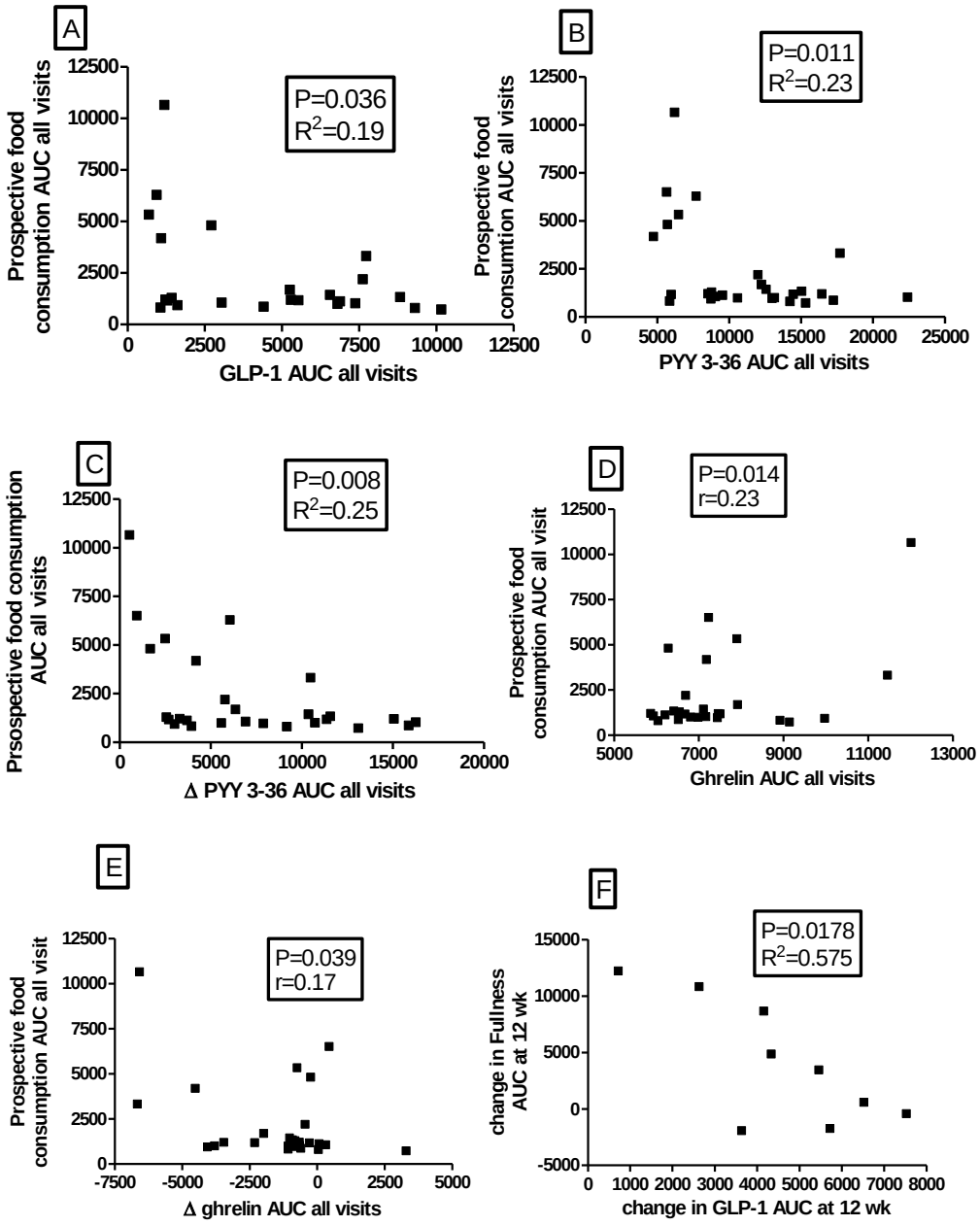


Figure-42; In the RYGBP group, prospective food consumption does correlate to GLP-1 (A), PYY3-36 (B), change in PYY3-36 from baseline (C), acyl-ghrelin (D) and change in

acyl-ghrelin from baseline (E). Further, the change in GLP-1 correlates to change in satiety. All VAS AUC is in mm. All plasma hormone measurements are in pM.

3.2.14.2 VAS and hormone correlation analysis in the SG group

By comparison in the SG group change in hunger did not correlate to change in any of the hormones studied. However, satiety after the liquid meal, did positively correlate to change in PYY3-36 from baseline (p=0.005, r=0.31) and GLP-1 (p=0.001, r=0.4) (figure-44). Further, prospective food consumption did negatively correlate to GLP-1 (p=0.004, r=0.33), change in PYY3-36 from baseline (p=0.043, r=0.17) and change in acyl-ghrelin from baseline (p=0.037, r=0.19). There was no correlation to amylin. Again low r values reflect small sample size, or a weak association. The p values indicate significant correlation (figure-44).

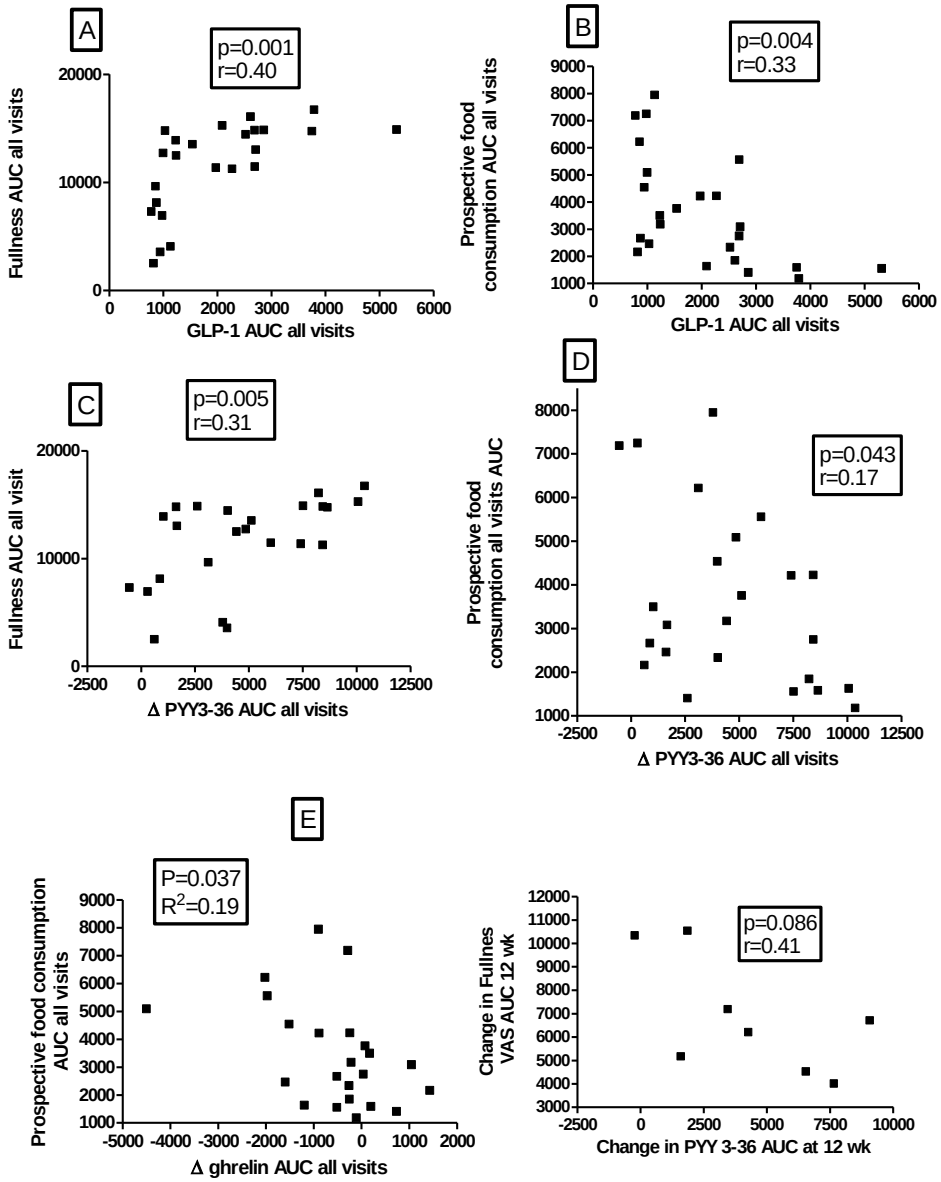


Figure-43; After SG; GLP-1 does correlate to Satiety (A) and prospective food consumption (B). The change in PYY3-36 from baseline does also correlate to satiety (C) and prospective food consumption (D). Further, change in acyl-ghrelin from baseline correlates to prospective food consumption (E). The change in PYY3-36 does also correlate to change in satiety (F). All VAS AUC is in mm. All plasma hormone measurements are in pM.

3.2.14.3 Change in hormone correlates to weight loss after RYGBP

In order to examine any causative link between change in gut hormones and weight loss correlational analysis was undertaken. The correlation between change in

hormone AUC and %EWL from baseline to corresponding time points were undertaken. The correlational analysis examined the change in PYY3-36, acyl-ghrelin, GLP-1 and amylin AUC from baseline to 6 weeks and 12 weeks and %EWL at those respective time points. I also examined the ability of hormone changes at 6 weeks to predict %EWL at 12 weeks. In the RYGBP group PYY3-36 did show a trend towards correlation to %EWL at 6 weeks (p=0.079, r=0.38) and 12 weeks (p=0.092, r=0.35) (figure-45). There was no significant correlation between change in acyl-ghrelin, GLP-1, amylin AUC from baseline to 6 weeks and 12 weeks and corresponding %EWL, nor change in AUC at 6 weeks to weight loss at 12 weeks. Further, the change in the composite islet hormone ratio correlated to weight loss. The insulin/ amylin ratio after RYGBP at six weeks (p=0.032, r=0.56) and twelve weeks (p=0.039, r=0.54) negatively correlated to %EWL at those time points (figure-45). The physiological significance of insulin/ amylin ratio after bariatric surgery is discussed in detail in section 4.3.14.

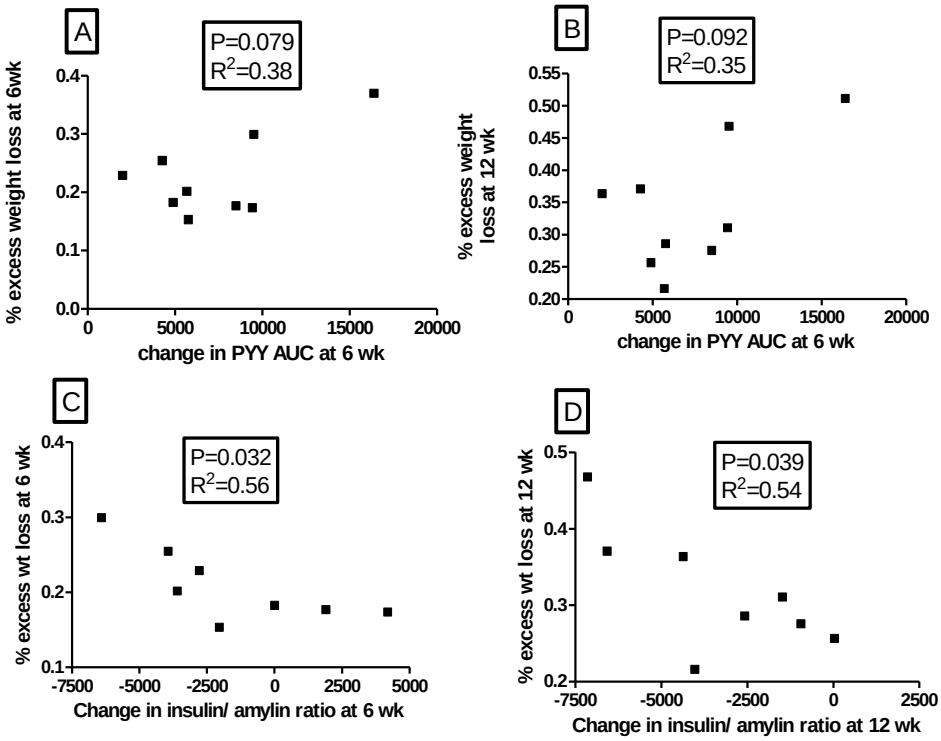


Figure-44; Scatter plots to display correlation between percentage excess weight loss (%EWL), and PYY AUC at 6 (A) and 12 (B) weeks, and insulin/ amylin ratio AUC at 6 (C) and 12 (D) weeks after RYGB.

3.2.14.4 Change in hormones correlates to weight loss after SG

Interestingly in the SG group the changes in PYY3-36, acyl-ghrelin, GLP-1 and amylin AUC correlated to weight loss after surgery. The change in hormone AUC from baseline to 6 weeks and 12 weeks correlated to change in %EWL at the corresponding time points. The change in PYY3-36 AUC from baseline to six weeks did show a trend towards positive correlation with %EWL at 6 weeks (p=0.056, r=0.48). This trend reached statistical significance (p=0.029, r=0.58) when change in PYY3-36 at six weeks was correlated to %EWL at 12 weeks (figure-46). The change in acyl-ghrelin at 6 weeks did correlate positively to %EWL at 6 weeks (p=0.042, r=0.6) and showed a trend towards correlation at twelve weeks (p=0.059, r=0.54) (figure-46). Also in this group, the change in amylin at 6 weeks does show a trend towards positive correlation with %EWL six weeks (p=0.068, r=0.45) and 12 weeks (p=0.075, r=0.44) (figure-46), change in amylin at 12 weeks did show a trend towards positive correlation with %EWL at 12 weeks (P=0.097, R2=0.40) (figure-46). The change in GLP-1 at 12 weeks after SG does correlate positively to %EWL at 12 weeks (p=0.044, r=0.52) (figure-46),

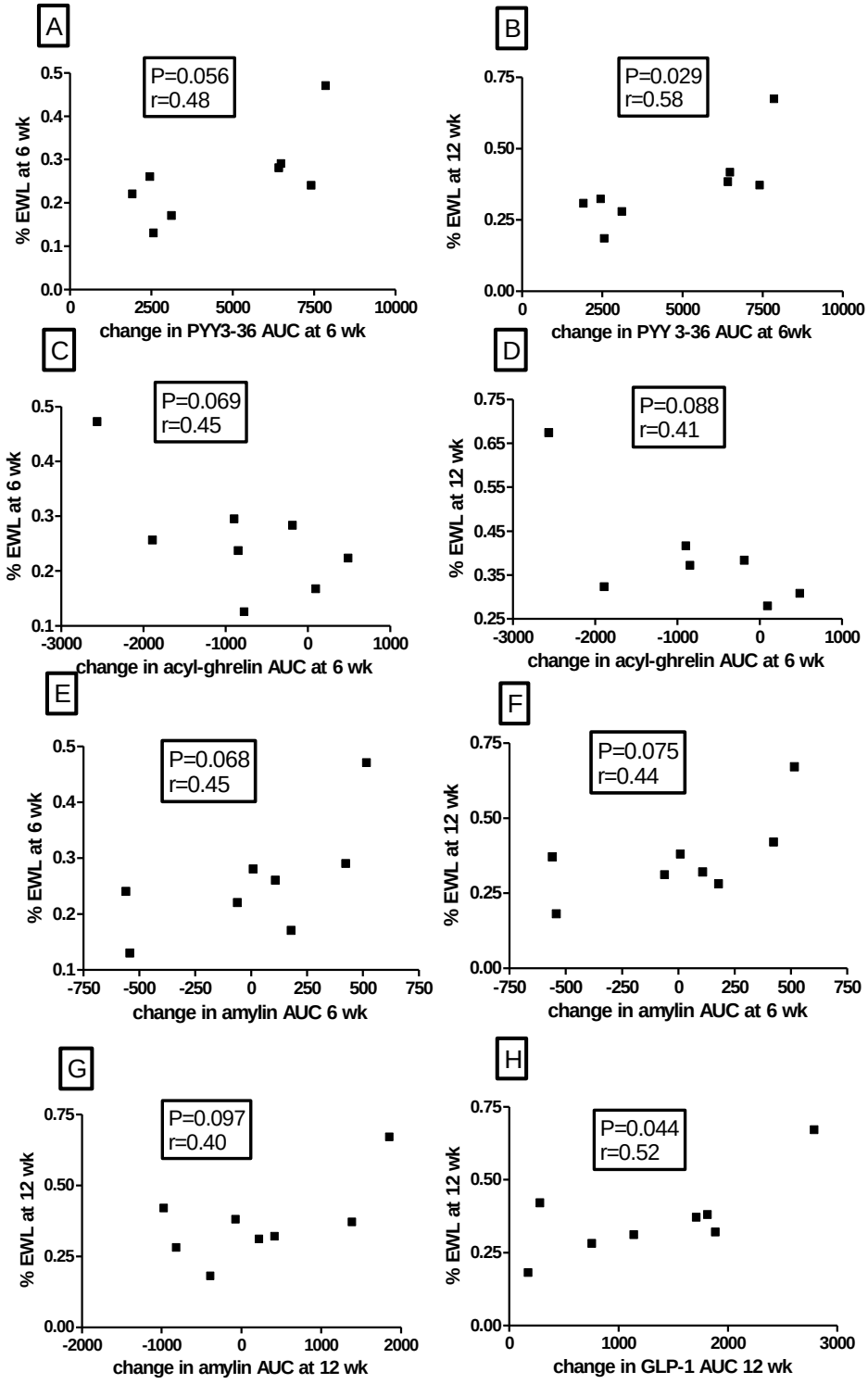


Figure-45; scatter plots to show correlation between change in PYY3-36 from baseline to six weeks and %EWL at 6 (A) and 12 (B) weeks. The change in acyl-ghrelin AUC at 6 weeks does correlate to %EWL and 6 (C) and 12 (D) weeks. Further, %EWL at 6 (E) and 12 (F) weeks correlates to change in amylin AUC at 6 weeks, also the EWL at 12

weeks correlate to change in amylin (G) and GLP-1 (H) AUC at 12 weeks in the SG group.

3.2.15 RYGBP and SG leads to correlation of PYY3-36 and GLP-1 secretion

Meal stimulated GLP-1 secretion across all visits correlated withPYY3-36 secretion in the RYGBP (p<0.0001, r=0.60) and SG (p=0.02, r=0.22) groups. However the strength of correlation is more pronounced in the RYGBP group.

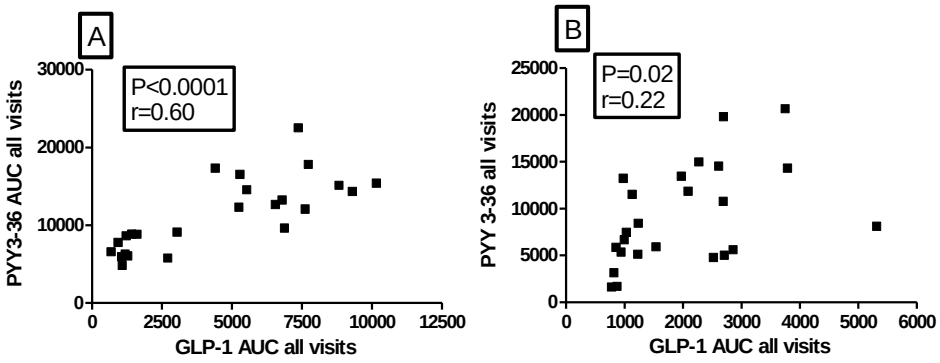


Figure-46; Scatter plots to show correlation between GLP-1 and PYY3-36 in the RYGBP (A) and SG (B) groups

3.2.16 Gut hormone changes precede failure to respond to surgery

One patient in the SG group did not lose any further weight between 3 and 12 months after surgery. This patient's meal stimulated PYY3-36, acyl-ghrelin and amylin response at 3 months after surgery did differ from those of others in the SG group (figure-48). The three month meal stimulated Δ PYY3-36 (change from baseline) response was below the pre-operative response. A comparison of the three month response with a two-tailed students t-test does show a significant (p=0.0002) difference between means, however the variance is not significantly different between the two temporal profiles (p=0.108). A comparison between this patient's Δ acyl-ghrelin response at three months and the mean of other patients did also show a trend towards significance (p=0.098). The variance was significantly (p<0.001) different between the two temporal profiles. This patient's amylin response was consistently below the pre-operative levels at 6 and 12 weeks after surgery and again analysis with Student's t-test comparing the 3 month responses did show a significant (p=0.002) difference in the means. The variance was also significantly different from the group (p=0.032) (figure-48).

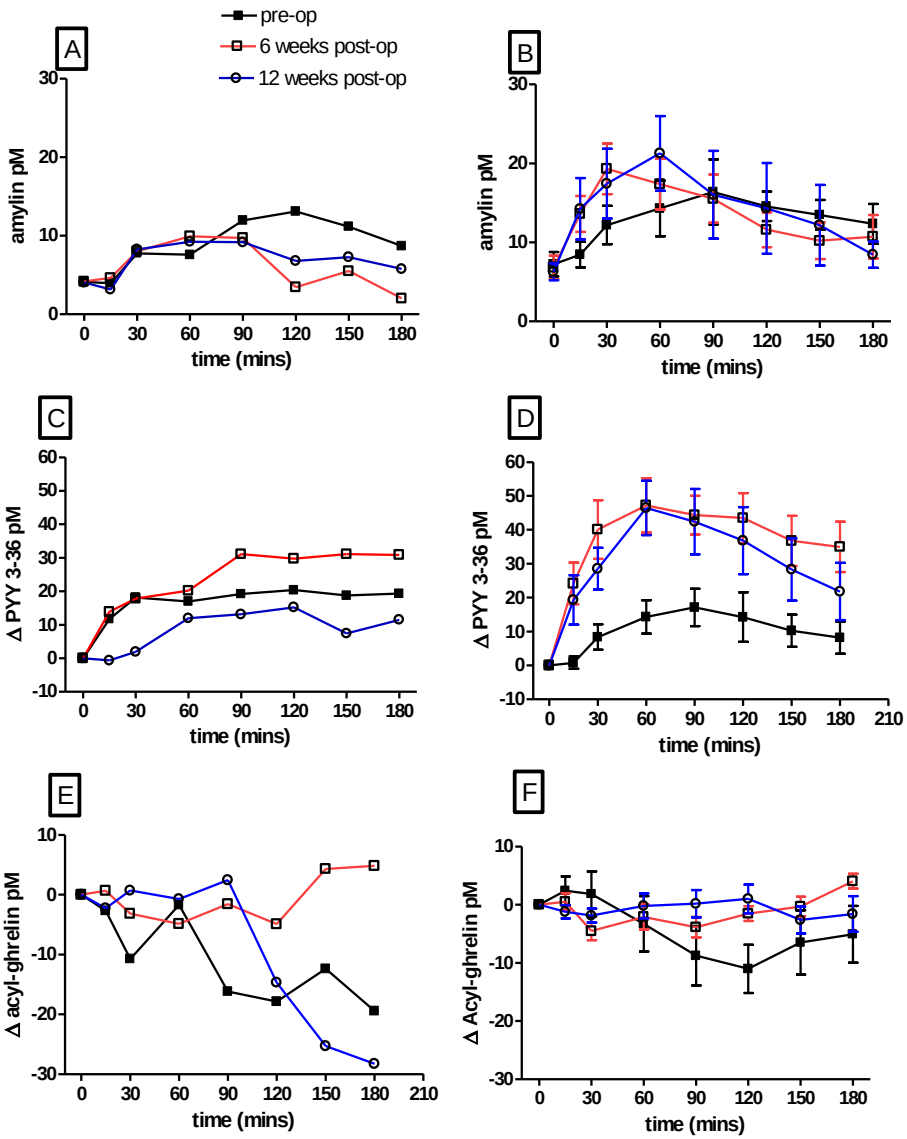


Figure-47; One patient in the SG group had a poor amylin (A), PYY3-36 (C) and acyl-ghrelin (E) response at 3 months after SG. This does contrast with changes seen in the respective hormones, in the rest of the group as shown opposite (B, D and F). This altered response was associated with a poor outcome after surgery.

3.3 Discussion

The two surgical interventions for morbid obesity had equivalent excess weight loss and BMI loss. This is in keeping with other recent studies (Karamanakos SN et al 2008 Peterli R et al 2009, Valderas JP et al 2010**,** Franco JVA et al 2011 and De Gordejuela AG et al 2011, Peterli R et al 2012). There was an equivalent change in leptin after both procedures, in keeping with loss of fat mass after surgery. Despite several studies

evaluating gut-hormone response in obese patients undergoing bariatric surgery (Cummings De et al 2002, Langer et al 2005, Korner J et al 2006, le Roux et al 2006, le Roux et al 2007, Whitson BA et al 2007, Karamanakos SN et al 2008, De Paula et al 2009, Y Wang et al 2009, Li F et al 2009, Peterli R et al, 2009, Bose M et al 2010, Abbatini F et al 2010, Bohdjalian A et al 2010, Basso N et al 2011, Chambers AP et al 2011, Peterli R et al 2012, Ramon J M et al 2012), none has been able to correlate gut hormone changes after surgery to outcome after surgery. This may be due to a lack of standardization prior to blood sampling for gut hormones and differences in blood sample processing (Chandarana K et al 2009). Others have compared post-surgical changes in gut hormones in patients against control groups (Cummings De et al 2002, Korner J et al, 2005Lopez PP 2009, Whitson BA 2007, Oliván B et al 2009, Bose M et al 2010, Valderas JP et al 2010), and not to their pre-operative state, making it difficult to draw conclusions on individual physiological changes and correlation to outcomes after surgery. Some studies have correlated poor response in a group to poor outcome after surgery (le Roux CW et al 2007). The change in gut hormones after RYGBP has been shown to correlate to weight loss in animal models (Shin AC et al 2010). We have shown that standardization of subjects, acclimatization and addition of protease inhibitors, DPP-4 and HCL to blood collected for gut hormone assays does influence plasma PYY3-36, GLP-1 and acyl-ghrelin (Chandarana K et al 2009), our study is the first to follow this standardization protocol to assay gut hormone changes after RYGBP and SG.

3.3.1 The role of PYY3-36

In our study, there was a similar and exaggerated meal stimulated PYY3-36 secretion after both procedures, but fasting PYY3-36 was only significantly reduced at 12 weeks after RYGBP, further; changes in PYY3-36 did correlate to perception of satiety and show a trend towards correlation to weight loss after RYGBP. This relationship between PYY3-36 and satiety and weight loss was also seen after SG, and the correlation to weight loss did reach statistical significance in this group. Recent publications have highlighted a pronounced PYY response after SG similar to that seen after RYGBP (Peterli R et al 2009), in keeping with our findings. Peterli and colleagues propose a quicker delivery of nutrients to the L-cells that secret PYY is thought to lead to a similar PYY response after RYGBP and SG, leading to earlier satiety and weight loss. A study to compare surgical intervention against medical treatment for obesity, was able to achieve similar weight loss after RYGBP, SG, and medical treatment, however favourable total PYY change was only seen after RYGBP and SG (Valderas JP et al 2010). In this study the meal stimulated total PYY AUC did increase

significantly after RYGB and SG, as with our study the magnitude of increase was significantly higher in the RYGBP group compared to the SG group and lean controls (Valderas JP et al 2010). Some studies have evaluated PYY3-36 after RYGBP in humans (Olivan B et al 2009, Bose M et al 2010). The study by Olivan and colleagues, and Bose and colleagues to investigate PYY3-36 in patients undergoing RYGBP did not employ VAS, and studied PYY3-36 response to a glucose tolerance test (GTT) (Oliván B et al 2009, Bose M et al 2010). A mixed liquid meal is more representative of a true meal than a GTT. Other studies to compare RYGBP and SG measured total PYY (Karamanakos SN et al 2008, Valderas JP et al 2010, Peterli R et al 2009, and Peterli R et al 2012). In the study by Karamanakos and colleagues fasting PYY levels increased significantly and progressively after surgery in both study groups. Furthermore, total PYY 2 hours after meal also increased significantly and equivalently in both study groups (Karamanakos et al 2008). A significant positive correlation between the change in AUC of total PYY level and satiety was seen in a study to compare RYGBP and SG (Valderas JP et al 2010)

3.3.2 The role of acyl-ghrelin

In our study fasting and meal stimulated acyl-ghrelin is decreased after SG, showing a trend towards significance, but not after RYGBP. This finding is in keeping with that of others studying RYGBP in rats (Shin AC et al 2010), further at 12 weeks there is a significant difference in fasting and meal stimulated acyl-ghrelin secretion between groups. A paradoxical rise in acyl-ghrelin at 6-12 months after surgery has recently been identified after RYGBP (Barazzoni R et al 2013). This is in keeping with the changes in acyl-ghrelin between 6 and 12 weeks, seen in our study. The significant decline in acyl-ghrelin after SG is thought to be due to the complete removal of the gastric fundus, the segment of the stomach, thought to produce the vast majority of acyl-ghrelin (Langer FB et al 2005). The change in acyl-ghrelin after RYGBP does significantly correlate to change in prospective food consumption after surgery, but not weight loss. This relationship between acyl-ghrelin and prospective food consumption is more pronounced in the SG group. Furthermore there is a positive correlation between change in acyl-ghrelin and weight loss after SG. Another study on weight regain after surgery, plasma ghrelin levels were higher in weight regain patients, but due to the small number of patients no significant differences were observed (Bohdjalian A et al 2010).

The suppression of ghrelin secretion seen after RYGBP is thought to be secondary to a permanent deprivation of nutrient stimulation to oxyntic gland cells responsible for the

production and release of acyl-ghrelin (Papailiou J et al 2010). The vagus nerve is also thought to play a part in this response (Papailiou J et al 2010). It is not yet clear if the reduced production of acyl-ghrelin seen after SG is temporary, and may be reversed over time, through post-surgical gastric hyperplasia or if other gastro-intestinal sites such as the duodenum take over acyl-ghrelin production, alternatively the central orexigenic effect of ghrelin may be restored by adaptations at the central sites of ghreln action (Papailiou J et al 2010). Whitson and colleagues also noted no significant contribution by acyl-ghrelin to weight loss after RYGBP (Whitson BA et al 2007). Further, they admit to poor collection practice. There has been much debate on the importance of ghrelin after SG (Langer FB et al 2005, Frezza EE et al 2008, Barazzoni R et al 2013). Total ghrelin is known to be elevated after diet induced weight loss (Cummings De et al 2002, Oliván B et al 2009) and it was initially thought that a decrease in total ghrelin after SG may explain the superior weight loss and maintenance of weight loss after SG (Langer FB et al 2005). However, a recent meta-analysis of several studies was unable to reach a conclusion (Frezza EE et al 2008). To date no study has measured acyl-ghrelin, the active octanoylated form collected under standardized conditions to prevent degradation (as recommended by the assay). A recent study points to the merits of measuring the active moiety, as the ration of the active moiety does rise with the passage of time, perhaps leading to weight regain (Barrazzoni et al 2013).

3.3.3 The role of GLP-1

Some studies have also examined the active GLP-1 response after RYGBP in humans (Peterli R et al 2009, Umeda L M et al 2011, and Peterli R et al 2012) and animals (Shin AC et al 2010). The human studies by Peterli and colleagues points to similar but smaller changes in GLP-1 after SG (Peterli R et al 2009 and Peterli R et al 2012), though no standardization protocol was followed in this study. It is worth noting that the baseline GLP-1 AUC are a third of the AUC in our study (Peterli R et al 2009 and Peterli R et al 2012), authors of the earlier study report an equivalent meal stimulated GLP-1 AUC after RYGBP and SG at three months after surgery (Peterli R et al 2009). In our study by contrast there is a significant increase in meal stimulated GLP-1 secretion after both procedures at 6 and 12 weeks, however, there is a more pronounced (3 fold) increase in meal stimulated GLP-1 secretion after RYGBP when compared to SG, that is maintained at 12 weeks, and is similar to the latter study profile (Peterli R et al 2012). This significant difference between RYGBP and SG meal stimulated GLP-1 secretion at both 6 and 12 weeks, the baseline difference in GLP-1 AUC, the four fold higher AUC of meal stimulated GLP-1 after RYGBP and the two fold increase seen after SG, when compared to the above study, even allowing for the

higher calorie content (400Kcal vs. 500Kcal) in our meal, does suggest differences in measurement protocol, and the importance of standardization. This may also explain why SG GLP-1 AUC levels approach that of RYGBP at 3 months in the first study. Furthermore the above study did not examine the role of GLP-1 in weight loss or satiety, nor the association of GLP-1 to other satiety hormones. Other studies that have assayed for active GLP-1 have done so without the addition of DPP-4 to samples (DePaula AL. et al 2009, Bose M et al 2010), making it difficult to interpret these results. In our study, there is a significant correlation between GLP-1 and prospective food consumption in the RYGBP group, though no correlation between change in GLP-1 and weight loss is observed in this group. However in the SG group there is correlation between GLP-1 and satiety, prospective food consumption and weight loss after surgery at 12 weeks, our study is the first study to show correlation between active GLP-1 change and outcome measures after SG. It is thought that a faster nutrient delivery to the hind gut after these procedures leads to a pronounced GLP-1 response after surgery (Peterli R et al 2009, Peterli R et al 2012).

3.3.4 The role of amylin

In our study there was a significant increase in meal stimulated active amylin secretion after RYGBP this is in keeping with recent reports on rats undergoing RYGBP (Shin AC et al 2010). No significant change in meal stimulated amylin secretion was seen after SG in our study. Others have found a significant increase in amylin when SG is combined with an ileal interposition on to the proximal duodenum and proximal jejunum (DePaula AL et al 2009). In our study the amylin changes on their own did not correlate to satiety, prospective food consumption or %EWL in the RYGBP group, a recent study on rats also found no correlation between increased amylin secretion and weight loss (Shin AC et al 2010).

3.3.5 Gut hormone changes precede weight loss

Recent studies on RYGBP and SG have shown that gut hormone changes occur independently of and precede weight loss (Korner J et al 2006 , Oliván B et al 2009, Peterli R et al 2009, Valderas JP et al 2010, Mousumi Bose et al 2010, Basso N et al 2011, Chambers AP et al 2011). In our study, patients continued to lose weight from the first post-operative study point at 6 weeks to the second study point at 12 weeks. However there was no significant change in the fasting or meal stimulated insulin, PYY3-36, acyl-ghrelin, GLP-1 and amylin from 6 to 12 weeks.

3.3.6 Gut hormone change correlates to weight loss after surgery

In the SG group changes in PYY3-36, acyl-ghrelin, GLP-1 and amylin independently correlate to weight loss. Taken together, these findings suggest that gut hormone changes alone could account for the weight loss seen after SG, this contrasts with RYGBP, where despite equivalent or even more pronounced gut hormone change, correlation of gut hormone change to weight loss is poor. This fundamental difference between the two surgical procedures may be due to alteration in neural "circuitry" that follows the more invasive RYGBP surgery. It is possible that RYGBP leads to other changes in neural signaling that favour weight loss, working alongside the endocrine changes that favour weight loss. Our findings, like that of others recently (Karamanakos et al 2008, Peterli R et al 2009, Oliván B et al 2009, Valderas JP et al 2010, Basso N et al 2011, Chambers AP et al 2011) also lend support to a hind gut factor mediating the effects of weight loss after RYGBP and SG surgery. It is also possible that local gut changes that occur after the two procedures promote a divergence in the metabolic outcome as outlined recently (Saeidi et al 2013). In the RYGBP group insulin/ amylin ratio alone correlated to weight loss after surgery, we also note that RYGBP patients continued to lose weight despite an increase in acyl-ghrelin secretion between 6 and 12 weeks. This is in keeping with recent findings at longer follow up after RYGBP leading to an increase in the active moiety of ghrelin (Barazzoni R et al 2013). It is also in keeping with other studies recently that have shown a significantly higher fasting and GTT stimulated total ghrelin AUC; an increase of 46% from a month after surgery to a year after RYGBP, despite a greater amount of weight loss after RYGBP (Bose M et al 2010).

3.3.7 Appetite and satiety correlate to hormone change

It is thought that gut hormones alter appetite and satiety after surgery and thus engender weight loss after RYGBP and SG surgery (Korner J et al 2005, Peterli R et al 2009, and Karamanakos SN et al 2008, Valderas JP et al 2010). No study to date had employed all three (hunger, satiety and prospective food consumption) questions on the VAS sheet, at several time points after a meal in conjunction with measurement of the active gut hormone. This does facilitate the assessment and correlation of the post meal active gut hormone response to VAS, and allow correlation of this hormone response to changes in VAS and weight loss; our study is unique in this respect. To date no correlation between changes in VAS and gut-hormones have been reported in the literature. The study by Valderas and colleagues (Valderas JP et al 2010) did also assay appetite at several time points following a meal and found appetite scores were significantly altered only after surgical intervention and not after medical treatment;

hunger AUC was significantly decreased only after RYGBP and satiety AUC significantly increased after RYGB and SG (Valderas JP et al 2010). This differential alteration in hunger and satiety after RYGBP and SG is similar to our study findings. However unlike our study they were unable to find any correlation between % EWL and change in plasma total PYY AUC in the whole sample or within each surgical group. However, as with our study, the PYY AUC did show positive correlation with satiety AUC in the three obese groups. This study did not add DPP4 inhibitor to blood samples and assays for total PYY and not PYY3-36 were done.

In our study, there is a significant decrease in prospective food consumption after RYGBP and SG at 6 and 12 weeks after surgery, further this decline is significantly lower after RYGBP at both time points after surgery. The change in acyl-ghrelin from baseline (Δ acyl-ghrelin) does show negative correlation to prospective food consumption in both groups, further, GLP-1, PYY3-36 and change in PYY3-36 from baseline (Δ PYY3-36) does also show negative correlation to prospective food consumption after RYGBP. In common with RYGBP, GLP-1 and Δ PYY3-36 do show a negative correlation to prospective food consumption after SG; conversely acyl-ghrelin does positively correlate prospective food consumption after RYGBP. RYGBP leads to a significant decrease in hunger after the meal at 6 and 12 weeks after surgery despite the non-significant change in acyl-ghrelin seen after RYGBP; the decrease in hunger after SG does show a trend towards significance at 12 weeks. A significant decrease in hunger after RYGBP relative to SG is seen after meals at both 6 and 12 weeks, despite the opposite in acyl-ghrelin changes. The meal related satiety response is very similar in the two groups at 6 weeks, but do differ at 12 weeks, where the increase remains significant only in the SG group. In the SG group, GLP-1 and Δ PYY does show positive correlation to satiety. These correlations between active gut hormones, hunger, prospective food consumption and satiety have not been reported before. Our study provides a link between the change in gut hormones and measures of appetite and satiety, and confirms gut hormone changes that occur after RYGBP and SG may lead to a decline in appetite and an increase in satiety, and therefore favour weight loss. We calculated the Δ hunger, Δ prospective food consumption and Δ satiety for all visits, and confirm that the meal leads to a significant decrease in Δ hunger and Δ prospective food consumption, and a significant increase in Δ satiety after RYGBP and SG.

In our study, RYGBP and SG seem to alter hunger, prospective food consumption and satiety differentially. RYGBP has a more pronounced influence on prospective food consumption and hunger, despite non-significant changes in acyl-ghrelin; whilst the converse is true of satiety. This variability does not fit with the overall gut hormone

changes seen after these procedures, RYGBP leads to a more pronounced PYY3-36, GLP-1 and amylin response and would be expected to alter satiety more, and SG by contrast does lead to a more pronounced and significant decline in acyl-ghrelin and thus expected to suppress hunger more.

3.3.8 Failure to respond to bariatric surgery

It is known that some patients fail to lose weight after RYGBP and SG, but the mechanisms behind this failure have yet to be explored. One patient in our SG group was noted to have lost no further weight between 3 and 12 months following surgery. This patient did have a three month meal stimulated amylin, Δ PYY3-36 and Δ acyl-ghrelin curve that was below the baseline curve for these hormones, this is in sharp contrast to all the other patients in the SG group, in other words a poor hormone response after surgery predicts failure to respond after SG. This altered meal stimulated response could be utilized to fast-track those patients predicted to fail to a second stage procedure. The correlation between weight loss; PYY3-36, acyl-ghrelin, active GLP-1 and active amylin, and the correlation between GLP-1, PYY3-36, acyl-ghrelin and VAS after SG together with the relationship between a poor 3 month amylin, Δ PYY3-36 and Δ acyl-ghrelin and poor outcome, does suggest that these gut hormones may account for the positive changes seen after SG. Whether poor gut hormone changes after RYGBP lead to a similar outcome is not clear.

3.3.9 Fasting plasma Leptin after bariatric surgery

Some authors have proposed a reduction in leptin in keeping with weight loss (Geloneze B et al 2001). However, others have proposed that the early decline in leptin is unlikely to be mediated by weight loss alone (Woelnerhanssen B et al 2011, Ramon J M et al 2012). In our study plasma leptin levels did not fall below the normal range in women. Further, there was no accelerated decline noted in the six week plasma leptin after surgery. The circulating plasma leptin was broadly in line with adiposity in our subjects. The significant correlation between plasma leptin and weight/ BMI/ fat mass/ VFA in both groups before and after surgery does confirm this. Our findings are in keeping with other recent published literature (Borg C M et al 2006, Jacobsen S H et al 2012).

3.3.10 Metformin in T2DM, and interference with gut hormone levels

One subject in the RYGBP group was on metformin therapy. This was stopped on the day of blood sampling before surgery, and patient was off metformin therapy at follow up visits at 6 and 12 weeks after surgery. Other comparative studies have also had this

discrepancy of diabetic patients in one group. In the study by Peterli and colleagues there were 3 T2DM patients in SG (2 patients were on insulin treatment and 1 on oral antidiabetic drugs) (Peterli R et al 2009). None of the patients of the RYGB group had T2DM. Also other published literature have included T2DM patients on metformin undergoing bariatric surgery, and examined PYY3-36, total ghrelin, total GLP-1, leptin, and amylin (Bose M et al 2010). In this study all but 3 were diabetic (Bose M et al 2010). In the comparative study by Karamanakos and colleagues 2 patients in the RYGBP group had diabetes. Both patients were on oral antidiabetic drugs, their diabetes resolved after surgery. One patient in the SG group had glucose intolerance in this study. Metformin is known to increase total PYY levels. However to date no study has examined PYY3-36 and metformin therapy in obese humans. Metformin administration is associated with an increase in fasting total PYY levels in normal women and women with PCOS (Tasoula T et al 2008). Acylated and total ghrelin levels were suppressed to a similar degree after a mixed meal in patients with type 2 diabetes treated with diet and metformin monotherapy (Kiyici S et al 2009). English and colleagues also found no effect of Metformin treatment on plasma PYY concentrations in type 2 diabetes (English PJ et al 2007). However, English and colleagues found subjects with T2DM treated with metformin to have a prolonged postprandial suppression of ghrelin, when compared to those treated with diet alone (English PJ et al 2007). In metformin-treated patients the plasma ghrelin was significantly below baseline concentrations and stayed low for an additional hour (English PJ et al 2007). Others have argued against this and have in fact shown the opposite with metformin therapy. Doogue and colleagues point to an increase in plasma total ghrelin concentrations in T2DM patients treated with metformin (Doogue MP et al 2009). However, they also point out that despite significant changes in ghrelin no change in either hunger or satiety in response meals was seen (Doogue MP et al 2009). Others point to significant reduction in ghrelin after an oral glucose tolerance test with metformin therapy (Kusaka I et al 2008). However, fasting ghrelin levels were unaltered with metformin therapy. The area under the curve for the 2-h ghrelin profile also decreased significantly (Kusaka I et al 2008). Metformin did not alter fasting amylin levels (Zapecka-Dubno B et al 1999). This remained similar to healthy individuals (Zapecka-Dubno B et al 1999). Metformin did interfere with the glucagon stimulated amylin secretion (Zapecka-Dubno B et al 1999). Mannucci and colleagues first reported the increase in plasma active GLP-1 in obese subjects and diabetic subjects (Mannucci E et al 2001, Mannucci E et al 2004). A recent publication suggests this to be an L-cell mediated effect of metformin (Mulherin AJ et al 2011). Metformin is thought to exert direct effects on the intestinal L cell as a GLP-1 secretagogue (Mulherin AJ et al 2011).

Our study confirms RYGBP and SG to be equally efficacious as metabolic surgical options. RYGBP and SG lead to a differential alteration in appetite. RYGBP alters hunger, and SG satiety. Further, prospective food consumptions were altered to a similar extent after both procedures. PYY3-36, GLP-1 and acyl-ghrelin does correlate to appetite in both surgical groups. RYGBP and SG led to equivalent fat mass loss and decline in plasma leptin. RYGBP leads to a more pronounced hind gut hormone response. However, SG also leads to a similar but less pronounced hind gut response. SG alone leads to a significant decline in acyl-ghrelin. RYGBP and SG lead to a divergent amylin response. There is no significant change in hormone profile between 6 and 12 weeks apart from acyl-ghrelin in the RYGBP group, where acyl-ghrelin does increase between these time points. This is in keeping with weight independent, and surgery mediated changes in the examined gut hormones. In the RYGBP group changes in PYY3-36 correlates to weight loss. In the SG group change in PYY3-36, acyl-ghrelin, GLP-1 and amylin correlate to weight loss after surgery. In the SG group a poor response in PYY3-36, acyl-ghrelin and amylin are associated with a poor outcome after surgery. RYGBP and SG seem to utilize different mechanisms to engender weight loss. The outcome after SG is dependent on the hormonal changes that ensue, whereas RYGBP may be dependent on other neuro-anatomical changes associated with surgery.

Chapter 4

Gut hormone changes after RYGBP and SG lead to improvements in glucose homeostasis

4.1 Introduction

4.1.1 T2DM and obesity are linked

The current epidemics of T2DM and obesity are thought to be related (Mokdad et al 2003*).* T2DM is thought to be linked to obesity by virtue of the insulin resistance that arises from an excess of body fat (Lazar 2005). Others propose the brain to play a central part as an insulin sensitive organ (reviewed by Schwartz and Porte Jr. 2005). Recent reviews put forward a model in which reduced neuronal insulin and leptin signalling contributes to the link between excess body fat and glucose homeostasis (reviewed by Schwartz and Porte Jr. 2005). The association of diet-induced obesity (DIO) with both higher serum levels of insulin and leptin and increased activation of inflammatory signalling pathways raises the possibility that the two are causally linked (De Souza et al 2005, Zhang X et al 2008, reviewed by Thaler and Schwartz, 2010). Another such factor is the increased levels of adipocyte-derived free fatty acids that have been shown to contribute to insulin resistance in liver and muscle in obesity (Bergman and Ad*er* 2000*,* Boden and Shulman 2002*).*

4.1.2 Bariatric surgery to treat T2DM

A recent statement on bariatric surgery as a treatment option for T2DM proposes that it be accepted as an option in patients with T2DM and BMI of at least 35 kg/m² (Zimmet P et al 2011). This statement by the International Diabetes Federation (IDF) proposes consideration of bariatric surgery where patients have failed to lose weight through weight-management programmes, and pharmacotherapy with an HbA1c of more than 7.5% (Zimmet P et al 2011). Further, it also proposes that bariatric surgery be considered as an option in patients with a BMI of 30–35 kg/m² when diabetes is inadequately controlled by pharmacotherapy, especially if other major co-morbidities are present (Zimmet P et al 2011). The HBA1c reduction was maintained for up to 4 years after RYGBP surgery (Kim S and Richards WO 2010). Hence surgery is now cautiously being considered as a treatment for T2DM in individuals with BMI's lower than the ranges prescribed by the current healthcare guidelines (Cummings and Flum, 2008). The international recommendations by the IDF are reflected in the recent National Bariatric Surgery Registry report in the UK (Welbourn R et al 2010). The improvement in T2DM has been confirmed in both morbidly obese and overweight group (Vidal et al 2008). However, by contrast the resolution rate in the over-weight group was halved a year after surgery (Lee et al 2010). A higher incidence of beta cell failure is present in the latter study group. The mean pre-operative fasting insulin levels were less than halved in the overweight group (Lee WJ et al 2010, Vidal J et al 2008).

RYGBP and SG do influence both beta cell failure and insulin resistance in the first week after surgery and maintained through the year of follow up (Lee et al 2010, Peterli R et al 2012, and Ramon J M et al 2012).

BMI (kg/m²)	**Eligible for surgery**	**Prioritised for surgery**
<30–35	Yes, conditional*	No
35–40	Yes	Yes, conditional*
>40	Yes	Yes

Figure-48; Eligibility and prioritisation for bariatric surgery in T2DM according to BMI (adapted from Zimmet P et al 2011). The statement proposes that the eligibility BMI lowered by 2·5 kg/m2 for Asians.

*HbA1c >7·5% on optimised pharmacotherapy or other weight responsive co-morbidities (blood pressure, dyslipidaemia, and obstructive sleep apnoea) not achieving targets on conventional therapies (adapted from Zimmet P et al 2011).

4.1.3 Bariatric surgery outcome in T2DM

Prospective (Sjostrom et al 2004, Buchwald et al, 2009) and retrospective studies (Rosenthal et al 2008) have shown resolution of T2DM after RYGBP. A meta-analysis of 135,246 patients in 621 studies by Buchwald and colleagues confirms 78.1% resolution, further 8.5% of patients showed improved glycaemic control after bariatric surgery (RYGBP, BPD and Gastric band) (Buchwald et al 2009). The mechanism underlying resolution of T2DM has been attributed to weight loss (Rosenthal et al 2008, Karamanakos SN, et al 2008), an improved incretin response (Peterli R et al 2009, Li F et al 2009, Dezaki K et al 2008, Peterli R et al 2012, Jacobsen S H et al 2012) and improvement in insulin resistance independent of weight loss (Pories et al 1995, Peterli R et al 2009, De Paula et al 2009, Umeda L M et al 2012, Jorgensen N B et al 2012). It has become clear that the improvement in T2DM and insulin resistance precedes weight changes and may be mediated by change in the hormone profile after RYGBP and SG but not after gastric band, despite equivalent weight loss (Oliván B et al 2009, Mousumi Bose et al 2010, Peterli R et al 2012, and Ramon J M et al 2012). RYGBP and SG led to the recovery in early-phase insulin secretion and an improvement in incretin levels (reviewed by Laferrère B. 2011, Ramon J M et al 2012, and Peterli R et al 2012).

A retrospective review of 262 T2DM patients after RYGB or SG over 8 years reveals similar numbers of patients remained off their diabetes medication (Bayham BE et al 2011). RYGBP and SG had similar effects on glucose homeostasis in morbidly obese T2DM patients at 3 years after surgery (Abbatini et al 2010). The rate of resolution of T2DM did not alter with the passage of time for up to 3 years in the RYGBP and SG group despite further significant weight loss (Abbatini et al 2010). RYGBP and SG at 3 years after surgery found similar resolution rates of 81.2% and 80.9% after (Abbatini et al 2010). However, others have shown a halving in resolution of T2DM over time (Sjostrom et al 2007). Also, several recent studies point to good T2DM resolution after SG (Silecchia G et al 2006, Cottam D et al 2006, and Shah S et al 2009, Vidal et al 2008, reviewed by Gill RS et al 2011). Further, some argue that SG may have a higher degree of T2DM resolution (Silecchia G et al 2006, Shah S et al 2009, and Abbatini F et al 2010). Baso and colleagues note an immediate restoration of first phase of insulin secretion and improved insulin sensitivity in diabetic obese patients with shorter duration of T2DM (Basso N et al 2011). A recent study by Rizzelo and colleagues confirm that pre-operative interventions and intra-abdominal surgery alone does not lead to changes in glucose homeostasis seen soon after SG (Rizzello M et al 2010).

4.1.4 Putative mechanisms for resolution of T2DM

Some studies have put the resolution of T2DM and the improvement in glucose homeostasis down to the improvement in weight (Karamanakos SN, et al 2008 and Rosenthal et al 2008). The mechanisms underlying the dramatic effects on insulin sensitivity and β-cell function have yet to be elucidated. However, several mechanisms including changes in gut hormones have been proposed (Cummings et al 2007, Peterli R et al 2012, Umeda L M et al 2012, Jorgensen N B et al 2012 and Jacobsen S H et al 2012). It has long been known that oral glucose stimulated insulin secretion is superior to intravenous glucose infusion (Elrick et al, 1964). This incretin effect accounts for between 50 and 70 % of total insulin secretion. Two major incretins have been characterized: glucose-dependant insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP- 1) (Drucker, 2006). Faster gastric emptying (Braghetto I et al 2009) and small bowel transit time (Shah S et al 2010) post SG surgery is thought to evoke a hind gut incretin hormone response not dissimilar to that seen following RYGBP (Peterli R et al 2009), leading to an improvement in insulin secretion (DePaula AL et al 2009, Peterli R et al 2012, Ramon J M et al 2012). This is in addition to the improvement in insulin resistance after surgery (Rizzello M et al 2010). The hind gut and foregut have been thought to play a part in the resolution of T2DM after bariatric surgery (Hickey et al 1998, Rubino Marescaux, 2004, Peterli R et al 2012, Ramon J M et al 2012). It is thought that procedures that eliminate the pyloric muscle control on gastric emptying

result in accelerated gastric emptying, stimulation of intestinal peristalsis and rapid nutrient delivery to the hindgut and an exaggerated hind gut response (Mason 2005, Aguirre et al 2008, and Rodriguez-Grunert et al 2008). A recent study to compare IT and duodenal-jejunal exclusion (DJE) in GK rats reported comparable weight loss, glucose tolerance and rise in GLP-1 in both groups post-operatively. Interestingly exendin 9-39, a GLP-1 receptor antagonist did reverse the improvement in glucose homeostasis seen after DJE indicating that the postoperative improvement in glucose homeostasis is mediated by enhanced GLP-1 signalling rather than from absence of a presumed foregut anti-incretin molecule (Kindel et al 2009, reviewed by Karra et al 2010).

Bariatric surgery (RYGBP and SG) leads to a 2-3 fold improvement in insulin sensitivity (Peterli R et al 2009, Papailiou J et al 2010, Basso N et al 2011 and Chambers AP et al 2011, Jorgenson N B et al 2012, Jacobsen S H et al 2012). This is seen early after surgery before any substantial weight loss has occurred (Peterli R et al 2009, Rizzello M et al 2010, Jorgensen N B et al 2012, Peterli R et al 2012). RYGBP and SG surgery lead to an absolute decline in insulin secretion (Kopp et al 2003, Wickremesekera et al 2005, Camastra et al 2005, Peterli R et al 2009 and Chambers AP et al 2011, Umeda L M et al 2012). RYGB and SG had comparable benefits in glucose metabolism in rodents. The insulin area-under the- curve (AUC) was greater when compared to controls (Chambers AP et al 2011). RYGBP and SG led to comparable loss of body weight and body fat and plasma insulin, and comparable improvements in glucose tolerance despite different anatomical rearrangement of the gastrointestinal system (Chambers AP et al 2011).

Our prospective parallel group study design enabled us to gather pilot data on plasma active gut hormone related changes in appetite, satiety, and weight loss. The short duration of the study also enabled us to study early influence on glucose homeostasis in relation to active gut hormone changes, after bariatric surgery. The lack of random treatment assignment may have led to systematic bias. The lack of standardization for calorie intake after surgery is one such bias. Recent publications on very low calorie diet mediated improvements in glucose homeostasis have challenged the initial proposed incretin mediated mechanisms (Knop FK and Taylor R 2013). Further recent reviews highlight the possibility of a ‘medical bypass' utilizing a multi-modal medical approach, though lacking all the clinical and physiological effects of surgery (Miras AD and le Roux CW 2014). The novel therapeutic targets identified in this multi-modal approach include food preferences, energy expenditure, gut microbiota, bile acid

signalling, inflammation, β-cell and hepatic glucose output (Miras AD and le Roux CW 2014). Initial studies in patients undergoing bariatric surgery pointed to weight independent changes in incretins after bariatric surgery (Laferre B 2011). This improvement is seen rapidly after the surgery, associated with recovery of the early phase insulin secretion and improved postprandial glucose levels not seen after an equivalent weight loss by diet, and blocked by the administration of a GLP-1 antagonist, demonstrating that the favorable metabolic changes after RYGBP are at least in part, GLP-1 dependent (Laferrère B 2011). Other studies comparing per oral route with gastric catheter related feeding after surgery note that the oral meal led to the typical postoperative exaggerated postprandial insulin and GLP-1 responses, while gastric catheter feeding resulted in (insulin/ GLP-1) responses similar to those seen preoperatively, along with postprandial glucose intolerance (Reviewed by Knop F K and Taylor R). It seems likely the greater exposure of L cells in the distal small intestine to ingested nutrients to have a direct beneficial effect on postprandial glucose metabolism after RYGBP (Reviewed by Knop F K and Taylor R). Supporting this, caloric restriction to 600 kcal/day for a week resulting in 2.1 kg weight loss and gastric banding had no effect on hepatic or peripheral insulin sensitivity (Laferrère B 2011). The recent Counterpoint study on very low calorie diets identified improvement in fasting glycaemia to normal in keeping with a fall in liver fat immediately, and a slower return of β-cell function mediated by the fall in pancreatic fat (Reviewed by Knop F K and Taylor R).

4.1.5 Insulin

The improvement in T2DM has been confirmed in both morbidly obese and overweight group (Vidal et al 2008). A study on patients undergoing SG to explore the role of incretins in patients with a lower BMI and advanced diabetes found SG in combination with proximal ileal inter-position led to an exaggerated incretin response, restoration of the first phase insulin secretion and resolution of T2DM in two thirds of patients (De Paula et al 2009). The pre-operative delayed insulin secretion pattern seen during GTT gradually changed through the year to an early secretion pattern (De Paula et al 2009). Further, a normal 30 minute early peak in insulin secretion was seen at 52 weeks after SG (Lee et al 2010, Peterli R et al 2012, and Ramon J M et al 2012). Insulin resistance and hyper-insulinaemia are common features in the morbidly obese Type-2 DM patients, but not in those with a lower BMI, leading to a higher incidence of B-cell failure in the latter. RYGBP and SG influence both beta cell failure and insulin resistance (Lee W J et al 2011, Peterli R et al 2012, and Ramon J M et al 2012)

4.1.6 Ghrelin

Several studies have demonstrated an inverse relationship between fasting ghrelin and fasting insulin levels (Purnell et al, 2003). Additionally, insulin resistance and T2DM are associated with reduced ghrelin levels, (Poykko et al, 2003) a correlation that has been shown to exist independently of bodyweight (McLaughlin et al, 2004). Ghrelin has been shown to inhibit insulin secretion both in vivo and in vitro (Dezaki et al, 2008; Reimer et al, 2003). Acyl-ghrelin is linked to insulin resistance through suppression of the insulin-sensitizing hormone adiponectin, blocking hepatic insulin signalling, inhibiting insulin secretion, increasing growth hormone secretion, increasing cortisol secretion and increasing epinephrine secretion. Therefore the decline in acyl-ghrelin secretion after SG may help restore insulin sensitivity (Peterli R et al 2009, reviewed by Yada et al 2008, Peterli r et al 2012, Ramon J M et al 2012). This has led some to speculate that the weight independent resolution of T2DM and improvement in glucose homeostasis seen after SG may in part be mediated by acyl- ghrelin (De Paula et al 2009, Abbatini et al 2010, Papailiou J et al 2010, Ramon J M et al 2012, Peterli R et al 2012). It is thought that the lack of a pronounced GLP-1 response after SG may be compensated for by the decrease in ghrelin seen after SG. This is thought to lead to improved insulin sensitivity after SG (Peterli R et al 2009, Li F et al 2009 and Papailiou J et al 2010, Peterli R et al 2012, Ramon J M et al 2012).

4.1.7 GLP-1

The incretin effect is severely reduced in T2DM patients compared to weight-matched controls, and is thought to contribute to the pathogenesis of T2DM (Nauck et al 1986). Patients with T2DM display a dose dependent response to exogenous GLP-1 (Kjems et al, 2003). RYGBP and SG lead to active GLP-1 plasma levels, 4 to 6 times higher than matched controls, after meals in humans (Peterli R et al 2009, Peterli R et al 2012, and Ramon J M et al 2012) and rodents (Chambers AP et al 2011). There were no differences in GLP-1 levels at any time point between the two groups in rodents (Chambers AP et al 2011). However, these studies were conducted five months after surgery. Studies in rodents and humans confirm a link between augmented GLP-1 secretion and insulin secretion (Shin AC et al 2010, Umeda L M et al 2011). They found significant correlation between the peaks in GLP-1 and insulin.

A recent study to examine GLP-1 antagonists on glucose homeostasis after bariatric surgery does point to reversal of the positive glucose homeostasis after DJB by GLP-1 receptor antagonism (Kindel TL et al 2009). This provides direct evidence that at least

some of the improvement after RYGBP is mediated by hind gut hormones (reviewed by Laferrère B. 2011). Recent evidence point to a persistently elevated postprandial GLP-1 at 4 and 20 years after RYGBP and DJB respectively (reviewed by Laferrère B. 2011 and Naslund et al 1998 respectively).

4.2 Aims of the study

We assessed fasting and meal stimulated glucose and insulin response along with HOMA IR and the incretin response one week before and at six and twelve weeks after RYGBP and SG. The post-operative changes in acyl-ghrelin in relation to the changes in insulin resistance seen after RYGBP and SG was also examined. Furthermore, we also analysed insulin/ amylin ratio and GLP-1/ insulin ratio after the two surgical procedures.

4.3 Results

4.3.1 Comparison of insulin resistance, glucose, insulin and GLP-1 between RYGBP and SG.

	Pre-operation		6 weeks post-op		12 weeks post-op	
	Bypass	Sleeve	Bypass	Sleeve	Bypass	Sleeve
Fasting Glucose (mmol/L)	5.6 ± 0.5	4.9 ± 0.2	5.4 ± 0.5	4.6 ± 0.2 *(P=0.087)	5.4 ± 0.4 #(P=0.098)	4.6 ± 0.2
Glucose AUC	1257± 143	1128 ± 47	1164±129 **	1027 ± 40 *(P=0.076)	1139 ± 140 **	1013 ± 49 *(P=0.075)
Fasting insulin (pM)	76.7± 12.9	180.2± 69.7	64.7± 10.0	84.3± 18.3	58.3 ± 8.0	74.6± 15.5
Insulin AUC	80759± 16167	111133± 35499	82696± 21232	166804± 60071	64523± 11133	151671± 54775
HOMA IR	1.48±0.27	3.10±1.10	1.22±0.20	1.51±0.32 *(P=0.095)	1.10 ± 0.15	1.34 ± 0.27 *(P=0.093)
Fasting active GLP-1 (pM)	4.4 ± 0.6	4.2 ± 0.1	5.6 ± 1.2	4.2 ± 0.1	5.1 ± 1.0	4.20 ± 0.1
GLP-1 AUC	1339 ± 220 #(P=0.089) (1diabetic)	931± 38	6095±1092 ** #	2804± 414 **	6106± 786 *** ###	2254± 307 **

*Figure-49; Comparison of glucose, insulin, HOMA IR and GLP-1 in the RYGBP and SG groups, mean ± SEM is shown, *p<0.05, ** p<0.01, ***p<0.001 when pre-operative values are compared with 6, 12 week post-operative values, and # p<0.05, ## p<0.01, ### p<0.001 for difference between RYGBP and SG groups.*

4.3.2 Glucose homeostasis after RYGBP and SG

The fasting baseline glucose was not significantly altered at 6 and 12 weeks after RYGBP and SG (figure-50/ 51). There was a significant decline in the temporal profile of glucose (two way ANOVA, p=0.0409) after RYGBP (figure-51). Bonferroni post test analysis confirmed significant declines at five time points at 6 weeks and at three time points at 12 weeks after surgery (figure-51). A late peak in post-prandial glucose was noted at t=120 mmol/L prior to RYGBP. This peak in glucose occured early at t=30 at 6 and 12 weeks (figure-51). There was also a significant (two way ANOVA, p=0.0014) decline in the temporal profile of glucose after SG. Bonferroni post test analysis

confirmed a significant decline in glucose at four time points both at 6 and 12 weeks after surgery (figure-50/ 51). Again the peak plasma glucose prior to surgery at t=90, was altered to t=30 at 6 and 12 weeks after SG. In the RYGBP group baseline glucose AUC was significantly decreased from 1257±143 to 1164±129 (p=0.008) at 6 weeks, and 1139 ±141 (p=0.001) at 12 weeks after surgery (figure-50). In the SG group this change in baseline glucose AUC from 1128±47 at baseline to 1028±40 at 6 (p=0.076) and 12 weeks 1013 ±49 (p=0.075) does not reach statistical significance (figure-50).

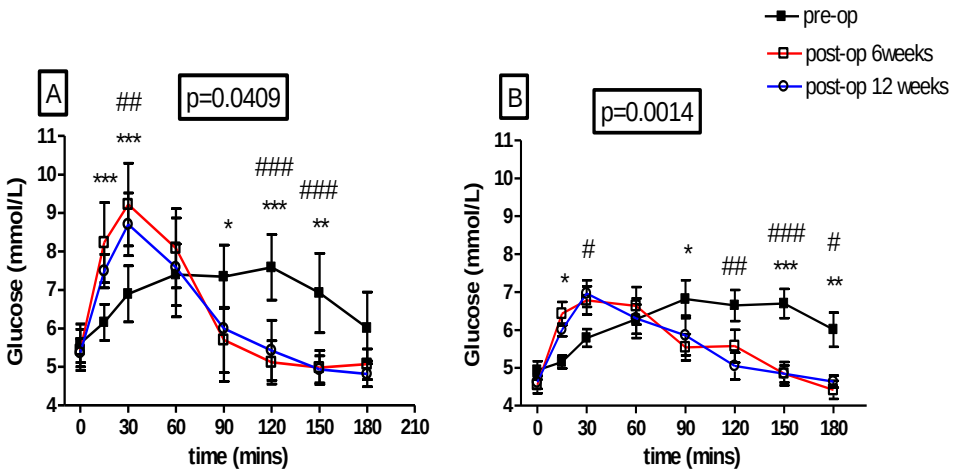


*Figure-50; Comparison of plasma glucose following the standard liquid meal in the RYGBP (A) and SG (B) groups are shown. There was a significant (two way matched ANOVA, p=0.0409) decline in the temporal profile of glucose, comparing pre-operative time point to post-operative time points after RYGBP. Bonferroni post test analysis did confirm significant decline at t=15 (p<0.001), t=30 (p<0.001), t=90 (p<0.05), t=120 (p<0.001), t=150 (p<0.01) at 6 weeks after surgery, and at t=30 (p<0.01), t=120 (p<0.001), t=150 (p<0.001) at 12 weeks after surgery. There was also a significant (two way matched ANOVA, p=0.0014) decline in the temporal profile of glucose after SG, comparing pre-operative time point to post-operative time points. Bonferroni post test analysis confirm a significant decline in glucose at t=15 (p<0.05), t=90 (p<0.05), t=150 (p<0.001), t=180 (p<0.01) at 6 weeks after surgery, and at t=30 (p<0.05), t=120 (p<0.01), t=150 (p<0.001), t=180 (p<0.05) at 12 weeks after surgery. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

4.3.3 Fasting and post-prandial insulin response after RYGBP and SG

RYGBP and SG led to no significant alterations in fasting plasma insulin at 6 and 12 weeks, compared to pre-surgery values. Analysis of the temporal profile of insulin after

RYGBP with a two way matched ANOVA did not reveal any significant increase in insulin secretion (p=0.178), However, Bonferroni post tests did confirm a significant increase at t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120 (p<0.01) at 6 weeks, but not at 12 weeks. The baseline peak insulin after RYGBP was observed earlier at t=60 from t=120 at 6 and 12 weeks after surgery. Analysis of temporal profiles with a two way matched ANOVA in the SG group did confirm a significant (p=0.0009) increase in meal stimulated insulin secretion after surgery. Bonferroni post-test analysis confirms an increase at three time points at 6 weeks and at one time point at 12 weeks after surgery (figure-52). There was also a shift in the peak plasma insulin to an earlier time point, from t=90 at baseline before surgery to t=30 at 6 and 12 weeks after surgery. There was no significant effect on insulin AUC after either procedure (figure-50).

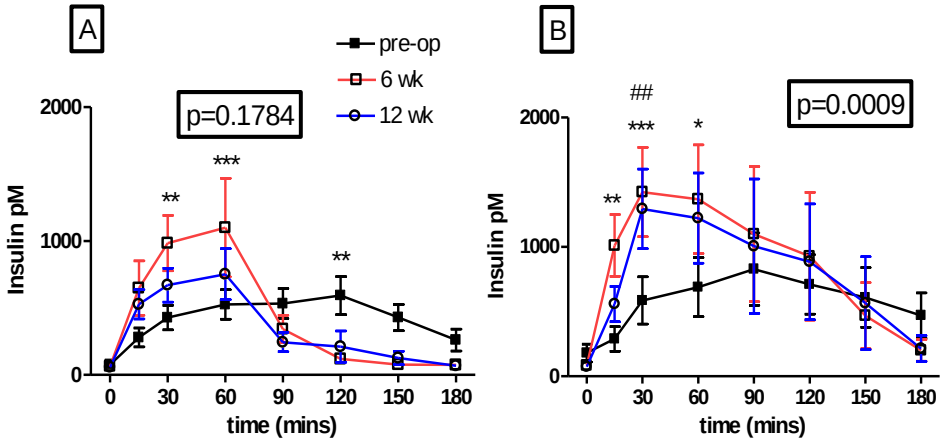


*Figure-51; Comparison of plasma insulin concentrations after the liquid meal following RYGBP (A) and SG (B) groups are shown. There is no change in the (two way matched ANOVA, p=0.1784) temporal profile of glucose, comparing pre-operative time point to post-operative time points after RYGBP. Bonferroni post test analysis did confirm a significant increase at t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120 (p<0.01) at 6 weeks after surgery. The analysis at 12 weeks after surgery did not identify any significant increase or decline at any time points. There is a significant (two way matched ANOVA, p=0.0009) increase in the temporal profile of glucose after SG, comparing pre-operative time point to post-operative time points. Bonferroni post-test analysis confirms an increase at t=15 (p<0.01), t=30 (p<0.001), t=60 (p<0.05) at 6 weeks after surgery and at t=30 (p<0.01) at 12 weeks after surgery. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

4.3.4 Fasting and post-prandial GLP-1 response after RYGBP and SG

There was no significant change in fasting active GLP-1 after RYGBP and SG. The temporal profile of meal stimulated active GLP-1 secretion was significantly altered after both RYGBP ($p<0.0001$) and SG ($p<0.0001$) (two way matched ANOVA). However the magnitude of change in the circulating active GLP-1 was three-fold higher after RYGBP. Bonferroni post-hoc analysis confirms significant increase at four time points at 6 and 12 weeks after RYGBP. Post hoc analysis following SG also point to significant increase at three time points at 6 weeks, maintained at two time points at 12 weeks (figure-53). There was an almost 8-fold increase in the peak active GLP-1 from t=30 9.9±2.4 pM at baseline to t=30 76.1±13.5 pM at 6 weeks and t=30 79.9±12.1 pM at 12 weeks after RYGBP. After SG there was a five-fold increase in peak active GLP-1 from baseline t=60 6.0±0.7 pM to t=30 29.7±4.2 pM at 6 weeks and t=30 27.2±5.4 pM at 12 weeks. There was no significant difference in baseline meal stimulated active GLP-1 AUC between RYGBP and SG groups despite the presence of a T2DM patient in the RYGBP group. There was a significant ($p<0.01$) increase in meal stimulated active GLP-1 AUC from 1339±220 at baseline to 6095±1092 at 6 weeks and to 6106±786 ($p<0.001$) at 12 weeks after RYGBP. After SG there was also a significant ($p<0.01$) increase in active GLP-1 AUC from 931±38 to 2804±414 at 6 weeks and to ($p<0.01$) 2254±306 at 12 weeks. Further there was a significant difference in the active GLP-1 AUC between the two groups at 6 ($p= 0.014$) and 12 ($p=0.0005$) weeks.

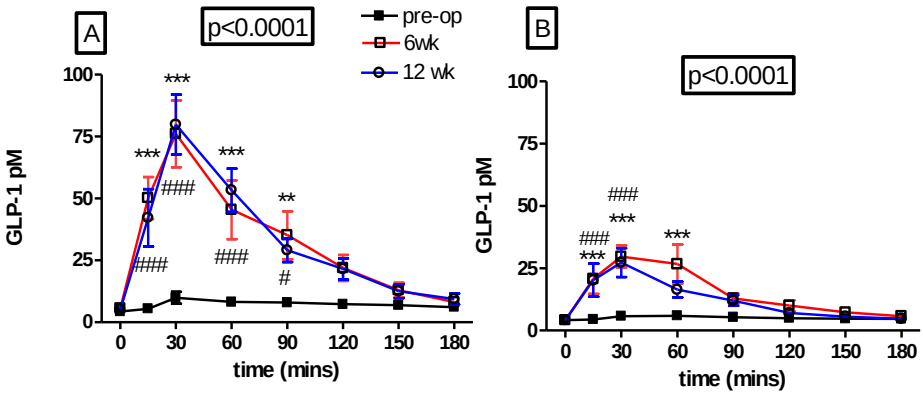


Figure-52; Comparison of meal stimulated GLP-1 response between RYGBP (A) and SG (B) groups. Analysis of the plasma temporal profile of GLP-1 after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points did show a significant increase after both RYGBP ($p<0.0001$) and SG ($p<0.0001$). Bonferroni post-hoc analysis did show significant increase at t=15 ($p<0.001$), 30 ($p<0.001$), 60 ($p<0.001$), and t=90 ($p<0.01$) at 6 weeks, and at t=15

*(p<0.001), 30 (p<0.001), 60 (p<0.001) and at t=90 (p<0.05) at 12 weeks after RYGBP. This analysis in the SG group did also show significant increase at t=15 (p<0.001), 30 (p<0.001), 60 (p<0.001) at 6 weeks, and at t=15 (p<0.001), t=30 (p<0.001) at 12 weeks (figure-40). Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

4.3.5 Change in insulin resistance after RYGBP and SG

There was no significant (p=0.152) difference between the RYGBP and SG HOMA IR at baseline. There was a decrease in HOMA IR after RYGBP surgery from 1.5 ± 0.3, to 1.2 ± 0.2 at 6 weeks and 1.1 ± 0.2 at 12 weeks after surgery (Turner RC et al 1979 and Levy JC et al 1998). This change did not reach statistical significance (p=0.2861) after RYGBP (one way matched ANOVA). There was a significant (p=0.05) (one way matched ANOVA) decline in insulin resistance measured by the homeostatic model of assessment after SG (Turner RC et al 1979 and Levy JC et al 1998). Post hoc analysis did not identify significant change at individual time points.

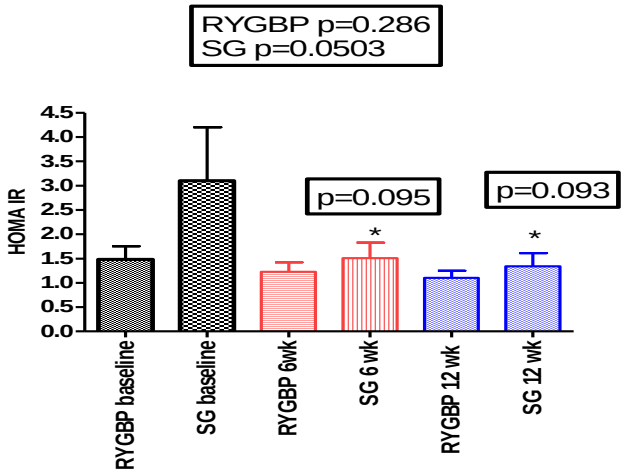


Figure-53; A comparison of change in insulin resistance measured by the HOMA IR model in the RYGBP and SG groups (one way matched ANOVA). There is a significant (p=0.0503) decline after SG but not RYGBP. There is also a trend towards significance at each time point in the SG group at 6 wk (p=0.095) and 12 weeks (p=0.093)

4.3.6 Acyl-ghrelin correlates to HOMA IR in the RYGBP and SG groups

There was a significant (p=0.025, r=0.19) negative correlation between HOMA IR and meal stimulated acyl-ghrelin AUC all visits, in the RYGBP group. There was no significant correlation between HOMA IR and meal stimulated acyl-ghrelin AUC all visits in the SG group (p=0.12, r=0.099).

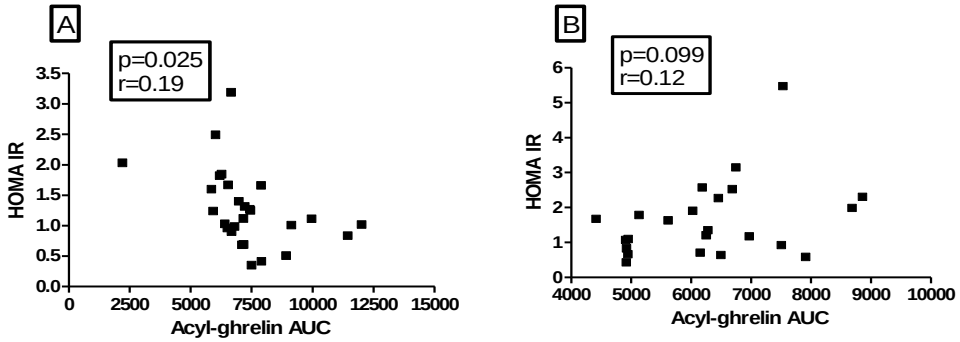


Figure-54; The negative correlation between HOMA IR and acyl-ghrelin in the RYGBP (A), and in the SG group (B)

4.3.7 Active GLP-1 secretion after RYGBP and SG does correlate to insulin

In the RYGBP group, change in active GLP-1 after surgery does positively correlate to change in insulin at 6 weeks (p=0.03, r=0.51) and 12 weeks (p=0.027, r=0.58) (figure-56). This correlation is not seen after SG. However, there was a significant and positive (p=0.005, r=0.31) correlation between meal stimulated active GLP-1 and meal stimulated insulin in all patient visits in the SG group (figure-57).

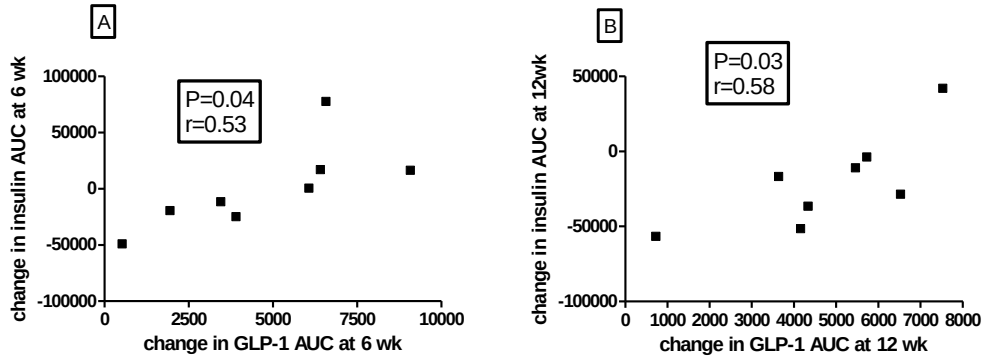


Figure-55; Scatter plots to show positive correlation between change in meal stimulated active GLP-1 and change in meal stimulated insulin at 6 (A) and 12 (B) weeks in the RYGBP group.

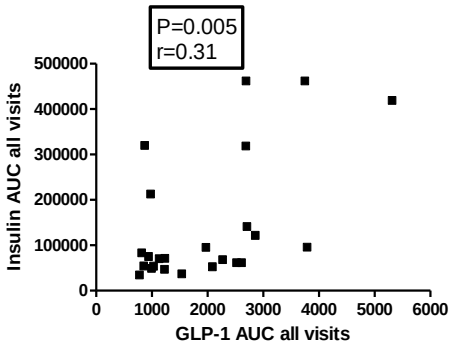


Figure-56; Scatter plots to show positive correlation between meal stimulated GLP-1 and meal stimulated insulin in all visits in the SG group

4.3.8 Insulin: GLP-1 ratio before and after RYGBP and SG

Recent studies have shown a reduction in insulin/ GLP-1 ratio after RYGBP (Hansen EN et al 2011). The meal stimulated insulin to active GLP-1 AUC ratio declined by around 60% after RYGBP (Hansen EN et al 2011). Further, there was no effect of gastrostomy tube feeding into the blind loop after RYGB. The active GLP-1 response after oral and gastrostomy tube delivered meal restored the aberrant preoperative active GLP-1 response (Hansen EN et al 2011). The authors propose that these findings are suggestive of more responsive distal L cells. Also both routes resulted in similar improvements in glucose tolerance, and argue against foregut exclusion as a primary mechanism (Hansen EN et al 2011). Despite the greater response in active GLP-1 after surgery insulin AUC/ active GLP-1 AUC declined after RYGB. There is a significant reduction in the amount of insulin secreted in response to an equivalent active GLP-1 stimulus after both procedures. However, the decline is more pronounced after RYGBP. The increased active GLP-1 release was not associated with an equipotent increase in insulin release. This may be related to a threshold effect, where beyond a certain concentration GLP-1 related augmentation of insulin secretion is seen to plateau.

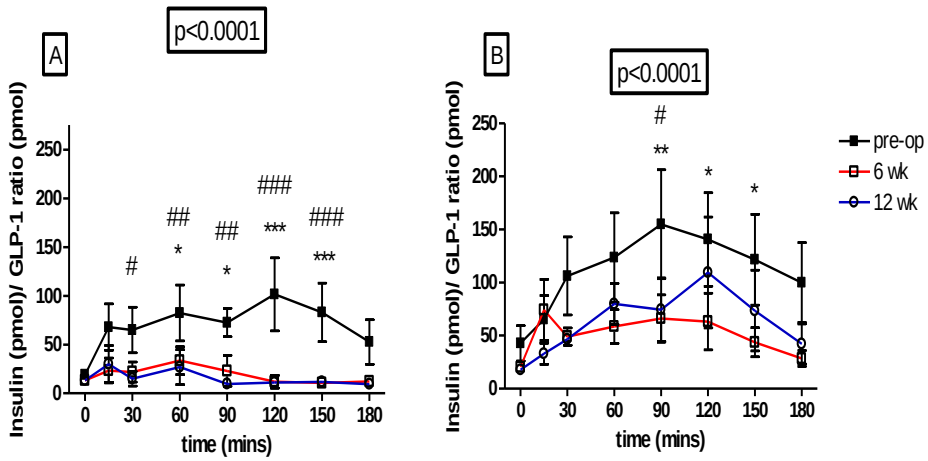


*Figure-57; comparison of meal stimulated temporal profile of Insulin/ GLP-1 ratio in the RYGBP (A) and SG (B) groups are shown. Analysis of the plasma temporal profile of insulin: GLP-1 ratio after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points did show a significant decline after both RYGBP (p<0.0001) and SG (p<0.0001). Bonferroni post-hoc analysis did show significant decline at t=60 (p<0.05), 90 (p<0.05), 120 (p<0.001), and t=150 (p<0.001) at 6 weeks, and at t=30 (p<0.05), 60 (p<0.01), 90 (p<0.01), t=120 (p<0.001) and at t=150 (p<0.001) at 12 weeks after RYGBP. This analysis in the SG group did also show significant decline at t=90 (p<0.01), 120 (p<0.05), and at t= 150 (p<0.05) at 6 weeks, and at t=90 (p<0.05) at 12 weeks. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

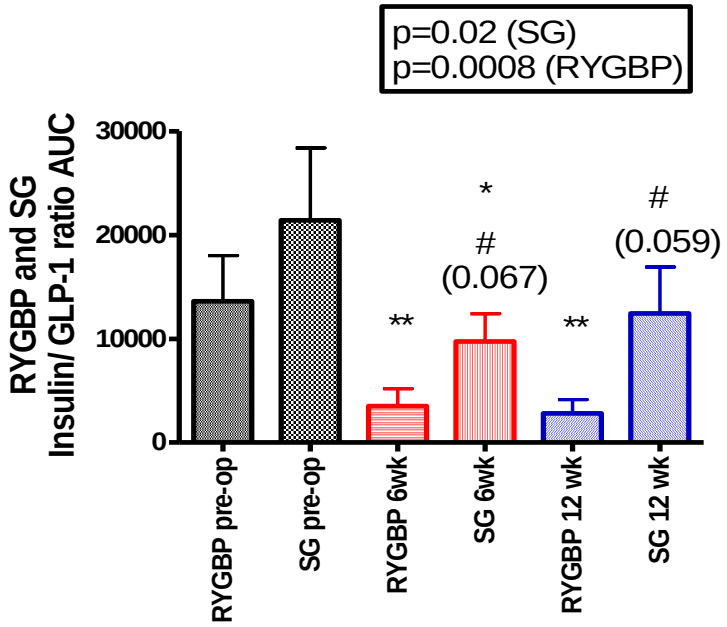


*Figure-58; A bar graph to compare pre-operative with 6 and 12 weeks post-operative insulin/ GLP-1 ratio (one way matched ANOVA). There was a significant decline after RYGBP (p=0.0008) and SG (p=0.02). A comparison between RYGBP and SG groups are also made at each time point. There was a significant (p<0.01) decline at 6 weeks and (p<0.01) 12 weeks after RYGBP, and after SG a significant (p<0.05) decline is noted at 6 weeks but not at 12 weeks. There was a trend toward significant (p=0.067) decline in the RYGBP group at 6 weeks and also (p=0.059) at 12 weeks, when compared to the SG group. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 when pre-operative values are compared with 6, 12 week post-operative values, and # p<0.05, ## p<0.01, ### p<0.001 for comparison between RYGBP and SG groups.*

4.3.9 Amylin: GLP-1 ratio before and after RYGBP and SG

Daily exenatide (GLP-1) treatment led to improved glucose and increased amylin/ insulin ratio in response to a mixed meal after islet graft dysfunction post islet transplantation (Faradji RN et al 2009). At three months after GLP-1 treatment a significant increase in amylin AUC and an increased baseline amylin/ insulin ratio were observed (Faradji RN et al 2009). At six months of treatment further increase in basal amylin/ insulin ratio was seen. It is thought that constant stimulation by exenatide may lead to supra-physiological amylin secretion made worse by hyperglycaemia (Rickels et al 2008). It is also possible that GLP-1 leads to amylin secretion from sites other than the islets (Zaki M et al 2002). The authors conclude that the effect of exenatide treatment in patients with islet allograft dysfunction is more metabolic than regenerative as the positive effects did not last long (Faradji RN et al 2009). In our study there is a

significant (p<0.0001) reduction in the amount of amylin secreted in response to an equivalent active GLP-1 stimulus after RYGBP and SG (analysis of the plasma temporal profile of amylin: GLP-1 ratio after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points). Again this may represent a threshold effect. Bonferroni post-hoc analysis did show significant decline at five time points at 6 and 12 weeks after RYGBP. This analysis in the SG group did also show significant decline at five time points at 6 weeks and at six time points at 12 weeks. The comparison of AUC of amylin: GLP-1 does also confirm a significant (p=0.0002) reduction after both RYGBP and SG surgery (matched one way ANOVA, comparison of pre-operative time point to post operative time points) (figure-61).

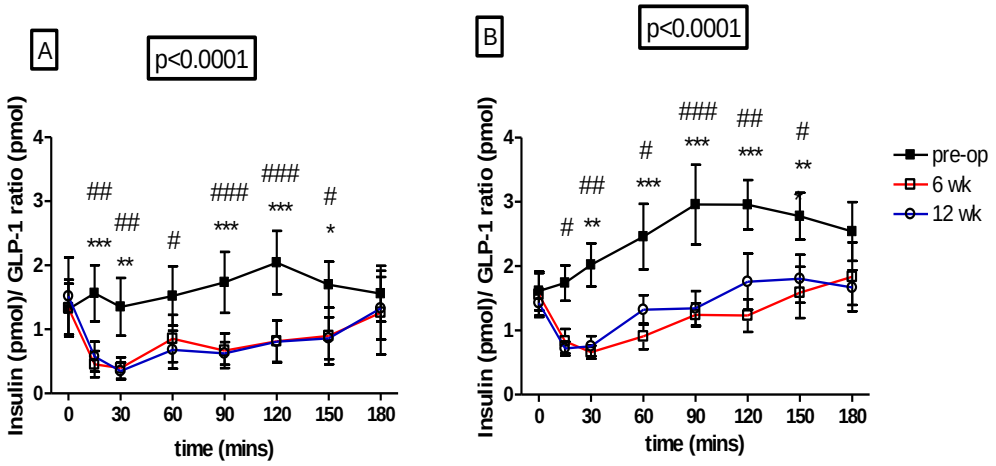


*Figure-59; Analysis of the plasma temporal profile of amylin: GLP-1 ratio after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points did show a significant decline after both RYGBP (p<0.0001) and SG (p<0.0001). Bonferroni post-hoc analysis did show significant decline at t=15 (P<0.001), t=30 (p<0.01), t=90 (p<0.001), t=120 (p<0.001), and t=150 (p<0.05) at 6 weeks, and at t=15 (p<0.01), t=30 (p<0.01), 60 (p<0.05), 90 (p<0.001), t=120 (p<0.001) and at t=150 (p<0.05) at 12 weeks after RYGBP. This analysis in the SG group did also show significant decline at t=30 (p<0.01), t=60 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), and at t= 150 (p<0.01) at 6 weeks, and at t=15 (p<0.05), t=30 (p<0.01), t=60 (p<0.05), t=90 (p<0.001), t=120 (p<0.01), and t=150 (p<0.05) at 12 weeks. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks*

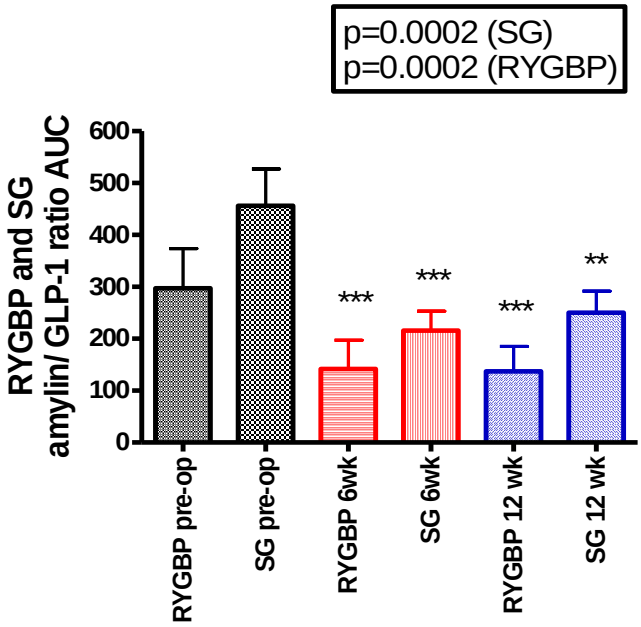


*Figure-60; Bar chart to compare amylin: GLP-1 pre-operative AUC to post operative time points in the RYGBP and SG groups. A comparison between RYGBP and SG groups are also made at each time point. *p<0.05, ** p<0.01, ***p<0.001 when pre-operative values are compared with 6, 12 week post-operative values, and # p<0.05, ## p<0.01, ### p<0.001 for difference between RYGBP and SG groups compared at each time point.*

4.3.10 Active GLP-1 secretion in the RYGBP and SG groups correlate to amylin secretion

The meal stimulated plasma active GLP-1 AUC from all visits does correlate to the corresponding plasma amylin AUC in the RYGBP (p<0.0001, r=0.82) and SG (p=0.043, r=0.18) groups.

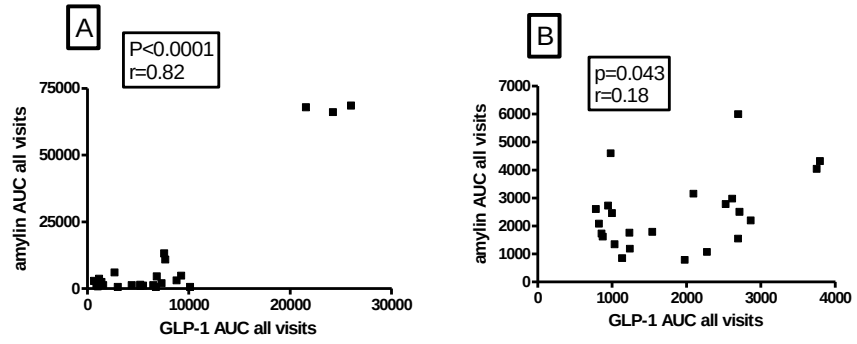


Figure-61; Scatter plots to highlight positive correlation between active GLP-1 (pM) and amylin (pM) for all visits in the RYGBP (A) and SG (B) groups.

4.3.11 Change in active GLP-1 secretion after SG does correlate to change in amylin secretion

The change in active GLP-1 secretion at 12 weeks after SG correlated to the change in amylin secretion at the corresponding time point (p=0.007, r=0.72) (figure-63). This correlation was not seen after RYGBP (p=0.213, r=0.244)

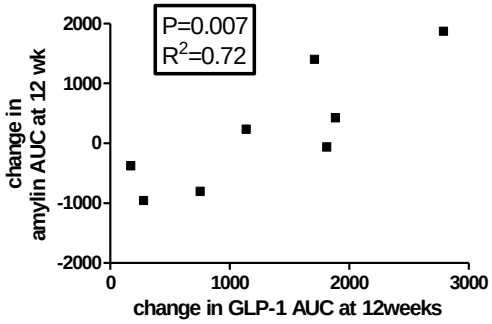


Figure-62; Scatter plot confirms positive correlation between change in plasma active GLP-1 and change in plasma amylin at 12 weeks after SG.

4.3.12 Change in insulin secretion and change in amylin secretion correlate after SG

There was a positive correlation between the change in plasma insulin and change in plasma amylin secretion at 12 weeks after SG (p=0.071, r=0.45) (figure-64). No correlation between the change in plasma insulin and the change in plasma amylin secretion was seen after RYGBP (p=0.68, r=0.03).

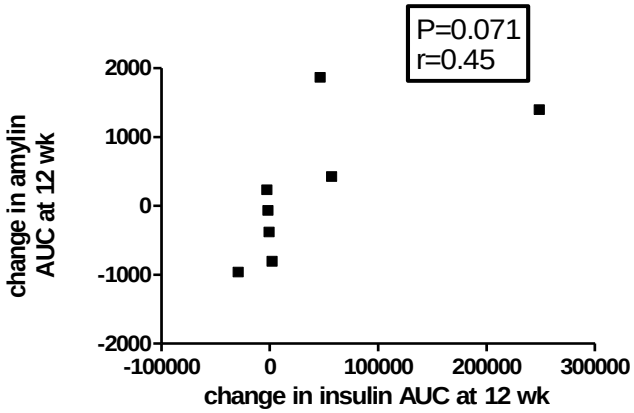


Figure-63; Scatter plot points to a positive correlation between change in plasma insulin and change in plasma amylin secretion at 12 weeks after SG

4.3.13 High active GLP-1 and correspondingly high amylin levels in a patient

One patient in the RYGBP group had meal stimulated active GLP-1 AUC levels markedly above the mean of the rest of the group (21615 vs 1339±622 at baseline, 26039 vs 6095±1168 at 6 weeks and 24254 vs 6106±840 at 12 weeks after surgery, p=0.0007). This patient's active GLP-1 response was also an outlier. Further, this patient's meal stimulated amylin AUC response was also markedly above the mean for the rest of the group (67762 vs 2123±697 at baseline, 68416 vs 3151±1592 at 6 weeks, and 65895 vs 3032±1271 at 12 weeks after surgery). This patient's active amylin temporal response to the mixed meal was also an outlier. The correlation analysis of the meal stimulated plasma active GLP-1 AUC and amylin AUC from all visits did include this patient and strengthened the correlation in the RYGBP group (p<0.0001, r=0.82).

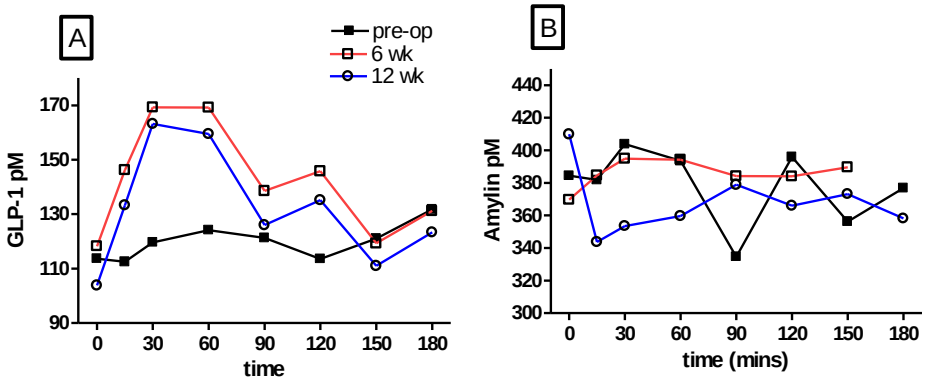


Figure-64; The mixed meal related temporal profile of GLP-1 and amylin in a patient, noted to be an outlier for GLP-1 and amylin profile and total AUC.

4.3.14 Insulin amylin ratio after bariatric surgery

It is thought that an increased ratio of amylin/ insulin expression may act as a marker for beta cell dysfunction (Weng HB et al 2008). Hyperglycaemia is thought to lead to the hypersecretion of amylin relative to insulin, and increase the amylin /insulin ratio in insulin-resistant rats (Leahy JL et al 1998). The amylin to insulin mRNA ratio is increased in these untreated rats (Weng HB et al 2008). A recent study implemented a 12 week regimen of recombined human GLP-1into spontaneously-diabetic rat related to an impairment of the glucose-induced release of insulin, to assess the effect on fasting and post-prandial amylin concentrations and islet amylin and insulin mRNA (Weng HB et al 2008). GLP-1 (7–36) stimulates the expression and secretion of amylin, whilst also increasing insulin protein expression in GK rats treated with GLP-1. GLP-1 (7–36) significantly increased the amylin and insulin mRNA levels, but markedly

decreased the ratio of amylin/insulin mRNA in spontaneous diabetic rats. GLP-1 may promote amylin gene expression separate from insulin gene expression (Weng HB et al 2008). In keeping with this GLP-1 elevated the levels of plasma amylin in response to an intraperitoneal glucose load (Weng HB et al 2008). However, it is not clear whether this is due to the direct effect of GLP-1(7–36) on stimulating amylin or due to the GLP-1 (7–36) stimulating insulin. It has been proposed that the amylin/insulin ratio may be a better measure than the absolute amylin mRNA level (Weng HB et al 2008). The content of insulin and amylin mRNA is known to correlate to the content of plasma insulin and amylin (reviewed by Cluck MW et al 2005). Amylin and insulin gene expression have usually been examined together. The independent regulation of these genes has not been examined in detail (reviewed by Cluck MW et al 2005). Under normal physiological conditions amylin and insulin are regulated in concert, but in pathological states such as diabetes and obesity their regulation may diverge (reviewed by Cluck MW et al 2005). The normal ratio of the amount of amylin mRNA and peptide to the amount of insulin mRNA and peptide can be altered in diabetes and obesity, where a marked increase in pancreatic amylin mRNA/ peptide are noted (Permert J et al 1994, Kautzky-Willer A et al 1994, Enoki S et al 1992). Second messengers utilised by GIP/ GLP-1, calcium and fatty acyl molecules can differentially regulate amylin, insulin secretion, and gene expression (reviewed by Cluck MW et al 2005). Several transcription factors such as HNF-1 are now implicated in the selective expression of the amylin gene (reviewed by Cluck MW et al 2005). Amylin and insulin mRNA content does increase in parallel following glucose challenge (Mulder H et al 1996). Supraphysiologic levels of exogenous amylin inhibit glucose-induced insulin secretion in humans, (Bretherton-Watt D, et al 1992). Further, physiologic concentrations of endogenous amylin may also effect insulin secretion (reviewed by Cluck MW et al 2005). Whilst the promoter elements and transcription factors that regulate rat and human insulin gene expression have been described, amylin gene expression is not well characterized (reviewed by Cluck MW et al 2005). The amylin promoter does contain elements similar to those present in insulin genes. Therefore a mechanism for parallel gene expression may exist (reviewed by Cluck MW et al 2005). It is thought that separate transcription factors regulate independent transcription of amylin and insulin (reviewed by Cluck MW et al 2005). Insulin secretion is inhibited by amylin both in vitro and in vivo, (Gebre-Medhin S et al 1998, Wang ZL et al 1993 and reviewed by Cluck MW et al 2005). Amylin has multiple physiologic effects on glucose homeostasis (Karlsson E 1999, Nyholm B et al 1999), including making GLP-I a less effective stimulus for insulin secretion (reviewed by Cluck MW et al 2005). Also recent studies have highlighted a role for amylin therapy in obesity (Ravussin E et al 2009, Smith SR et al 2008).

Our study is the first to examine changes insulin: amylin ratio after bariatric surgery, and its relationship to weight loss post RYGBP and SG surgery. In our study, there is a significant decrease in insulin: amylin ratio after RYGBP. Insulin secretion is not significantly altered after RYGBP. However there is a significant increase in amylin secretion after surgery. This does lead to a decrease in insulin: amylin ratio after surgery at 6 and 12 weeks. This change in ratio did correlate to %EWL at those time points after RYGBP. We did not find a correlation between insulin: amylin ratio and plasma glucose after RYGBP and SG. There have been no studies on meal stimulated amylin secretion after SG. We found no significant difference in amylin secretion after SG. The change in amylin secretion after SG did correlate to weight loss at 6 and 12 weeks after surgery. In keeping with this there was a significant increase in meal stimulated insulin secretion after SG. This led to lower insulin: amylin ratio after SG surgery. This contrasting alteration in ratio did not correlate to satiety, prospective food consumption or weight loss.

In our study GLP-1 secretion does show a positive correlation to amylin secretion in both groups, before and after surgical intervention. It is interesting that the change in meal-stimulated amylin does show a negative correlation to the change in meal stimulated insulin at six weeks and a positive correlation at 12 weeks after SG. This is due to the amylin AUC largely remaining unchanged but the insulin secretion AUC is increased from baseline at six weeks but declines between six and twelve weeks. The insulin secretion is significantly improved after SG and does not change significantly between 6 and 12 weeks. However, the meal stimulated insulin AUC is lower at 12 weeks when compared to 6 weeks. Also, the meal stimulated GLP-1 does decline from 6 to 12 weeks, again not reaching statistical significance. The amylin secretion is unchanged between 6 and 12 weeks. Therefore it is likely that other factors such as GIP, fatty acyl molecules that can differentially regulate amylin, insulin synthesis and secretion lead to an alteration in the relationship between insulin and amylin after SG, between these time points. In the RYGBP and SG groups there was a significant correlation between the AUC for GLP-1 and amylin for all visits ($p<0.0001$, $r=0.83$) in the RYGBP and ($p=0.043$, $r=0.18$) SG groups. The markedly high GLP-1 and amylin response seen in one patient adds further weight to this correlation. The post operative GLP-1 response in the SG group at 12 weeks correlated with the amylin response at that time point ($p=0.0075$, $r=0.72$). Therefore some of the change in correlation may be due to the non-significant reduction in GLP-1 secretion between these time points. In support of this, others have proposed that amylin synthesis and secretion may be

under the influence of GLP-1 (Ahrén B et al 1997), and amylin in turn may mediate some of the biological actions of GLP-1 (Asmar M et al 2010).

4.3.15 Differential change in insulin/ amylin ratio after RYGBP and SG

Amylin secretion is regulated by cAMP and (protein kinase A) PKA. GLP-1 signals through PKA. It is thought that GLP-1 promotes amylin and insulin gene expression via different intracellular pathways and result in a dissociation of their secretion (Asmar M et al 2010). Others propose that changes in the activity of the respective convertase enzymes may lead to the dissociation of these two peptides. Also, the biosynthesis and secretion of insulin is inhibited by amylin, both in vitro and in vivo (Furnsinn C et al 1994). Improved glycaemic control in T2DM did not change insulin response to glucose, but did significantly improve GLP-1 potentiation of glucose-induced insulin secretion- (Hojberg PV et al 2008). Plasma amylin is decreased in T2DM (van Jaarsveld BC et al 1993). The ability of GLP-1 on glucose-induced amylin secretion was significantly increased after improved glycaemia in T2DM. The amylin/C-peptide ratio was also significantly higher with GLP-1 (Asmar M et al 2010). This may explain the change in insulin amylin ratio between the two groups in our study. The relative amylin content would be improved by better glycaemic control after RYGBP and SG surgery. Further as RYGBP leads to a more pronounced GLP-1 response, a more pronounced amylin response will be seen after RYGBP. The change in amylin is disproportionate to change in C-peptide (Asmar M et al 2010). Insulin/ amylin ratio is altered differentially after RYGBP and SG. There is a significant ($p<0.0001$) decrease in the ratio after RYGBP surgery, post hoc test show significant decrease at t=120 and t=150 at 6 weeks and at t=90, 120, 180 at 12 weeks after RYGBP (figure-66). In the SG group there is a significant ($p=0.0002$) increase in insulin amylin ratio after surgery, post-hoc test show significant increase at 15 minutes after the meal at 6 weeks after surgery (figure-66).

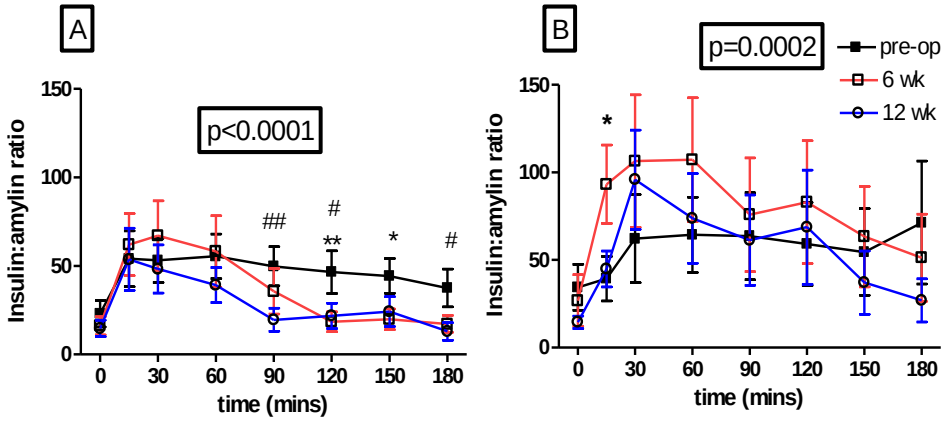


*Figure-65; Analysis of the plasma temporal profile of insulin :amylin ratio after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points does point to a differential response after RYGBP (A); where it was significantly (p<0.0001) decreased, and significantly (p=0.0002) increased after SG (B). Bonferroni post-hoc analysis did show significant decline at t=120 (p<0.01), and t=150 (p<0.05) at 6 weeks, and at t=90 (p<0.01), t=120 (p<0.05) and at t=180 (p<0.05) at 12 weeks after RYGBP. This analysis in the SG group did not show any significant change at a time point. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks.*

4.3.16 Change in active GLP-1 correlates to change in insulin/ amylin ratio after RYGBP

The change in active GLP-1 AUC at 6 and 12 weeks after RYGBP did also correlate to the change in insulin/ amylin ratio after RYGBP at 6 weeks (p=0.029, r=0.58) and 12 weeks (p0.057, r=0.48).

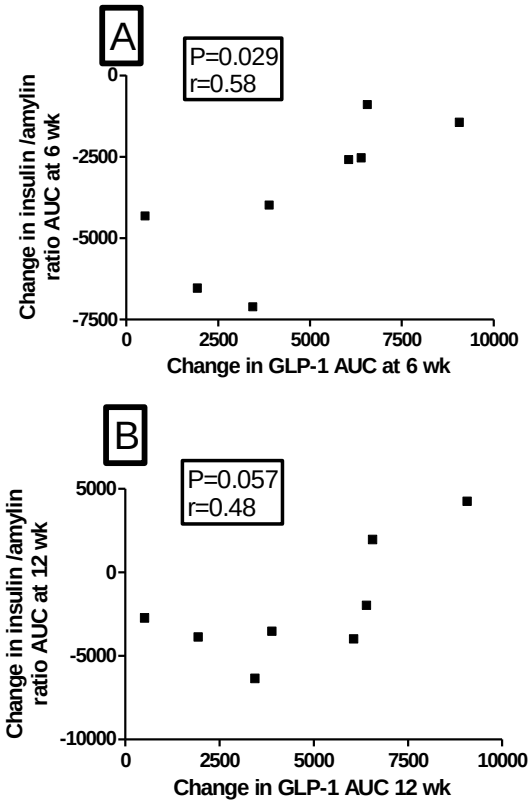


Figure-66; Scatter plots to display correlation between change in GLP-1 and change in insulin/ amylin ratio at 6 (A) and 12 (B) weeks after RYGBP.

4.3.17 Analysis of RYGBP insulin profile excluding Type-2 DM patient

The RYGBP group had one T2DM patient. We undertook comparative analysis of insulin and glucose excluding this patient. RYGBP led to a non-significant decline in fasting plasma insulin from 76.7±12.9 pmol/L to 64.7±10.3 pmol/L at 6 weeks, and 58.3±8.0 pmol/L at 12 weeks. This did not alter significantly when the T2DM patient was excluded (p=0.59, paired t-test). After excluding the T2DM patient the baseline fasting plasma insulin declined from 67.3±10.1 pmol/L to 66.3±11.2 pmol/L at 6 weeks and to 59.4±9.0 pmol/L at 12 weeks. Analysis of the temporal profile of insulin after RYGBP with the diabetic patient did not reveal any significant increase in insulin secretion (p=0.178), However, Bonferroni post tests did confirm a significant increase at t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120 (p<0.01) at 6 weeks after surgery. The analysis at 12 weeks after surgery did not identify any significant increase or decline at any time points. This was altered after excluding the T2DM patient. The temporal profile after surgery did now show a trend towards significance (p=0.06). Further, Bonferroni post test analysis did confirm a significant increase at t=15 (p<0.05), t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120

(p<0.01) at 6 weeks after surgery. The analysis at 12 weeks after surgery also confirmed a significant decline at t=120 (p<0.05). The baseline peak insulin in the RYGBP group was observed at t=120, 593.5±142.1 pmol/L. This did alter and occur earlier at t=60 1100.8±367.7 pmol/L at 6 weeks after surgery, and at t=60 753.3±191.1 pmol/L at 12 weeks after surgery. The peak did alter significantly (p=0.045, paired t-test) when the T2DM patient was excluded. However the time at which the peak insulin concentrations occur did not alter between the two groups. The baseline peak insulin in the RYGBP group excluding the T2DM patient was observed at t=120, 634.7±154.3 pmol/L. Again this did alter and occur earlier at t=60 1154.1±412.6 pmol/L at 6 weeks after surgery, and at t=60 776.8±215.1 pmol/L at 12 weeks after surgery. There was a non-significant increase in plasma insulin AUC from 80759±16167 at baseline to 82696±21232 at 6 weeks after RYGBP surgery. This increase was reversed by 12 weeks after RYGBP surgery to 64523±11133. This did not alter significantly when the T2DM patient was excluded (p=0.82). The AUC in the group excluding the T2DM patient was 83742.8±18017 at baseline increasing to 85882±23803 at 6 weeks after RYGBP surgery. As with the RYGBP group this increase was reversed by 12 weeks after RYGBP surgery to 60253±11659. In summary the inclusion of a T2DM patient did not alter fasting insulin, time of peak insulin nor insulin AUC. However it did alter the peak plasma insulin concentration.

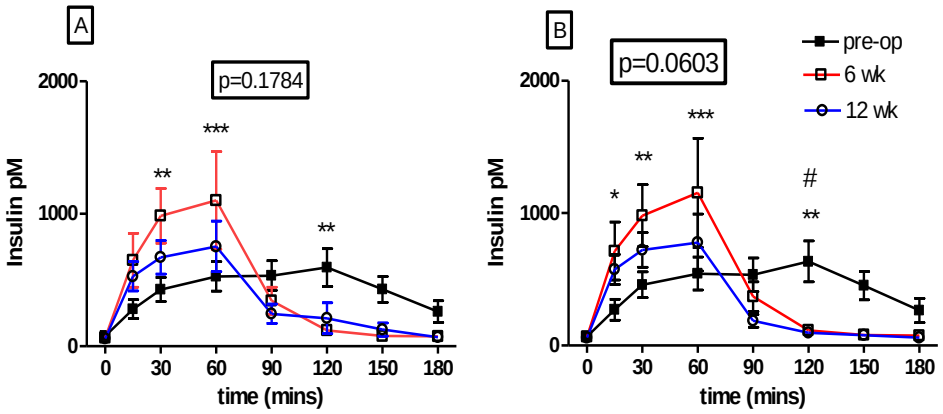


Figure-67; Comparison of plasma insulin concentrations after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points in the RYGBP (A) and RYGBP excluding T2DM patient (B) groups. Analysis of the temporal profile of insulin after RYGBP with the diabetic patient did not reveal any significant increase in insulin secretion (p=0.178), However, analysis with Bonferroni post Hoc tests did confirm a significant increase at t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120 (p<0.01) at 6 weeks after surgery. The

*analysis at 12 weeks after surgery did not identify any significant increase or decline at any time points. This was altered after excluding the T2DM patient. The temporal profile after surgery did now show a trend towards significance (p=0.06). Further, Bonferroni post test analysis did confirm a significant increase at t=15 (p<0.05), t=30 (p<0.01), t=60 (p<0.001), and a significant decline at t=120 (p<0.01) at 6 weeks after surgery. The analysis at 12 weeks after surgery also confirmed a significant decline at t=120 (p<0.05). Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks.*

4.3.18 Analysis of RYGBP group glucose profile excluding Type-2 DM patient

The fasting baseline glucose of 5.6±0.5 mmol/L was not significantly altered to 5.4±0.5 mmol/L at 6 weeks and to 5.4±0.4 mmol/L at 12 weeks after RYGBP. Again this baseline glucose of 5.14±0.2 mmol/L was not significantly altered to 4.98±0.3 mmol/L at 6 weeks and 5.03±0.17 mmol/L at 12 weeks after RYGBP when the T2DM patient is excluded. However, the mean fasting glucose was significantly (p=0.0096) altered when the two groups are compared. There was a significant decline in the temporal profile of glucose (two way ANOVA, p=0.0409) after RYGBP (figure-69). Bonferroni post test analysis confirms a significant decline at t=15 (p<0.001), t=30 (p<0.001), t=90 (p<0.05), t=120 (p<0.001), t=150 (p<0.01) at 6 weeks after surgery, and at t=30 (p<0.01), t=120 (p<0.001), t=150 (p<0.001) at 12 weeks after surgery (figure-69). Again when the T2DM patient is excluded, there was a significant decline in the temporal profile of glucose (two way ANOVA, p=0.0168) after RYGBP (figure-69). Bonferroni post test analysis confirms significant decline at t=15 (p<0.001), t=30 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.01) at 6 weeks after surgery, and at t=15 (p<0.01), t=30 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.01) at 12 weeks after surgery (figure-69). A late peak in post-prandial glucose was noted at t=120 7.6±0.9 mmol/L prior to RYGBP. This peak in glucose occurs early at 6 weeks t=30, 9.2±1.1 mmol/L, and at 12 weeks t=30, 8.7±0.8 mmol/L (figure-69). There was a significant decline (p=0.0061) in the peak plasma glucose when the T2DM patient is excluded. However, the timing of the peak remains the same. The baseline peak glucose occurs late at t=120 6.74±0.16, this was brought forward to t=30 8.26±0.54 at 6 weeks and t=30 7.97±0.41 at 12 weeks in the RYGBP group when the T2DM patient was excluded. In the RYGBP group baseline glucose AUC was significantly decreased from 1257±143 to 1164±129 (p=0.008) at 6 weeks, and 1139 ±141 (p=0.001) at 12 weeks after surgery (table-x). The exclusion of the T2DM patient led to the baseline AUC to decline from 1117.7±37.6 to 1037.8±31.2 at 6 weeks and 1000.3±26.1 at 12 weeks. Further, there was a significant (p=0.0009, paired t-test) decline in the mean plasma glucose AUC when the T2DM patient is excluded. In summary excluding the

T2DM patient did lead to a significant decline in mean fasting, mean peak plasma glucose, and mean glucose AUC. The temporal profile of glucose is also significantly altered.

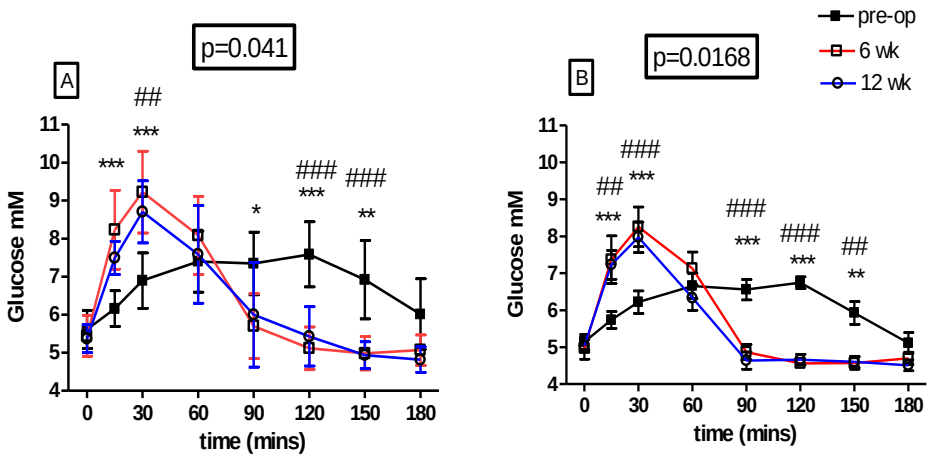


*Figure-68; Comparison of plasma glucose concentrations after a mixed meal test utilising a matched two-way ANOVA, comparing pre-operative time point to post-operative time points in the RYGBP (A) and RYGBP excluding T2DM patient (B) groups. There was a significant decline in the temporal profile of glucose after RYGBP. Bonferroni post test analysis did confirm significant (p=0.041) decline at t=15 (p<0.001), t=30 (p<0.001), t=90 (p<0.05), t=120 (p<0.001), t=150 (p<0.01) at 6 weeks after surgery, and at t=30 (p<0.01), t=120 (p<0.001), t=150 (p<0.001) at 12 weeks after surgery (figure-69). Again when the T2DM patient is excluded, there was a significant decline in the temporal profile of glucose (p=0.0168) after RYGBP. Bonferroni post test analysis did confirm significant decline at t=15 (p<0.001), t=30 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.01) at 6 weeks after surgery, and at t=15 (p<0.01), t=30 (p<0.001), t=90 (p<0.001), t=120 (p<0.001), t=150 (p<0.01) at 12 weeks after surgery. Over the three visits: *p<0.05, ** p<0.01, ***p<0.001 at 6 weeks, # p<0.05, ## p<0.01, ### p<0.001 at 12 weeks.*

4.4 Discussion

4.4.1 Remission of T2DM diabetes after bariatric surgery

In our study there is a decline in HOMA IR after SG. The decline after RYGBP did not reach statistical significance. This discrepancy can partly be explained by the significant decline in acyl-ghrelin seen only after SG but not after RYGBP, both in our

study and in the study by Karamanakos and colleagues. Karamanakos and colleagues showed significant decrease in fasting and post-prandial ghrelin after SG but not after RYGBP (Karamanakos SN et al 2008). Samat and colleagues have also shown a correlation between suppression of total ghrelin and insulin sensitivity at 12 months after RYGBP (Samat A et al 2013). However, others have shown change in insulin resistance measured by HOMA IR to be significantly lower after RYGBP and SG surgery (Korner J et al 2005, Buchwald H et al 2009, Peterli R et al 2012, Ramon J M et al 2012). But these were cross sectional studies. In our study there is an improvement in post-prandial glucose after RYGBP and SG. This improvement is more pronounced after SG than RYGBP. Others have also shown similar fasting and post prandial glucose AUC in the two groups at 12 months after surgery (Karamanakos SN et al 2008, Peterli R et al 2012, and Samat A et al 2013).

Gill and colleagues point out that the duodenal exclusion hypothesis is unlikely to be a viable explanation given the remission of diabetes after sleeve gastrectomy in a large percentage of patients despite of a functional duodenum (Gill RS et al 2010, and reviewed by Laferrère B. 2011). In our study RYGBP and SG leads to comparable loss of body weight, body fat and a reduction in plasma insulin. This is in keeping with recent comparative studies (Karamanakos N et al 2008, Peterli R et al 2009, Chambers AP et al 2011, Basso N et al 2011, Peterli R et al 2012, and Ramon J M et al 2012). In our study RYGBP and SG led to greater initial insulin secretion from baseline followed by rapid return toward baseline secretion. The insulin area-under the- curve (AUC) was greater at 6 weeks after both procedures when compared to pre-operative levels. This rise was reversed at 12 weeks with improved insulin resistance. These findings are in keeping with recent studies (Lee et al 2010, Chambers AP et al 2011, Ramon J M et al 2012, and Peterli R et al 2012).

4.4.2 The role of active GLP-1

It is known that surgery and not weight loss mediates an increase in GLP-1 (B. Laferrere et al 2008, B. Ahren et al 2003). A significant and similar decline in fasting glucose, insulin and HOMA-IR was seen after diet and RYGBP induced 10 kg weight loss (Oliván B et al 2009). However, a significant increase in glucose-stimulated GLP-1 occurred only after RYGBP (Oliván B et al 2009). In our study there is no significant change in fasting active GLP-1 after either procedure. The temporal profile of meal stimulated active GLP-1 secretion is similarly and significantly altered after both RYGBP ($p<0.0001$) and SG ($p<0.0001$). Further the active GLP-1 response after RYGBP is almost three-fold higher than that after SG. There is a significant difference in the active GLP-1 AUC between the two groups at 6 ($p= 0.014$) and 12 weeks

(p=0.0005). The significant increase in active GLP-1 seen at six weeks is not altered at 3 months after surgery. A similar parallel group study was done by Peterli and colleagues (Peterli R et al 2009, and Peterli R et al 2012). In the initial study, patients were studied at pre-operative, 1 week and 3 months after surgery after RYGBP and SG. The SG group had three diabetic patients but none in the RYGBP group. The fasting insulin concentrations and HOMA indices were significantly reduced, before any significant weight loss had occurred (Peterli R et al 2009). As with our study, the impaired postprandial active GLP-1 and insulin response was reversed in both groups, at a week after surgery. The authors argue that this points at endocrine mediators (Peterli R et al 2009, Peterli R et al 2012). As with our study, a marked increase in postprandial active GLP-1 and insulin concentrations was observed after RYGB and SG. As with our study the RYGBP patients had an exaggerated postprandial active GLP-1 response at 1 week that was significantly higher than that of the SG group (Peterli R et al 2009, and Peterli R et al 2012), but this difference in active GLP-1 response was no longer significant at 3 months after surgery (Peterli R et al 2009, and Peterli R et al 2012). It is also noteworthy that the baseline active GLP-1 AUC are a third of the AUC in our study. By contrast, in our study, there is a significant increase in meal stimulated active GLP-1 secretion after both procedures at 6 and 12 weeks, but a more pronounced (3-fold) increase in meal stimulated active GLP-1 secretion after RYGBP when compared to SG that is maintained at 12 weeks. These discrepancies may be explained by a lack of standardization.

The study by Kindel and colleagues to examine GLP-1 antagonists on glucose homeostasis after bariatric surgery does point to reversal of the improved glucose homeostasis by GLP-1 receptor antagonism (Kindel TL et al 2009). This does provide direct evidence that at least some of the improvement after RYGBP is mediated by hind gut hormones (reviewed by Laferrère B. 2011). A recent study in rodents confirms a link between augmented GLP-1 secretion and insulin secretion (Shin AC et al 2010). The discrepancy in meal stimulated active GLP-1 in our study does not seem to adversely effect insulin secretion or plasma glucose levels after SG when compared to patients that underwent RYGBP, in fact insulin secretion is significantly increased after SG in our study. The more pronounced decline in GLP-1 to insulin ratio after RYGBP may explain someof this discrepancy. This threshold effect of GLP-1 has been reported by others (Hansen E N et al 2011). In the SG group the change in active GLP-1 did correlate to change in amylin and also account for the improvement in glucose disposal. GLP-1 and insulin did show positive correlation after RYGBP that led to significantly lower post-prandial plasma glucose reaching that of lean controls (Shin AC et al 2010, Jacobsen S H et al 2012). In our study the meal stimulated active GLP-1

response after RYGBP correlates to PYY3-36. This correlation has been reported in rats after RYGBP (Shin AC et al 2010). However, our study is the first to report this in humans. Our study is also the first to report that meal stimulated active GLP-1 does also correlate to PYY3-36 and insulin after SG. In the RYGBP group, we are the first to report that the change in meal stimulated active GLP-1 after RYGBP does correlate to change in meal stimulated insulin and insulin: amylin ratio at 6 and 12 weeks after surgery. Others have identified a correlation between peak active GLP-1 and insulin after RYGBP (Shin AC et al 2010, Jacobsen S H et al 2012). Further, in our study the change in active GLP-1 after SG did correlate to change in amylin. The above correlations may help explain the improvement in glucose disposal and remission of T2DM reported after SG. The change in GLP-1 from baseline did negatively correlate to prospective food consumption in the RYGBP group ($p=0.036$, $r=0.19$). By comparison in the SG group satiety after the liquid meal did positively correlate to change in GLP-1 ($p=0.001$, $r=0.4$). Further, prospective food consumption also negatively correlate to GLP-1 ($p=0.004$, $r=0.33$).

4.4.3 Plasma insulin, glucose homeostasis after RYGBP and SG

In our study a similar post-meal temporal glucose profile is seen after both RYGBP and SG. However the improvement in post-meal plasma glucose after RYGBP is statistically significant. There is an equivalent fasting, meal stimulated insulin response after both RYGBP and SG. The disparate GLP-1 response seen after RYGBP and SG suggest different mechanisms at play in the two groups to produce an equivalent meal stimulated insulin secretion and similar plasma glucose profile after the two procedures. The pronounced GLP-1 response seen after RYGBP is thought to promote insulin secretion in this group (Whitson BA et al 2007, Dezaki K et al 2008, Peterli R et al 2009, Li F et al 2009, Shin AC et al 2010, Chambers AP et al 2011, Jacobsen S H et al 2012). It is thought that the lack of such a pronounced GLP-1 response after SG may be compensated for by the decrease in ghrelin seen after SG and improved insulin sensitivity (Karamanakos SN et al 2008, Peterli R et al 2009, Li F et al 2009, Papailiou J et al 2010, Rizzelo M et al 2010, Abbatini et al 2010, and Peterli R et al 2012). This points to a combination of foregut and hind gut hormones leading to equivalent clinical outcome after these procedures (Peterli R et al 2012). In our study there was no significant change in insulin AUC after both procedures. Others have shown greater insulin AUC (Chambers AP et al 2011). In contrast to a 100% resolution of T2DM in morbidly obese patients (Vidal et al 2008), the resolution rate in over weight advanced T2DM is much lower at 50% a year after surgery (Lee et al 2010). The authors suggest the discrepancy may be due to the type of patients studied, highlighting a higher incidence of B-cell failure is present in the later study group (Lee et al 2010). It seems

therefore that bariatric surgery does influence both beta cell failure and insulin resistance.

4.4.4 Acyl-ghrelin and HOMA IR

In our study SG alone leads to a significant decline in fasting and meal stimulated acyl-ghrelin. Further there is a rise in acyl-ghrelin between 6 and 12 weeks after RYGBP. This late paradoxical rise has also been identified in a recent study on acyl-ghrelin at 6 to 12 months after surgery (Barazzoni R et al 2013). In contrast to our findings, Shin and colleagues found a significant suppression in acyl-ghrelin after RYGBP. This study utilized multiplex assay's, with high inter-assay variability (<24%), and rats underwent one assessment at three months after surgery, therefore introducing both high variability in plasma hormone levels, and perhaps missing immediate physiological changes (Shin AC e al 2010). Other studies that have examined the role of ghrelin, conducted assays for total ghrelin in the absence of HCL and protease inhibitors, and despite this, yielding similar results to our active acyl-ghrelin results (Karamanakos SN et al 2008). The decrease in acyl-ghrelin secretion after SG may help restore insulin sensitivity (Peterli R et al 2009, Peterli R et al 2012). Some speculate that the weight independent resolution of type-2 DM and improvement in glucose homeostasis after bariatric surgery may in part be mediated by acyl- ghrelin (Papailiou J et al 2010). The decline in acyl-ghrelin is thought to facilitate maximal capacity in the islets enabling the islets to respond adequately to the hyper-glycaemia and meet the increased demand associated with obesity (Papailiou J et al 2010 and Abbatini et al 2010). Ghrelin is also known to decrease insulin secretion in vitro and in vivo ((Dezaki et al, 2008; Reimer et al, 2003). In another study, the greatest improvement from preoperative values in HOMA IR occurred in the SG group, the authors suggest that this may be due to the large drop in ghrelin seen after SG (Abbatini et al 2010), also in keeping with our study findings.

4.4.5 Summary

In our study SG and RYGB markedly improved glucose homeostasis. Comparative analysis excluding the T2DM patient in the RYGBP group does not point to significant changes in the insulin profile. However as expected the glucose homeostasis is improved in the RYGBP group when the T2DM patient is excluded. The improvement in insulin secretion is thought to be through the augmented GLP-1 response, reduction in acyl-ghrelin and weight loss. The decrease in ghrelin secretion seen after SG may lead to improved insulin sensitivity, leading some to propose that the proximal small intestine may not mediate any of the improvement in glucose homeostasis (Peterli R et

al 2009, Karra et al 2010). The rise in meal stimulated GLP-1 seen in our study may lead to changes in glucose homeostasis through all the above effects. The rise in GLP-1 after RYGBP and SG do not lead to equivalent glucose dependent insulin secretion. This may be related to a threshold phenomenon. The differential insulin amylin ratio after RYGBP and SG is noteworthy. The relatively lower amylin in the SG group may also contribute to the improved glucose homeostasis after SG, and this may further compensate for the relatively lower GLP-1. This may in part be due to the different GLP-1 responses after the respective procedures. The GLP-1 stimulated amylin response does also show a threshold phenomenon. However, there does not seem to be any difference between the two groups.

Statistical analysis is based on assumption of independence between the model residuals, with no correlation in time or space. A study with repeated measures in individual subjects does therefore contain potential sources of non-independence, and negated when individuals are only measured once. It is postulated that for time-series data, unmeasured factors can produce correlations or temporal auto-correlation. In our study the temporal correlation between weight loss before and after surgery, low calorie consumption before and after surgery does make it difficult to isolate these changes. In light of recent publications on low calorie mediated improvement in glucose homeostasis, further work to undertake studies on active gut hormone changes standardized for calorie consumption through-out the study period would help isolate the effects of calorie consumption. Recent publications have identified areas that require clarification. It is well established that surgically induced direct early delivery of nutrients to the small intestine results in an enhanced GLP-1 response, in turn enhancing the insulin response. This response can be augmented by GLP-1 analogues and blocked by GLP-1 receptor blockers. It has also emerged that sudden negative calorie balance in type 2 diabetes normalizes plasma glucose levels within days. It is not yet clear as to what extent these two competing mechanisms play in the resolution or improvement in diabetes, nor the role of these two mechanisms on enhanced meal-related insulin secretion. The role of GLP-1 secretion on long-term β-cell function is also yet to be elucidated.

Chapter 5

Long term and short term metabolic signals influence risk-sensitive reward in humans.

5.1 Introduction

The procurement of food and food intake is regulated by a complex neuro-endocrine network (Lenard NR and Berthoud HR 2008). The neural network regulating food intake can be divided into homeostatic and non-homeostatic pathways (Gao Q and Horvath TL 2008). The non homeostatic pathway is thought to mediate the rewarding aspects of food (Lenard NR and Berthoud HR 2008, Gao Q and Horvath TL 2008). The two pathways are thought to interact to govern feeding behaviour (Morton GJ et al 2006). More recently, a number of studies have begun to explore the importance of non-hypothalamic and cortical regions in feeding behaviour (Berthoud, 2007). The homeostatic and non-homeostatic elements within the nervous system respond to information concerning internal state and external environment to maintain energy balance. Critical brain regions have been identified through experimental lesioning, electrical/chemical stimulation, targeted gene deletions and functional brain imaging studies (Berthoud, 2003). Neuronal tracing studies demonstrate the hypothalamus to be well connected to many other regions in the brain, resulting in a complex circuit that allows adaptation and coordination in an unpredictable environment (Berthoud, 2002). Imaging studies have begun to demonstrate that circulating appetite signals can modulate brain activity in areas beyond the hypothalamus and brainstem such as the OFC (Malik et al 2008); (Batterham et al 2007). Peripheral signals relaying information regarding nutrient status appear to be essential for appetite regulation. A number of hormones released from the GI tract have been isolated and investigated for their roles in energy balance. In addition, the receptors of several of these hormones have been located in areas of the brain characterised for their involvement in appetite and bodyweight regulation (Chaudhri et al 2006). In the current calorie rich environment, it is clear that socio-economic and sensory influences such as availability, palatability, variety, social context and meal timing impact upon feeding behaviour (de Castro and Stroebele, 2002). Hence brain regions involved in the processing of the psychological features of appetite, such as liking, wanting, pleasure, hedonic value and reward as well as the memories of these features, have been under investigation recently (Berthoud, 2003).

The concept that reward perception is subject to homeostatic regulation derives from evidence that food deprivation strongly augments the reward value. One mechanism to explain this effect proposes that metabolic signals such as leptin and insulin tonically inhibit brain reward circuitry and that, by lowering circulating levels of these hormones, energy restriction increases the sensitivity of reward circuits (Fulton et al 2000 and Figlewicz *et al* 2004). More recently evidence from animal studies and functional

magnetic imaging has suggested that primary re-inforcers such as food (Beaver JD et al 2006 and Batterham RL et al 2007) and secondary re-inforcers such as psycho-active drugs (Volkow ND and Wise RA 2005) and monetary rewards (Ernst et al 2004 and Matthews et al 2004) are all thought to mediate their rewarding effects through the dopaminergic reward pathway.

Animals take risks when foraging for food. Risk-sensitive foraging theory states that this risk is dependent on the animal's baseline energy state, the energetic benefit of the food reward and the risks involved in achieving this energetic benefit (Caraco, T et al 1980, Joseph M et al 1988, JM McNamara, AI Houston 1992). Many foraging animals have been shown to respond to food variability, by selecting the risk averse source or the least variable source when expected energy intake exceeds the caloric needs of the animal, and the more variable/ risky option when expected energy intake is less than that required for survival (Caraco T et al 1980, Joseph M et al 1988, JM McNamara and AI Houston 1992). This effect is seen across many species when energy reserves are depleted by fasting, or energy requirements are increased by altering ambient temperature (A Kacelnik and M Bateson 1996), such that when a meal has a small effect on metabolic state, and the animal is in a relatively low-energy state, here a greater risk-taking approach is taken so as not to fall below the metabolic target (Kacelnik A and Bateson M, 1996). The metabolic reference point is often taken in ecology as the intake required for survival. Baseline risk will depend upon baseline energy reserves, and energy requirements (Kacelnik A and Bateson M, 1996), and an increase in baseline risk-aversion is seen as energy reserves exceed a metabolic threshold. In other words animals that are energy-replete after a meal, do not need to indulge in risky behaviour around predators, and can do so without the danger of falling below a metabolic target. Foraging animals have developed sensitivity to environmental variation in food sources. Ecological theories on the feeding behaviour of foraging animals does account for the daily risks they take in searching for food sources; risk-sensitive foraging theory describes an integration of risk and food reward in ecology (JM Mcnamara, AI Houston 1992). Baseline risk will depend upon baseline energy reserves, and energy requirements (Kacelnik A and Bateson M, 1996). Risk-sensitive foraging theory states that this risk is dependent on the animal's baseline energy state, the energetic benefit of the food reward and the risks involved in achieving this energetic benefit (Caraco, T et al 1980, Joseph M et al 1988, JM Mcnamara, AI Houston 1992).

It is known that activity in the reward pathway is related to presentation of conditioned stimuli linked to natural rewards in animals (Berridge, 1994). A study in monkeys,

showed activity linked to tasting food reward only initially, however after cues were introduced, the greatest activity in the reward pathway was elicited by the conditioned stimuli in anticipation of the reward (Berridge 1994). The reward pathway does not differentiate between rewarding experiences provoked by natural reinforcers like food, illicit drugs like cocaine, or behaviours like gambling (Kelley et al 2005). An individual's approach to risk-sensitive financial reward is not dissimilar to a foraging animal's approach to risk sensitive food reward (Lee D 2005). Economic theories on risk-sensitive monetary reward date back to the eighteenth century. The expected utility theory proposed by Daniel Bernoulli states that individuals place subjective values or utilities on monetary outcomes. Utility is the product of the subjective value and the probability of that outcome (Lee D 2005). Modern financial and economic theories account for risk-sensitivity in humans (Chris Starmer 2000, Platt ML and Huettel SA 2008). When a comparison between an individual's decision making and animals that make risk-sensitive foraging decisions is made; like animals that have sufficient energy for the day, humans are risk averse when they face potential monetary gains and risk prone when the choice involves potential monetary loss, as when an animal faces inadequate energetic benefit (Lee D 2005). Further, there is some evidence to point towards similarities between monetary and sugary fluid rewards in humans, a uniform pattern of risk sensitive decision making was seen in both humans and non-human primates (Hayden BY and Platt ML 2009). Recent fMRI studies have highlighted the link between risk-sensitive reward and the dopaminergic reward pathway; a study with a simple gambling paradigm did show total winnings correlated with hemodynamic response in the reward pathway (Elliott et al, 2000), other fMRI studies have also adopted this risk-sensitive reward paradigm (Ernst et al 2004 and Matthews et al 2004) and confirm increased activity in the reward pathway during selection of the high-reward/risk option than during selection of low-reward/risk option.

Further, there is some evidence to point towards similarities between monetary and sugary fluid rewards in humans. A recent human study to compare and assess choices made for sugary fluid rewards and monetary gains on gambling tasks, revealed a consistent pattern of decision making for both food and monetary rewards (Hayden BY and Platt ML 2009). Here a uniform pattern of risk sensitive decision making was seen in both humans and non-human primates (Hayden BY and Platt ML 2009). However, metabolic state is not known to play a part in economic theories on decision making in humans, this is in contrast to ecological theories on animal foraging behaviour. Risk-sensitive reward in humans can be formally quantified with the aid of a variety of methods (G. W. Harrison, E. E. Rutström, 2008). We employed the paired lottery task (Hey and Orme 1994).

Takahashi attempts to link endocrine markers of energy homeostasis and feeding behavior with obesity utilizing mathematical modeling, utilizing economic theories as a framework. He proposes that neuroeconomic studies can examine the link between endocrine mediators of energy metabolism; ghrelin, leptin and satiety; amylin, GLP-1 and PYY, and obesity using complex mathematical models based in economic theories, to study complex behavior linked to obesity. He points to the investigation of obesity attracting attention in several inter-related disciplines of neurobiology, psychiatry, and neuroeconomics, and suggests that therefore studies incorporating these disciplines into a mathematical model may yield insight into how neurobiological substrates can be integrated to predict outcome (Takahashi, 2010).

5.2 Aims of the study:

We conducted a single blind within subject randomised study to assay an individual's risk sensitive reward seeking behavior in three different feeding states: fasted, fed or immediately, and 60 minutes post-meal. To assess the influence of metabolic state on risk sensitive monetary reward seeking behavior.

5.3 Methods

5.3.1 Ethics

Ethical approval was obtained from University College London Research Ethics Committee. This study was carried out in accordance with the principles of the Declaration of Helsinki. All subjects attended a screening session where they received oral and written information about the study and were given the opportunity to ask any questions about the study. An informed consent form was signed on the first day of the study. Data collected was stored in accordance with the Data Protection Act 1998.

5.3.2 Subject recruitment

Healthy, normal weight male volunteers between the ages of 18 -60 were recruited through advertisements on the University College London campus. Exclusion criteria were the use of regular medications, smoking, food allergies and presence of any medical or psychiatric illnesses. All subjects were weight-stable for at least 3 months prior to recruitment. On the day before each study day subjects were asked to maintain a similar schedule of activities and refrain from alcohol consumption. One subject dropped out of the study after the first visit, citing travel abroad, another was excluded due to fasting hyper-glycaemia. Randomisation errors led to three other

subjects being excluded from the analysis and a further subject was excluded from the biochemical analysis as some of his plasma samples were haemolysed. Eighteen subjects were included in the final analysis.

5.3.3 Subject standardisation and acclimatization

Subjects were asked to follow a standardization protocol on the day prior to the study morning. This involved refraining from alcohol and strenuous exercise (Chandarana, K et al, 2009). They consumed a standard 774 kcal meal between 19:30 and 20:30 on the evening before the study morning (Chandarana, K et al, 2009). Subjects then fasted and drank only water until attending our clinical facility the following morning. Details of the standard meals are listed (figure-71)

5.3.4 Cognitive tasks undertaken in three metabolic states.

We assayed a subject's risk sensitive reward behaviour through the ordered paired lottery (Hey and Orme, 1994). Subjects were given two options of monetary reward per scenario and 200 scenarios at each visit. Each option had four monetary values. These scenarios were given in the same order on each subsequent visit, but unknown to the subjects, the placement of the monetary values in the two options in a scenario was changed from week to week. In each scenario, one option was defined as the safe option and the other risky. We calculated an individual's risk averse score per visit, by calculating the percentage of times an individual chose the safe option. In order to make the presented scenarios of monetary risk taking as real as possible, subjects were told that the choices they made will be stored, and one of his choices played out at random, at the end, to determine payout. The subjects also did two other control computer tasks. In order to make these control tasks as real as possible, subjects were also paid monetary rewards according to their performance in these tasks. These control tasks were temporal discounting, where subjects were asked to make a choice between payment of a higher monetary value at a later date against a sooner payment of less monetary value; and a learning rate task, where subjects were asked to recognise patterns encrypted in a pixel maze. A single blind within subject randomised study was performed, with each subject tested on three separate occasions. Tasks were undertaken in three different feeding states: fasted (t=0 to t=60), fed/ immediately post-meal (t=90 to t=150) and 60 minutes post-meal (t=150-t=210). Subjects performed one of three tasks at each time point. This design was undertaken to ensure that equal attention being paid to each task throughout the experimental session. Each task was performed once in each week in randomised order

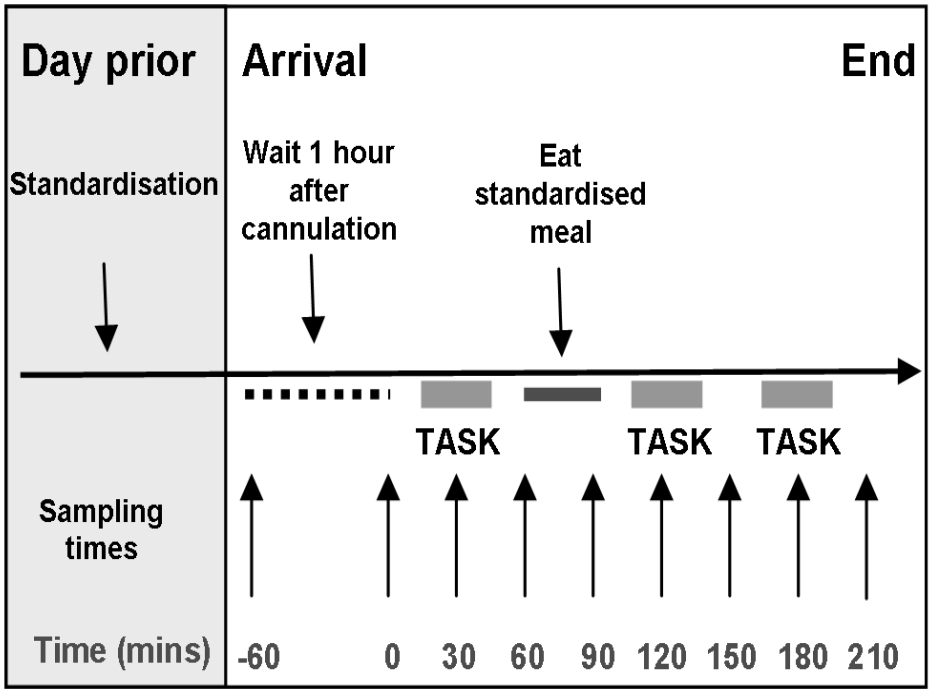


Figure-69; A schematic time line diagram of the decision making study

Subject number	Age	BMI	Percentage body fat	glucose	Leptin
1	22	24.5	14	4.9	40.06
2	20	22.2	12.5	4.9	59.06
3	22	20.7	10.5	4.5	132.42
6	32	21.2	11	5.2	248.62
7	20	24.7	16.5	4.9	504.9
9	20	21.6	12.5	4.5	116.83
11	25	21.2	11.5	4.8	2.49
12	22	22.8	13	4.7	99.46
13	22	20.3	8.5	4.8	9.33
14	22	20.4	9.5	4.7	1.17
15	27	25	16	4.7	204.49
18	22	20.3	10	4.9	180.49
19	21	21.9	15.5	4.6	700.13
20	22	23.1	15	4.9	79.81
21	23	25	14.5	4.5	457.34
22	34	24.8	19	4.5	268.47
23	46	23.3	11.5	5.1	186.40
24	20	22.9	12	4.8	117.30

Figure-70; table of baseline anthropometric characteristics and fasting plasma hormone measurements.

5.3.5 Blood collection

This is as described in detail in section 2.2.5.

5.3.6 Reagents added to blood to preserve active hormones

This is as described in 2.2.7.

5.3.7 Visual analogue score

This is as described in 2.2.8

5.3.8 Standard meal

Type of food	Chicken Wrap	100g Pringles crisps	Chocolate drink	Trifle	***Cheese & tomato pizza***
Energy (kcal)	535	540	620	371	***774***
Protein (g)	20.6	4.1	21.6	3.8	***36.4***
Carbohydrate (g)	46.4	49	74.6	26.7	***109.6***
Sugar (g)	4.2	1.9	72	19.7	***11.2***
Fat (g)	30	36	26	28.3	***21***
Saturated fat(g)	6.1	10	11.6	18	***11.2***
Fibre (g)	4.4	3.6	3.6	1.56	***6.6***
Na (g)	0.61	0.53	0.36	0.06	***1.02***
Equivalent salt (g)	1.53		0.9		***1.28***

Table-71; Nutritional composition of the standard meal is shown. The meal in bold italics was consumed on the night before the study day, all other components were consumed as the study day meal.

5.3.9 Payment

Payment for the first two tasks (risk preference and inter-temporal choice) was through random lottery incentive mechanism. One choice from the three weeks was selected at random and played out for real to determine an individual's winnings. One of either the risk elicitation task or the inter-temporal choice task was played out. This was chosen by random number generation on a computer. Winnings from the risk preference task ranged from £0-80. A baseline payment of £40/week was made for participation on completion of all three weeks.

5.3.10 Hormone assays

This is as described in 2.2.11

5.3.11 Radioimmuno assay

This is as described in 2.2.12

5.3.12 ELISA

This is as described in 2.2.13

5.3.13 Statistical analysis

This is as described in 2.2.17

5.4 Results

5.4.1 Feeding alters risky choices

Feeding significantly ($p=0.008$) altered a subject's risk averse score from the fasted ($t=0$) to the fed state ($t=90$), and showed a trend towards significance ($p=0.137$) from the fasted ($t=0$) to the 1-hour post-meal time point ($t=150$) (figure 72/ 73). Subjects became more risk seeking after the meal. There was a significant change in plasma ghrelin with feeding. Our temporal ghrelin profile is similar to that of other published studies on meal related change in ghrelin. For correlation analysis with change in risk averse scores, we calculated the change in plasma ghrelin from baseline ($t=0$ min). The baseline ghrelin value was defined as zero, the change from the baseline value to the end of the study ($t=210$ min), was calculated for each visit- delta ghrelin.

We also found a statistically significant correlation between the change in risk averse score from baseline to the satiety state (an hour past feeding, t=150min), and change in delta ghrelin for the corresponding time points (figure-8). Therefore an individual's risk averse score for monetary rewards, an hour after feeding was significantly correlated to change in the ghrelin level. This was a negative correlation. Therefore a lesser change in ghrelin led to a higher risk seeking. Therefore both leptin and ghrelin did influence monetary reward seeking behaviour.

	Percentage safe choices made			Change in safe choices made	
subject	fasted	Fed	1 hour post meal	Ä(fast-fed)	Ä (fast-1hour post meal)
1	45.2	44.9	49.2	0.3	-4
2	83.9	83.4	76.9	0.5	7
3	71.4	75.9	70.9	-4.5	0.5
6	75.4	71.4	69.8	4	5.5
7	53.3	45.2	53.8	8	-0.5
9	48.2	44.7	47	3.5	1.3
11	44.9	35.6	40.2	9.3	4.7
12	48.2	46.7	46.7	1.5	1.5
13	69.3	67.8	66.3	1.5	3
14	59.3	63.5	65.7	-4.2	-6.4
15	79.4	78.4	78.4	1	1
18	68.7	70.9	64.8	-2.2	3.9
19	75.4	68.7	69.8	6.7	5.5
20	69.2	65.8	56.3	3.4	12.9
21	67.3	59.3	65.3	8	2
22	52.3	48.2	61.8	4	-9.5
23	76.9	69.8	71.9	7	5
24	47	43.4	46.7	3.5	0.2

Figure-72; Percentage of safe choices made in each of the metabolic states, and the change in safe choices made from the fasted state, in the paired lottery task are shown for all subjects included in the analysis.

	Fasted	Fed	1 hour post meal
Number of values	18	18	18
Mean	63.07	60.2	61.19
Std. Deviation	13.21	14.42	11.53
Std. Error	3.114	3.398	2.718
Normality Test			
KS distance	0.1811	0.1862	0.1783
P value	P > 0.10	P > 0.10	P > 0.10
Passed normality test (alpha=0.05)?	Yes	Yes	Yes
P value summary	Ns	ns	ns

Figure-73; table to summarise descriptive statistics from the decision making study. we undertook a normality test to ensure gausian distribution prior to undertaking statistical analysis. The analysis summary is shown.

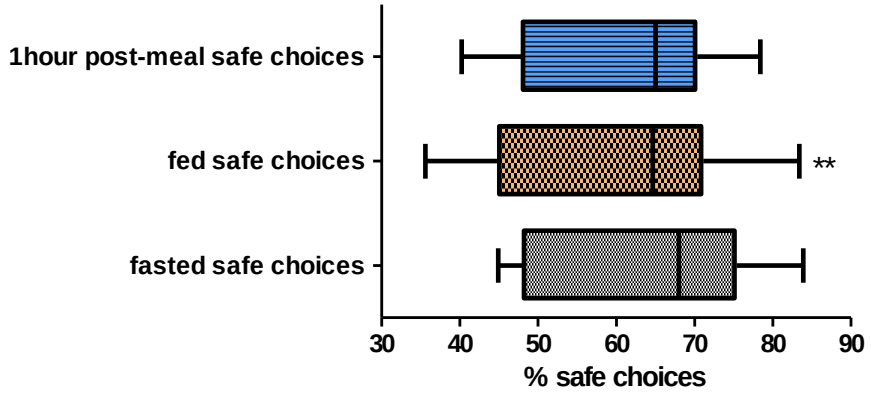


Figure-74; A box-plot to show the change in safe choices made after the meal, and an hour after the meal

5.4.2 Body fat mass correlates to plasma leptin and BMI

There is a significant positive correlation between circulating plasma leptin concentrations and the body fat mass measured by impedence (p = 0.058, r=0.21). There is also a significant correlation between BMI and body fat percentage (p=0.0002, r = 0.59) in our subjects. Given this correlation, BMI and leptin both did correlate to change in risk averse choices made from the fasted to the fed state.

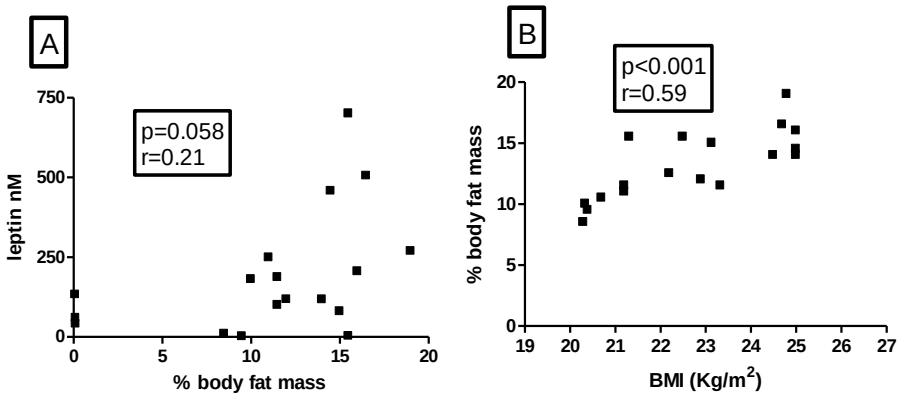


Figure-75; A scatter plots to show positive correlation between measured body fat percentage and plasma leptin (A), and BMI.

5.4.3 Leptin and BMI correlate to change in risky choices from fasted to fed state

There was a positive correlation between both leptin (p=0.034, r=0.25) and BMI (p=0.038, r=0.24), and the increase in risky choices made from the fasted to fed state.

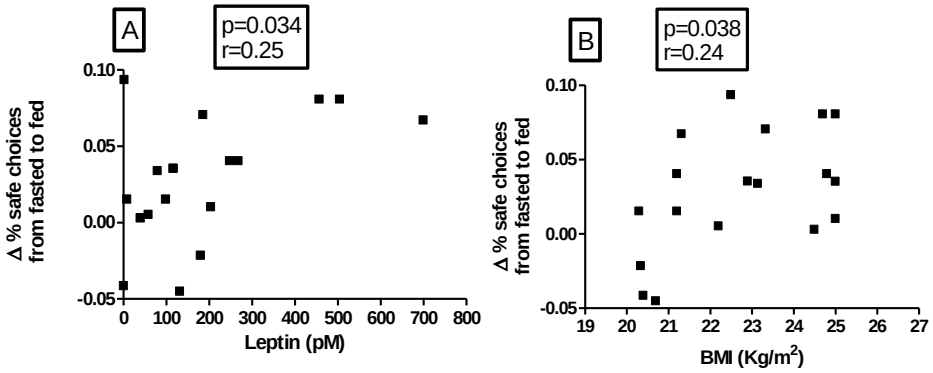


Figure-76; scatter plots to show positive correlation between change in safe choices made from the fasted to fed state, and plasma leptin (A), and BMI (B)

5.4.4 Temporal profile of acyl-ghrelin

Consumption of the meal caused a significant decrease in plasma acyl-ghrelin (two-way repeated-measures ANOVA, $p < 0.001$), plasma acyl-ghrelin did peak just before the meal, showing an increase from t = 0 to t = 60 min, of 63.1 ± 12.2 pmol/L, $p<0.001$, falling to trough level at t = 120 min, decreasing from t=0 to t=120 min by 98.7 ± 12.0 pmol/L, $p < 0.001$. There was no significant within-subjects difference in acyl-ghrelin profiles across weeks ($p = 0.237$).

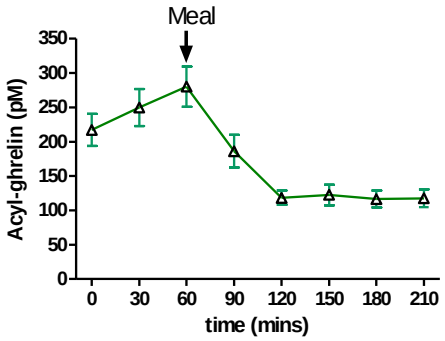


Figure-77; A graph to show the post-prandial temporal change in plasma acyl-ghrelin concentration from fasted to fed, and at an hour after meal

5.4.5 Temporal appetite and satiety profiles

There was a significant decrease in subjective hunger score (VAS) from the fasted to the fed state, through the course of the study period, and across subjects (two-way repeated measures ANOVA- week, time point), there was a significant decrease across time point ($p<0.001$), and this effect was consistent across weeks, ($p=0.48$), however there was also a significant difference in hunger between weeks ($p=0.0006$), hunger scores were higher on subsequent visits, further, Bonferroni post-hoc test did show significant ($p<0.001$) increase in baseline ($t=0$) hunger from week-1 to week-3. Hunger increased from baseline to administration of the meal ($t = 0$ to $t = 60$) 10.7 ± 1.7, $p <0.001$, then fell immediately after the meal, reaching a nadir at $t =120$ min, decreasing by ($t=0$ to $t=120$ min) 52.1 ± 3.3, $p <0.001$. There was also a significant ($p<0.001$) decrease in prospective food consumption ratings over the course of each session (two-way repeated measures ANOVA- week, time point) again there was a significant decrease across time points $p<0.001$, but there was no significant difference between weeks, $p=0.084$. An increase in prospective food consumption occurred from $t = 0$ to $t = 60$ min: 9 ± 1.5, $p <0.001$; which then decreased after the meal ($t=0$ to $t=120$) by 49.1 ± 2.8, $p < 0.001$.

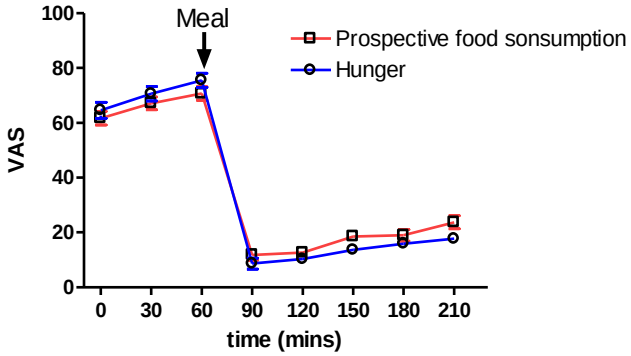


Figure-78; The temporal profile of VAS hunger and prospective food consumption

5.4.6 Acyl-ghrelin correlates to hunger

The mean hunger score at each time point across all visits did show a highly significant positive correlation (p=0.003, r=0.80) to the corresponding mean plasma acyl-ghrelin (figure-78).

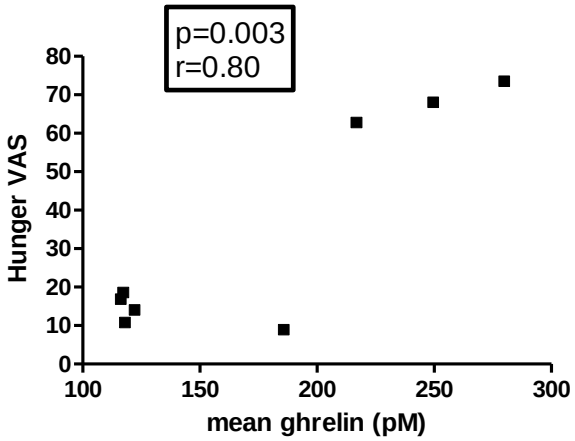


Figure-79; A significant positive correlation between the mean hunger VAS and mean plasma acyl-ghrelin across all visits for all subjects

5.4.7 Prandial Change in acyl-ghrelin correlates to change in risky choices

We calculated changes in acyl-ghrelin from the t=0 min time point (Δ-ghrelin) to all other time points throughout a session, for each individual, to controls for small variations in fasting acyl-ghrelin level between weeks. The differences in Δ acyl-ghrelin between states was then calculated across weeks by calculating the change from the end of the interval in which each subject performed the task (t=30/60 min; t=120/150 min; t=180/210 min). There was a significant negative correlation between increase in

risky choices from the fasted to the 1-hour post meal stage and the decrease in Δ acyl-ghrelin at the corresponding time point (p=0.03, r=0.26).

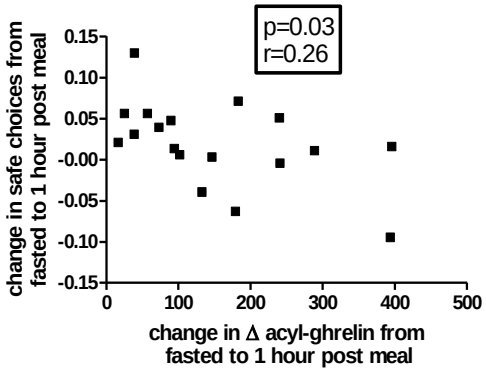


Figure-80; scatter plot to confirm correlation between change in delta acyl-ghrelin and change in safe choices made an hour after the meal.

5.4.8 Baseline leptin and acyl-ghrelin do not correlate

There was no correlation between baseline acyl-ghrelin and leptin levels (p = 0.19) (figure-80). Additionally, there was no correlation between leptin, body mass index, or body fat percentage and mean risk averse score across across all sessions p=0.48, 0.23 and 0.25.

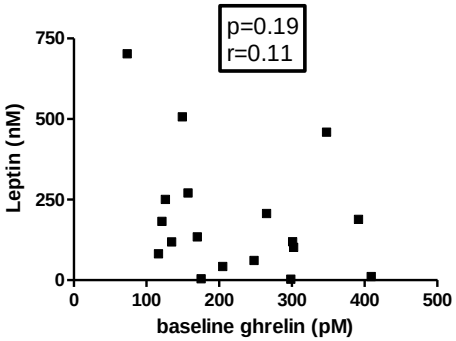


Figure-81; Scatter plot to confirm no correlation between acyl-ghrelin and leptin

5.5 Discussion

According to the prospect theory, normal subjects tend to assign greater weight to loss than to gain (Hahnemann and Tversky, 1979 and Tversky and Kahneman, 1981). Furthermore, preferences are typically risk-averse in the gain domain and risk-seeking in the loss domain (Kahneman and Tversky, 1979 and Tversky and Kahneman, 1981).

Subjects in the decision making study were risk averse at baseline. A study with a simple gambling paradigm did show total winnings correlated with haemodynamic response in the reward pathway ((Elliott et al, 2000). A further study confirmed roles for the reward pathway, with an on-off pattern of response in the reward pathway and the amygdala in relation to expectation, detection and occurrence of reward. In other words the neural substrates, responsive to monetary reinforcement overlap extensively with those responsive to primary reinforcers such as food in animals (Elliott et al 2003). Other fMRI studies have adopted a risk-sensitive reward paradigm (Ernst et al 2004 and Matthews et al 2004) similar to ours and confirm increased activity in the reward pathway during selection of the high-reward/risk option than during selection of low-reward/risk option. Further, in the study by Ino et al (Ino et al 2010), that evaluated fMRI activity whilst undertaking a monetary task in which subjects were endowed with money initially, and required to choose either high-reward/risk or low-reward/risk option, showed that the reward pathway was a main region activated when selecting the high-risk/reward option compared to selecting the low-risk/reward option, and is consistent with the previous studies, showing that the reward pathway is a major neural substrate where alteration in activity is seen between the two options (Ino et al 2010).

Results from the decision making study suggests that an individual's metabolic state does influence risk-sensitive monetary reward. The change in an individual's risk-sensitive reward from the fasted to fed state is significantly correlated to his baseline energy stores indexed by the metabolic hormone leptin, further the change in risk sensitive reward at an hour after the meal (when acyl-ghrelin has reached its nadir) is correlated to ghrelin a hormone that indexes acute nutrient intake.

The mid brain dopaminergic neural reward pathway is thought to mediate the rewarding aspects food, drugs of addiction and money (Elliott R et al 2003, Volkow ND and Wise RA 2005, Palmiter RD 2007, Platt ML and Huettel SA 2008). Recent evidence from *in vivo* studies confirms the presence of functional receptors to leptin and ghrelin in the dopaminergic reward pathway (Palmiter RD 2007). Metabolic hormones can activate (acyl-ghrelin) or inhibit (leptin) this dopaminergic reward pathway (Morton GJ et al 2006, Palmiter RD 2007, Lenard NR and Berthoud HR 2008). The dopaminergic reward pathway is interconnected to other brain areas concerned with learning and memory (hippocampus), energy balance (hypothalamus and brainstem), motivation (amygdala), reward value (orbitofrontal cortex) and executive function (prefrontal cortex) (Beaver JD et al 2006, Morton GJ et al 2006, Lenard NR and Berthoud HR 2008, Stoeckel LE et al 2008). The reward pathway is therefore able to assay metabolic signals and afferent neural inputs from other regions of the brain, and in turn

inform neural pathways to bring about an individual's desired behavioural response (Lenard NR and Berthoud HR 2008). The dopaminergic reward pathway is thought to play a significant role in feeding behaviour in our current calorie abundant environment (Volkow ND and Wise RA 2005, Morton GJ et al 2006, Palmiter RD 2007, Lenard NR and Berthoud HR 2008, Stoeckel LE et al 2008).

An individual's approach to risk-sensitive monetary reward and a foraging animal's approach to risk-sensitive food reward are known to share common characteristics (Lee D 2005), further, as the vast majority of human studies on risk sensitive reward involve monetary rewards, a review of human risk preference and animal food reward, suggests that risk-sensitive reward in humans and animals share common characteristics, both are better predicted by a measure of risk that relates variability of outcomes to expected returns (EU Weber 2004).

5.5.1 Metabolic state does influence human risk-sensitive reward

In our subject population, a calorie rich meal significantly increased an individual's risk sensitive monetary reward seeking behaviour, from the fasted to the fed state and showed a trend towards significance at an hour after the meal ($p=0.137$). It could be argued that the high calorie meal in a safe environment increased their reward seeking behaviour to gain other, namely monetary reward. In other words one rewarding experience increased their appetite for other rewarding experiences. The "priming" effect of a small amount of a palatable food on binge eating described as the 'priming' effect is also seen in addiction behaviour, where even a small dose tends to elicit a strong 'craving' and compulsion for further use, hence in our study food may have caused a 'priming' effect (Davis et al 2004) This concept is also supported by the dopamine hypothesis, which states that dopamine promotes the wanting of rewards, making animals work harder and faster to keep up the elevated levels of dopamine, which in turn makes the animals feel rewarded (Palmiter RD 2007). Recent evidence linking addiction behaviour and obesity to the reward pathway (Volkow ND and Wise RA 2005, Engelmann JB 2006, Palmiter RD 2007, Stoeckel LE et al 2008) also lends support to this concept of transfer of effect between food and money. Other evidence from *in vivo* studies link food reward to drugs of abuse; food deprivation augments the rewarding value of drugs of abuse and food reward (Morton GJ et al 2006), further, animal studies have shown a link between food deprivation and relapse of drug seeking behaviour, after a prolonged drug free period (Shalev U et al 2000). This effect was attenuated by leptin infusion, suggesting that food deprivation may augment

reinstatement of drug seeking, by its actions on the dopaminergic reward pathway (Shalev U et al 2000).

5.5.2 Baseline energy stores influence the change in risk sensitive reward in humans

In our study, BMI and plasma leptin is positively and significantly correlated to the change in monetary risk-sensitive reward from the fasted to fed state. This positive association between baseline energy stores and risk sensitive reward is similar to the risk-sensitive foraging for food seen in animals (Caraco T et al 1980, Lee D 2005). The lipostatic theory on energy homeostasis states that humoral signals generated from body fat stores, act through homeostatic centres to maintain body fat stores (Mayer J 1955). In other words, the higher an individuals body fat stores, the more energy they need to sustain their current energy state. Therefore subjects with a higher percentage body fat and plasma leptin levels, would be expected to be relatively more risk seeking for food reward after the same meal. However, it is interesting that in our subjects this increase in risk-sensitive reward was not to food reward but to monetary rewards. This transfer of effect from food to monetary reward has not been reported before. The transfer of effect from one rewarding experience to another is supported by the dopamine hypothesis, and recent evidence suggesting similarities between decisions made for food reward and monetary reward in humans. A recent study compared an individual's approach to risk sensitive monetary reward and sugary liquid treats in a gambling task, and found similarities in choices made for both sugary fluid rewards and monetary gains. There was no difference in an individual's pattern of decision making for both food and monetary rewards (Hayden BY et al 2009).

However, the positive correlation between leptin and risk-sensitive reward does also raise further questions. The dopaminergic reward pathway is known to have functional leptin receptors that have an inhibitory effect on these neurons (Palmiter RD 2007, Lenard NR and Berthoud HR 2008). A review of *in vivo* studies on leptin and the reward pathway also point to an inhibitory effect of leptin (Morton GJ et al 2006, Fulton S 2006). In our study, the higher leptin level did not lead to an inhibitory effect on the reward pathway, and make those individual's with higher adiposity less likely to seek further monetary reward, in fact the opposite occurred. Subjects with a higher leptin level displayed a risky approach to reward.

A coherent model to explain the effects of leptin on reward seeking behaviour, at an organism level, will need to take account of the effects of leptin on both the homeostatic

pathways governing energy homeostasis, and the dopaminergic reward pathway. The homeostatic pathway and the reward pathway are interconnected and inter-related (Morton GJ et al 2006). The idea that reward perception is subject to homeostatic regulation is now accepted. A lack of availability of food exerts a global stimulatory effect on reward perception, including food reward (Morton GJ et al 2006). One proposed mechanism suggests that leptin and insulin tonically inhibit the reward circuitry, and food deprivation leads to a lower circulating level of these hormones, increasing the sensitivity of the reward circuitry. However, the opposing scenario, which occurs in obesity, when an individual's abundant of energy store will be expected to lead to the opposite effect and decreased sensitivity, is still debated (Morton GJ et al 2006). Therefore it could be argued that in our subject pool, individuals with a higher body fat mass and plasma leptin would need more energy to sustain their fat mass, and as suggested by the lipostatic theory, they will be more likely to seek further food reward to sustain their energy stores.

There are also other possible explanations for this discrepancy. It is possible that like in obesity and drug addiction, where supra-physiological stimulation of the reward pathway leads to stimulus preferences, a high calorie meal led to the preference of a more rewarding stimulus (Volkow ND and Wise RA 200510), further, it is also possible that higher centres in the prefrontal cortex that are known to exert executive control over the reward pathway (Lenard NR and Berthoud HR 2008, Figlewicz DP and Benoit SC 2008) , may have played a role in choosing the higher gain monetary incentives on offer, in a predominantly student population.

5.5.3 Post-prandial changes in acyl-ghrelin influence risk-sensitive reward seeking behaviour

The temporal profile of acyl-ghrelin in our study, confirms that the plasma acyl-ghrelin level is significantly altered from fasting to an hour after feeding, this change in acyl-ghrelin does also show positive and significant correlation to hunger VAS. The magnitude of this change in plasma acyl-ghrelin is negatively correlated to the change in risk-sensitive reward seeking in our subject population. In other words, an individual with a small change in plasma acyl-ghrelin level became more risk seeking to monetary reward.

The plasma acyl-ghrelin response after a meal is associated with the inter-meal interval in normal weight men (Blom WA et al 2009). It has also been shown that the change in plasma acyl-ghrelin from baseline to an hour after ingestion is proportional to calorific

intake (Callahan HS et al 2004). Acyl-ghrelin is able to promote feeding behaviour by its actions on the homeostatic and reward pathways; orexigenic neurons in the homeostatic pathway have functional ghrelin receptors that mediate feeding behaviour (Cummings DE 2006). Therefore acyl-ghrelin is able to relay information about energy gains from food intake to the homeostatic centre and regulate feeding behaviour. Ghrelin is also able to promote food intake by acting on the dopaminergic reward pathway (Ghigo E et al 2005, Cummings DE 2006, Palmiter RD 2007), where it is known to have an excitatory effect, on these neurons (Abizaid A et al 2006, Palmiter RD 2007), and the dopaminergic reward pathway is thought to mediate the rewarding aspects of food and financial reward (Volkow ND, and Wise RA 2005, Palmiter RD 2007, Platt ML and Huettel SA 2008). This may explain the transfer of effect from food to money seen in our study. In those individuals with a relatively low suppression, ghrelin will be expected to promote further food intake by its action on the homeostatic pathway to maintain energy homeostasis, and acyl-ghrelin will also be expected to promote food intake through the reward pathway, in these same individuals. The risky approach to monetary reward, in individuals with low acyl-ghrelin suppression is in agreement with risk-sensitive foraging behaviour in animals, when an animal is not able to meet its daily energetic requirement with the "safe source" of food on offer, it would seek more variable and risk prone food sources **(**A Kacelnik and M Bateson 1996). In our subjects, as with leptin, a transfer of effect from food to monetary reward is seen, again this has not been reported before. However our findings of an increase in risky choices after feeding in the cohort does contradict the decline in risk-sensitive monetary reward seeking behaviour with greater suppression of acyl-ghrelin.

5.5.4 Leptin and acyl-ghrelin interact to signal energy stores

It was initially thought that leptin regulated ghrelin levels (Barazzoni R et al 2003). Though more recent evidence points towards multiple factors regulating ghrelin secretion; nutrients (carbohydrate and protein suppress ghrelin more than lipid), insulin, intestinal osmolarity, enteric neural signalling and vagal response, have all been shown to suppress ghrelin secretion after a meal (Ghigo E et al 2005, Cummings DE. 2006, Klok MD et al 2007). There is some evidence pointing at insulin communicating information on both short term energy gains and long term energy stores, to ghrelin producing cells in the oxyntic mucosa (Cummings DE, Foster KE 2003, Klok MD et al 2007). It is thought that adiposity related changes in insulin, but not leptin may convey information on long term energy stores to ghrelin producing cells (Cummings DE, Foster KE 2003, Cummings DE 2006). Ghrelin producing cells appose the basement membrane, in close proximity to the vascular compartment and are not known to be in

direct contact with the gastric lumen (Cummings DE 2006). Therefore ghrelin producing cells are more likely to respond to blood borne signals and less likely to respond to luminal contents.

It is now thought that leptin and ghrelin act in parallel on the homeostatic and reward pathways (Cummings DE 2006, Cummings DE, Foster KE 2003, Klok MD et al 2007). They are metabolic counterparts with opposing actions. Ghrelin is an evolutionarily conserved protein, conveying information on intestinal energy stores, in lower non vertebrate organisms (Cummings DE, Foster KE 2003).

5.5.5 Baseline energy stores and feeding alter reward behaviour

The decision making study suggests that an individual's metabolic state does influence his monetary decisions, risk-sensitive monetary decisions were influenced by both long-term metabolic signals indexing energy stores and short-term metabolic signals that index energy gains. This is not surprising, given that the homeostatic mechanisms that regulate body energy stores also influence reward pathways (Morton GJ et al 2006).

At the neurobiological level, our results suggest an overlap between food and monetary reward. This has significant implications for all decisions that incorporate risk and monetary reward. The implications of the results from the decision making study are wide ranging, given that all individual's make assessment of risk and reward in many aspects of our daily lives, from crossing the road to placing a bet at the grand-national. An individual's body mass index and his nutritional intake could alter behavioural patterns, In the financial services industry; as long term energy stores will influence risk sensitive reward seeking, should all who take risks to attain monetary reward be encouraged to adhere to a ideal body weight, further, after a meal, as the energetic value of the meal in relation to his energetic requirement will influence risk sensitive reward, should we also encourage these individuals to be satiated, though it could be argued that all aspects of human behaviour does assess risk and reward, perhaps we should all take note of our hunger when it comes to pursuing any task that involves an assessment of risk and reward

Chapter 6
Summary and discussion

6.1 RYGBP and SG lead to equivalent weight loss

There is equivalent %EWL after both RYGBP and SG at 6 and 12 weeks. Despite starting with a lower BMI, the SG group lost similar BMI points to the RYGBP group. This is in keeping with other recent short term (Karamanakos et al 2008, Peterli R et al 2009, Valderas JP et al 2010, Benaiges D et al 2011, Peterli R et al 2012, Ramon J M et al 2012) and long term (Morales MP et al 2010, de Gordejuela AG et al 2011, and Peterli R et al 2012) human studies. This is also seen in rodent studies (Chambers AP et al 2011). More recently conversion of both procedures to the opposite has been successfully undertaken for failure of weight loss. RYGBP was converted to SG and was seen to alter dietary behaviour (Dapri G et al 2011). Revision after SG for weight gain, gastric reflux and other complications, to RYGB also revealed sustained weight loss (Morales MP et al 2010).

6.2.1 Differential change in hunger, satiety and prospective food consumption, and gut hormone levels

With regard to changes in appetite following bariatric surgery, no study to date has found correlation between changes in active gut hormones after RYGBP and changes in perception of hunger, satiety or prospective food consumption. So far only a few studies have looked at appetite scores alongside gut hormones (Korner J et al, 2005, Korner J et al 2006, Buchwald et al 2007, Karamanakos SN et al 2008, DePaula AL et al 2009, Valderas JP et al 2010), and have shown significant decrease in hunger and increase in satiety, after RYGBP, and SG. However, none had employed a comprehensive VAS recording. Our study was unique in this respect. To date no correlation between changes in an individual's VAS and an individual's gut-hormones have been reported in the literature. Some studies utilised only two time points per visit (Korner J et al, 2005, Korner J et al 2006, Karamanakos et al 2008). Therefore correlation analysis between changes in gut hormones has not been feasible. Other studies with multiple time points, point to gut hormones altering appetite and satiety after surgery and thus engender weight loss after RYGBP and SG, though have been unable to correlate these outcomes in an individual (Korner J et al 2005, Valderas JP et al 2010, and Karamanakos SN et al 2008). The total PYY AUC did show positive correlation with satiety AUC (Valderas JP et al 2010).

In our study, RYGBP and SG seem to alter hunger, prospective food consumption and satiety differentially. RYGBP has a more pronounced influence on prospective food consumption and hunger, despite non-significant changes in acyl-ghrelin; whilst the converse is true of satiety. This variability does not fit with the overall gut hormone changes seen after these procedures. RYGBP leads to a more pronounced PYY3-36, GLP-1 and amylin response and would be expected to alter satiety more. The meal related satiety response is very similar in both groups at 6 weeks, but do differ at 12 weeks, where the increase remains significant in the SG group alone. In the SG group, GLP-1 and Δ PYY does show positive correlation to satiety. SG by contrast does lead to a more pronounced and significant decline in acyl-ghrelin and thus expected to suppress hunger more than RYGBP. The Δ acyl-ghrelin does show negative correlation to prospective food consumption in both groups. Further, GLP-1, PYY3-36 and Δ PYY3-36 does also show negative correlation to prospective food consumption after RYGBP. In common with RYGBP, GLP-1 and Δ PYY3-36 do show a negative correlation to prospective food consumption after SG. Conversely acyl-ghrelin does positively correlate prospective food consumption after RYGBP. These correlations between active gut hormones, hunger, prospective food consumption and satiety have not been reported before. Our study provides a link between the change in gut hormones and measures of appetite and satiety, and confirms gut hormone changes that occur after RYGBP and SG may lead to a decline in appetite and an increase in satiety in an individual, and therefore favour weight loss.

6.2.2 RYGBP and SG lead to a differential change in Δ hunger, Δ satiety and Δ prospective food consumption

There was a pronounced decline in the Δ hunger in the SG group when compared to the RYGBP group. This is in keeping with acyl-ghrelin changes, and may point to Δ hunger being a better marker of change in plasma hormones. This does contrast with the VAS for hunger where there was a pronounced decline after RYGBP. There was a pronounced decline in Δ prospective food consumption after SG when compared to RYGBP. The Δ delta satiety was similar, but more pronounced after SG.

6.3 RYGBP and SG lead to equivalent leptin decline which correlates to change in BMI, fat mass and VFA

Fasting plasma leptin does decline significantly in keeping with adiposity after RYGP and SG. Further, there was no significant difference between the two groups. In the RYGBP and SG groups weight, BMI, fat mass and visceral fat area correlate to

circulating leptin. This is the first study to show such correlation, and argues against any other neuro-humoral cause in the first three months after surgery. It is known that fat mass is not the sole determinant of plasma leptin levels (Dubuc GR et al 1998). Nutritional factors such as recent energy intake are also involved in the regulation of leptin production (Dubuc GR et al 1998). Negative energy balance does also influence leptin production (Havel PJ 2001). Others have also demonstrated the correlation between circulating leptin and adiposity before and after RYGBP (Faraj M et al 2003). Paradoxically circulating leptin levels of obese subjects undergoing weight loss after RYGBP does fall below normal reference values despite them remaining obese. In other words there was a greater magnitude decline in leptin in this study (Faraj M et al 2003). This is thought to be due to adiposity-independent energy balance changes (Faraj M et al 2003). In a recent study where RYGBP and SG led to similar weight loss a year after surgery, the fasting leptin levels were halved at 1 week after surgery, and plasma leptin continued to decline for up to 12 months after surgery (Woelnerhanssen B et al 2011). The early decline in leptin is unlikely to be mediated by weight loss alone. In our study plasma leptin levels did not fall below the reference range for women. The circulating plasma leptin was broadly in line with adiposity in our subjects, this is in keeping with other recent studies (Lee W J et al 2011, Dimitriadis E et al 2013). However others have suggested a significantly lower leptin after RYGBP in comparison to SG (Ramon J M et al 2012). The significant correlation between plasma leptin and weight/ BMI/ fat mass/ VFA in our groups argues against this. This is the first study to show correlation between several measured adiposity indices and fasting plasma leptin after SG.

6.4 RYGBP and SG lead to similar significant improvement in meal stimulated PYY3-36

The mean postprandial peak in PYY3-36 is increased by 2.5-fold after RYGBP and 1.7 fold after SG. The meal stimulated PYY3-36 AUC is significantly increased after RYGBP and SG. There is no significant difference between the RYGBP and SG PYY3-36 AUC at all time points. There have been no studies to investigate meal stimulated PYY3-36 secretion after SG. It is already known that the meal stimulated total PYY (Korner J et al 2005, Karamanakos SN et al 2008, Peterli R et al 2012, and Ramon J M et al 2012) response following SG is similar to that seen after RYGBP. However, fasting PYY3-36 is only significantly reduced at 12 weeks after RYGBP. Further, changes in PYY3-36 did correlate to perception of satiety and show a trend towards correlation to weight loss after RYGBP. This relationship between PYY3-36 and satiety and weight loss was also seen after SG, and the correlation to weight loss did reach

statistical significance in this group. Recent publications have also highlighted a pronounced hind-gut response after SG similar to that seen after RYGBP (Peterli R et al 2009, Peterli R et al 2012, and Ramon J M et al 2012). This is in keeping with our findings. In our study the secretion of the two distal gut hormones after surgery does correlate after both procedures adding weight to an exaggerated distal gut response.

6.5 RYGBP leads to a significantly higher post-prandial GLP-1 response

The temporal profile of meal stimulated active GLP-1 secretion is significantly increased after both RYGBP and SG. However the magnitude of change is three fold higher after RYGBP. This is maintained at 12 weeks. Further, there is a significant difference in the active GLP-1 AUC between the two groups at 6 and 12 weeks. A recent study reports an equivalent meal stimulated active GLP-1 AUC after RYGBP and SG at three months after surgery (Peterli R et al 2009, Peterli R et al 2012, Lee W J et al 2011, and Ramon J M et al 2012). The baseline difference in active GLP-1 AUC, the three fold higher AUC of meal stimulated active GLP-1 after RYGBP, even allowing for the higher calorie content (400Kcal vs. 500Kcal) in our meal, does suggest difference in measurement protocol and the importance of standardization (Petrli R et al 2009). There is a significant correlation between active GLP-1 and prospective food consumption in the RYGBP group though no correlation to weight loss is observed. However, in the SG group there is correlation between active GLP-1 satiety, prospective food consumption and weight loss at 12 weeks. The correlation between active GLP-1 changes and outcome measures after SG has not been shown before.

6.6 SG but not RYGBP leads to significant decline in acyl-ghrelin

At 12 weeks after surgery the fasting plasma acyl-ghrelin is significantly lower in the SG group in comparison to the RYGBP group. There is a significant decrease in the meal stimulated temporal profile of acyl-ghrelin after both RYGBP and SG. This finding is in keeping with that of others studying RYGBP (Shin AC et al 2010, Barazzoni R et al 2013, and Dimitriadis E et al 2013), but not all (Samat A et al 2013). Further at 12 weeks the comparison of groups reveals a significant difference in AUC between RYGBP and SG groups. Recent studies in total ghrelin have reproduced our findings of superior suppression after SG (Peterli R et al 2012, Ramon J M et al 2012). Others have shown an increment in acyl-ghrelin with time after RYGBP (Barazzoni R et al 2013).

Total ghrelin is known to be elevated after diet induced weight loss (Oliván B et al 2009) and it was initially thought that a decrease in total ghrelin after SG may explain the superior weight loss and maintenance of weight loss after SG (Langer FB et al 2005). However, a recent meta-analysis of several studies was unable to reach a conclusion (Frezza EE et al 2008). To date no study has measured acyl-ghrelin, the active octanoylated form collected under standardised conditions to prevent degradation (as recommended by the assay). The significant decline in acyl-ghrelin after SG is thought to be due to the complete removal of the gastric fundus, the segment of the stomach, thought to produce the vast majority of acyl-ghrelin (Langer FB et al 2005). However, the change in acyl-ghrelin after RYGBP does correlate significantly to change in prospective food consumption after surgery, but not weight loss. This relationship between acyl-ghrelin and prospective food consumption is more pronounced in the SG group. Further there is a positive correlation between change in acyl-ghrelin and weight loss after SG. This study is the first report that changes in acyl-ghrelin correlate to outcome measures in humans. These changes do contrast with the higher acyl-ghrelin reported after diet induced weight loss and gastric banding (Cummings D E et al 2002, Langer FB et al 2005, Oliván B et al 2009). However, the above studies did not look at meal stimulated acyl-ghrelin, following standardization.

6.7 There is significant increase in amylin after RYGBP but not after SG

There is a significant increase in the meal stimulated temporal profile of active amylin secretion after RYGBP but not SG. This is in keeping with active amylin secretion in rats undergoing RYGBP (Shin AC et al 2010). However this is contrary to others who reported a decrease in total amylin after RYGBP (Mousumi Bose et al 2010) and and active amylin after SG (Dimitriadis E et al 2013). Others have reported no change in total amylin after RYGBP (Jacobsen et al 2012). No significant change in meal stimulated active amylin secretion was seen after SG in our study. Others have found significant increase in amylin when SG is combined with an ileal interposition on to the proximal duodenum and proximal jejunum (DePaula AL et al 2009). This study does not state if total or active amylin was measured, further this study does not specify inter-assay variations. However, the finding of this study does suggest that the exclusion of the duodenum may lead to an increase in amylin secretion. Further, the amylin changes on their own did not correlate to satiety, prospective food consumption or %EWL in the RYGBP group. This was mirrored in the only other study on active amylin in rodents, where no correlation between active amylin and weight loss was seen (Shin AC et al 2010). Interestingly in the SG group change in amylin AUC at 6 and 12 weeks does correlate to %EWL at these corresponding time points. This is seen despite non-significant change in amylin after surgery in this group.

6.8.1 Gut hormone changes after RYGBP and SG correlate to weight loss

The correlation between changes in gut hormone secretion and weight loss in an individual has not yet been shown after either procedure in humans, but has been shown in rats after RYGBP (Shin AC et al, 2010). This discrepancy as highlighted before, may be related to study design and sample processing. Several studies have shown a blunted hind gut hormone (PYY and GLP-1) response in the morbidly obese patients that is reversed by bariatric surgery (RYGBP and SG) (Karamanakos SN et al 2008, Peterli R et al 2009, Basso N et al 2010, Chambers AP et al 2011, Umeda L M et al 2011, and Peterli R et al 2012, Dimitriadis E et al 2013). In a similar comparative study of RYGBP and SG, equivalent total PYY and active GLP-1 changes were noted at three months and one year with equivalent weight loss (Peterli R et al 2012). Others have shown a poor total PYY and GLP-1 response after SG led to poor weight loss and the opposite after RYGBP (Ramon J M et al 2012). In our study PYY3-36, GLP-1 and acyl-ghrelin correlate to measures of appetite in the RYGBP group. The Change in insulin/ amylin ratio after RYGBP did show correlation with %EWL. The change in PYY3-36 did also show a trend towards significance with EWL. In the SG group PYY3-36, GLP-1 and acyl-gherlin correlate to measures of appetite. In the SG group the change in acyl-ghrelin at 6 weeks did correlate positively to %EWL at 6 weeks, and show a trend towards correlation at twelve weeks. The converse was true of PYY3-36, where there was a trend towards correlation at 6 weeks and significant correlation at 12 weeks. The change in GLP-1 at 12 weeks after SG does also correlate positively to %EWL at 12 weeks. The change in amylin at 6 and 12 weeks does show a trend towards positive correlation with %EWL six weeks and 12 weeks. Further, the meal stimulated GLP-1 and PYY3-36 secretion do correlate after both procedures, pointing towards a similar exaggerated hind gut response after both procedures. However the strength of this correlation is more pronounced in the RYGBP group.

In the SG group, changes in several gut hormones (PYY3-36, acyl-ghrelin, GLP-1 and amylin) independently correlate to weight loss, taken together, these findings suggest that gut hormone changes alone could account for the weight loss seen after SG, this contrasts with RYGBP, where despite equivalent or even more pronounced gut hormone change, correlation of gut hormone change to weight loss is poor. This fundamental difference between the two surgical procedures may be due to alteration in neural “circuitry” that follows the more invasive RYGBP surgery. It is possible that

RYGBP leads to other changes in neural signaling that favour weight loss, working alongside the endocrine changes that favour weight loss. This perspective was further advanced by Saeidi and colleagues recently, they point to local gut changes after anatomical changes in the gut leading to improved glucose homeostasis (Saeidi N et al 2013, and reviewed by Berthoud R H 2013).

6.8.2 Gut hormone changes after bariatric surgery predict failure of sleeve gastrectomy

It is known that some patients fail to lose weight after RYGBP and SG. One patient in our SG group was noted to have lost no further weight between 3 and 12 months following surgery. This patient's meal stimulated PYY3-36, acyl-ghrelin and amylin response at 3 months after surgery did differ from those of others in the SG group. The three month meal stimulated Δ PYY3-36 (change from baseline) response was below the pre-operative response. This altered meal stimulated response could be utilized to fast-track those patients predicted to fail to a second stage procedure. The correlation between weight loss; PYY3-36, acyl-ghrelin, GLP-1 and amylin, and the correlation between GLP-1, PYY3-36, acyl-ghrelin and VAS after SG together with the relationship between a poor 3 month amylin, Δ PYY3-36 and Δ acyl-ghrelin and poor outcome, does suggest that these gut hormones may account for the changes seen after SG. Whether poor gut hormone changes after RYGBP lead to a similar outcome is not clear.

6.9 RYGBP leads to better glucose disposal in comparison to SG

In keeping with our findings others have also shown improvement in glucose homeostasis within weeks of surgery (Peterli R et al 2009, Abbatini et al 2010, Basso N et al 2011, Umeda L M et al 2011, Peterli R et al 2012, Jacobsen et al 2012). It has become clear that the improvement in T2DM and insulin resistance precedes weight changes and may be mediated by change in the gut hormone profile (Mousumi Bose et al 2010, Jacobsen S H et al 2012, Jorgensen N B et al 2012, Peterli R et al 2012). In our study there is improvement in post-prandial glucose profile after RYGBP and SG. This improvement is more pronounced after RYGBP when the glucose AUC is compared. When temporal profiles are compared SG leads to a more pronounced decline after surgery. However, this can partly be explained by the lone T2DM patient in the RYGBP group. The late post-prandial peak is also reversed after RYGBP and SG. Baseline glucose AUC is significantly decreased at 6 and 12 weeks only after RYGBP.

Analysis of the temporal profile of insulin revealed no significant change after RYGBP but a highly significant increase after SG. Time to peak insulin did occur early in both groups. There was a non-significant change in plasma insulin AUC after RYGBP and SG surgery. There is an equivalent fasting, meal stimulated insulin response after both RYGBP and SG, given the disparate GLP-1 response; different mechanisms are at play in the two groups. Others have shown that the delayed pre-operative insulin secretion pattern does gradually change to an early secretion pattern after SG (Lee et al 2010). The pronounced GLP-1 response seen after RYGBP is thought to promote insulin secretion in this group (Peterli R et al 2009, Li F et al 2009, Dezaki K et al 2008). It is thought that the lack of such a pronounced GLP-1 response after SG may be compensated for by the decrease in ghrelin seen after SG, this is thought to lead to improved insulin sensitivity after SG (Peterli R et al 2009, Li F et al 2009 Papailiou J et al 2010, Peterli R et al 2012, Ramon J M et al 2012). Further, others have shown an equivalent GLP-1 response after RYGBP and SG (Lee W J et al 2011, Chambers A P et al 2011, Peterli R et al 2012). Peterli and colleagues point to similar active GLP-1 changes at 3 months and 1 year, after both RYGBP and SG. These discrepancies may be accounted for by the timing of sampling, inter-species variation in stomach emptying and lack of standardization in sampling. Barazzoni and colleagues also point to the rise in acyl-ghrelin after RYGBP limiting the improvement in insulin resistance (Barazzoni R et al 2013).

6.10 Acyl ghrelin and HOMA IR

A comparison of SG, RYGBP on glucose homeostasis in morbidly obese T2DM patients did point to restoration of insulin resistance to normal values in all patients (Abbatini et al 2010, Umeda et al 2011, Ramon J M et al 2012, Peterli R et al 2012). Chambers and colleagues adapted RYGBP and SG in humans to a rat model in order to study the mechanisms underlying the improvements in weight and glucose metabolism (Chambers AP et al 2011). RYGBP and SG had comparable benefits. They led to comparable loss of body weight and body fat and a reduction in plasma insulin. They also caused comparable improvements in glucose tolerance (Chambers AP et al 2011). The two surgical procedures had similar metabolic effects despite different anatomical rearrangement of the gastrointestinal system (Chambers AP et al 2011). However these studies were undertaken five months after surgery, when rats were in a weight stable position. The greatest improvement in insulin resistance was noted in the SG group in humans and may be due to the large drop in ghrelin seen after SG (Abbatini et al 2010, reviewed by Yada et al 2008, and Peterli R et al 2012).

Our data does also suggest this but the correlation is not significant. Other studies that have examined the role of ghrelin had conducted assays for total ghrelin in the absence of HCL and protease inhibitors. Despite this these studies have yielded similar results to our active acyl-ghrelin results (Karamanakos SN et al 2008, De Paula et al 2009, Peterli R et al 2012, and Barazzoni R et al 2013). This has led some to speculate that the weight independent resolution of T2DM and improvement in glucose homeostasis seen after bariatric surgery may in part be mediated by acyl- ghrelin (Peterli R et al 2009, Li F et al 2009 and Papailiou J et al 2010, Peterli R et al 2012). There was a significant decline in HOMA IR at 6 and 12 weeks in the SG group. There was no significant decline in HOMA IR after RYGBP. There was a significant negative correlation between HOMA IR and meal stimulated acyl-ghrelin AUC all visits, in the RYGBP group. There was an expected positive correlation between Acyl-ghrelin and HOMA IR in the SG group. This did show a trend towards significance. The positive correlation between post-prandial acyl-ghrelin and HOMA IR seen in our SG group is in keeping with the growth hormone secretagogue receptor activation of acyl-ghrelin, and a decrease in this activity may lead to the improvement in insulin resistance seen in the SG group after surgery (reviewed by Yada et al 2008). The negative correlation between acyl-ghrelin and HOMA IR seen in the RYGBP group is difficult to explain, as the opposite would be expected. However, in our study there is a decrease in HOMA IR after surgery in this group, despite no significant change in acyl-ghrelin from pre-operative to six weeks, and a trend towards increase between 6 and 12 weeks. This suggests that the changes in HOMA IR in this group occur despite the opposite change in acyl-ghrelin. In keeping with our findings other comparative studies between RYGBP and SG found lower acyl-ghrelin and des-acyl ghrelin in the SG group but similar glucagon-like peptide-1, PYY and leptin after these procedures (Lee WJ et al 2011, Peterli R et al 2012, Ramon J M et al 2012), and rising acyl-ghrelin after RYGBP (Barazzoni R et al 2013). The fasting insulin concentrations and HOMA indices were significantly reduced, before any significant weight loss had occurred (Peterli R et al 2009, Peterli R et al 2012, Umeda L M et al 2011, Reed M A et al 2011, Jacobsen S H et al 2012, Jorgensen N B et al 2012). An improvement in glucose disposal occurs despite a decline in insulin secretion (Reed M A et al 2011). Recent evidence also points to a more direct effect of PYY3-36 on insulin sensitivity (van den Hoek et al 2007). PYY3-36 is known to be co-secreted with GLP-1 by intestinal L cells in response to food intake. The role of PYY3-36 on insulin sensitivity independent of food intake is not confirmed. The T2DM patient in the RYGBP group could also have skewed the results. However, analysis of plasma glucose and plasma insulin excluding the T2DM patient did not suggest large discrepancies.

6.11 GLP-1 is likely to mediate improved glucose homeostasis after RYGBP

SG and RYGBP are associated with similar T2DM remission rates (Romero F et al 2012). The GLP-1 AUC was significantly and comparably improved after SG and RYGB (Romero F et al 2012, Peterli R et al 2012, and Ramon J M et al 2012). Enhanced insulin sensitivity and improved GLP-1 secretion contribute to the early control of glucose homeostasis after RYGBP (Falkén Y et al 2011, Samat A et al 2013). A progressive decrease in HOMA IR was noted after 2 months (Falkén Y et al 2011), and 12 months (Samat A et al 2013). This is in contrast to our findings. RYGBP and SG led to greater initial insulin secretion from baseline followed by rapid return toward baseline. The insulin area-under the- curve (AUC) was greater when compared to controls (Chambers AP et al 2011, Umeda L M et al 2011, Jacobsen S H et al 2012, Jorgensen N B et al 2012). These findings are in keeping with our study. Despite the discrepancy in peak active GLP-1, SG leads to restoration of first phase insulin secretion. In fact insulin secretion is significantly increased after SG in our study. However, there is a significant difference in the active GLP-1 AUC between the two groups at 6 and 12 weeks. A similar parallel group study on patients undergoing RYGBP and SG at pre-operative, 1 week and 3 months after surgery where the SG group had three diabetic patients was conducted by Peterli and colleagues. As with our study, the impaired postprandial active GLP-1, insulin response was reversed in both groups, at a week after surgery. As with our study, a marked increase in postprandial active GLP-1 and insulin concentrations was observed after RYGB and SG (Peterli R et al 2009). Recent evidence points to GLP-1 mediating some of the effects of bariatric surgery. However comparable results after RYGBP and SG have led some authors to propose alternative mechanisms (Chambers AP et al 2011). The study by Kindel and colleagues does provide direct evidence that at least some of the improvement after RYGBP is mediated by GLP-1 (reviewed by Laferrère B. 2011). Gill and colleagues also point out that the duodenal exclusion hypothesis is unlikely to be a viable explanation given the recent results on sleeve gastrectomy leading to diabetes remission in a large percentage of patients, accompanied by an increase in gut hormones not dissimilar to RYGBP, in spite of a functional duodenum- (Gill RS et al 2010) (reviewed by Laferrère B. 2011). Further, a recent study points to local adaptive effects after surgery playing a significant part in improved glucose homeostasis (Saeidi et al 2013, reviewed by Berthoud R et al 201

6.12 Hind gut stimulation, not the foregut exclusion theory

It is proposed that incompletely digested nutrients to the ileum and colon leads to an exaggerated PYY and GLP-1 response (reviewed by Karra, and Batterham, 2010, Peterli R et al 2012). Surgical procedures that increase nutrient delivery to the distal gut such as BPD, JIB and RYGB result in rapid resolution of T2DM (Buchwald et al 2004). Faster gastric emptying (Braghetto I et al 2009) and small bowel transit time (Shah S et al 2010), and increased foregut hormone secretion (Peterli R et al 2012) post SG surgery is thought to lead to quick delivery of nutrients to the hindgut and in-turn evoke a hind gut incretin hormone response not dissimilar to that seen following RYGBP (Peterli R et al 2009, Peterli R et al 2012, Ramon J M et al 2012), and improve insulin secretion (DePaula AL et al 2009, Peterli R et al 2012, Ramon J M et al 2012). The equivalent PYY response at 3 months and 1 year after RYGBP and SG (Peterli R et al 2012) does lead to equivalent weight loss after both procedures. This is in addition to the improvement in insulin resistance (Rizzello M et al 2010, Peterli R et al 2012, Ramon J M et al 2012, Lee W J et al 2011). These findings have led some to argue that the hindgut plays a major role in mediating anti-diabetic effects of bariatric surgery (Karra E et al 2010). In our study PYY3-36 and (GLP-1 mediated) insulin: amylin ratio correlates to weight loss after RYGBP. After SG PYY3-36, GLP-1, acyl-ghrelin and amylin all correlate to weight loss. These results and recent results of others (Peterli R et al 2009, Oliván B et al 2009) lends support to a hind gut factor mediating the effects of weight loss after RYGBP and SG surgery. We also note that RYGBP patients continued to lose weight despite an increase in acyl-ghrelin secretion between 6 and 12 weeks. This finding is in keeping with a greater amount of weight loss after RYGBP despite a higher fasting and GTT stimulated total ghrelin in this group (Bose M et al 2010, Barazzoni R et al 2013). Others have recently proposed a balance in foregut and hind gut hormones mediate outcome after RYGBP and SG (Peterli R et al 2012). In support of the foregut theory, some have shown differential effects of oral versus gastrostomy glucose loading after RYGBP with exclusion of the proximal small bowel from glucose passage inducing greater plasma insulin, GLP-1, and PYY responses with glucose loading by way of the gastrostomy tube (Pournaras DJ et al 2012).

6.13 GLP-1 correlates to insulin, amylin and PYY3-36

The meal stimulated active GLP-1 response after RYGBP correlates to PYY3-36. This correlation has been reported in rats after RYGBP (Shin AC et al 2010). However our study is the first to report this in humans. Our study is also the first to report that meal stimulated active GLP-1 does also correlate to PYY 3-36 and insulin after SG. Further,

in our study the change in active GLP-1 after SG did correlate to change in amylin. The change in insulin and amylin after SG also show positive correlation. The above correlations may help explain the improvement in glucose disposal reported in our study and by others (Karamanakos SN et al 2008, Peterli R et al 2009, Peterli R et al 2012, and Ramon J M et al 2012). Our study does confirm correlation between GLP-1 and insulin secretion after RYGBP. In the RYGBP group, change in GLP-1 after surgery does also positively correlate to change in insulin at 6 and 12 weeks. This has been reported by others recently (Umeda L M et al 2011). The meal stimulated plasma GLP-1 AUC does correlate to the corresponding plasma amylin AUC in the RYGBP and SG groups. The meal stimulated plasma GLP-1 AUC from all visits does correlate to the corresponding plasma amylin AUC after RYGBP. In the RYGBP group, we are the first to report that the change in meal stimulated active GLP-1 after RYGBP does correlates to change in insulin, amylin and insulin: amylin ratio after surgery. These correlations point towards GLP-1 mediated changes in insulin and amylin secretion after RYGBP.

6.14 Analysis of RYGBP glucose and insulin profile excluding Type-2 DM patient

The baseline mean fasting glucose is significantly reduced when the T2DM patient is excluded, and there is a significant decline in the temporal profile of glucose after RYGBP. Bonferroni post test analysis does confirm significant decline in glucose at similar time points after the meal at six weeks but at 12 weeks more early and late time points are significant when the T2DM patient is excluded. There is also a significant decline in the peak plasma glucose when the T2DM patient is excluded. However the timing of peak remains the same. There is a significant decline in the mean plasma glucose AUC when the T2DM patient is excluded. In summary excluding the T2DM patient did lead to a significant decline in mean fasting, mean peak plasma glucose, and mean glucose AUC. The temporal profile of glucose is also significantly altered.

Fasting insulin did not alter significantly when the T2DM patient was excluded. The temporal profile was significantly altered after excluding the T2DM patient. There was now a trend towards significance in meal stimulated insulin profile after RYGBP. Further, Bonferroni post test analysis did confirm a significant increase at early time points and significant decline at late time points. The baseline peak insulin did alter significantly when the T2DM patient was excluded. However, the time at which the peak insulin concentrations occur did not alter between the two groups. The plasma insulin AUC did not alter significantly when the T2DM patient was excluded. In

summary the inclusion of a T2DM patient did not alter fasting insulin, time of peak insulin nor insulin AUC. However it did alter the peak plasma insulin concentration.

6.15 A differential insulin amylin ratio after RYGBP and SG

Insulin/ amylin ratio is altered differentially after RYGBP and SG. There is a significant decrease in the ratio after RYGBP surgery, In the SG group there is a significant increase in insulin amylin ratio after surgery. Further, physiologic concentrations of endogenous amylin may also effect insulin secretion (reviewed by Cluck MW et al 2005). Insulin secretion is inhibited by amylin both in vitro and in vivo, (Gebre-Medhin S et al 1998, Wang ZL et al 1993 and reviewed by Cluck MW et al 2005). Recent studies have highlighted a role for amylin therapy in obesity (Ravussin E et al 2009, Smith SR et al 2008). In our study the increase in amylin content could explain the poor GLP-1 to insulin ratio after RYGBP surgery (Hansen EN et al 2011). The meal stimulated insulin to active GLP-1 AUC ratio declined by around 60% after RYGBP. There is a significant reduction in the amount of insulin secreted in response to an equivalent active GLP-1 stimulus after both procedures. However, the decline is more pronounced after RYGBP. This may also be related to a threshold effect.

This change in insulin: amylin ratio did correlate to %EWL at those time points in the RYGBP group. The superior GLP-1 response seen after RYGBP may have contributed to this. The change in GLP-1 after surgery does correlate to change in insulin/ amylin ratio after RYGBP. However, relative increase in amylin secretion did not adversely influence glucose homeostasis in that group. One previous study examined the role of portal amylin: insulin ratio at time of gastric by-pass surgery, and found an inverse relationship to glucose disposal rate 7 months after surgery (Blackard WG et al 1994). We did not find a correlation between insulin: amylin ratio and plasma glucose after RYGBP and SG. There have been no studies on meal stimulated active amylin secretion after SG. Others have measured total amylin (Dimitriadis E et al 2013). This study points to a reduction in fasting and meal stimulated total amylin. We found no significant difference in amylin secretion after SG. The change in amylin secretion after SG did correlate to weight loss at 6 and 12 weeks after surgery. This contrasting alteration in ratio did not correlate to satiety, prospective food consumption or weight loss after SG. Daily exenatide (GLP-1) treatment led to improved glucose and increased amylin/ insulin ratio in response to a mixed meal (Faradji RN et al 2009). At three months after GLP-1 treatment a significant increase in amylin AUC and an increased baseline amylin/ insulin ratio were observed (Faradji RN et al 2009). It is also possible that GLP-1 could increase amylin secretion from sites other than the islets

(Zaki M et al 2002). In our study there is a significant reduction in the amount of amylin secreted in response to an equivalent active GLP-1 stimulus after both procedures. Again this may represent a threshold effect.

In our study GLP-1 secretion does show a positive correlation to amylin secretion in both groups, before and after surgical intervention. It is interesting that the Change in meal stimulated amylin does show a positive correlation to the change in meal stimulated insulin at 12 weeks after SG. The insulin secretion is significantly improved after SG and does not change significantly between 6 and 12 weeks. The amylin secretion is unchanged between 6 and 12 weeks. Therefore it is likely that other factors such as GIP, fatty acyl molecules that can differentially regulate amylin, insulin synthesis and secretion leading to an alteration in the relationship between insulin and amylin after SG, between these time points. There was a significant correlation between the AUC for GLP-1 and amylin for all visits in the RYGBP and SG groups. The outlying markedly high GLP-1 and amylin response seen in one patient adds further weight to this correlation. Also, post operative GLP-1 response in the SG group at 12 weeks did correlate to amylin response at that time point. In support of this others have proposed that amylin synthesis and secretion may be under the influence of GLP-1 (Ahrén B et al 1997), and amylin in turn may mediate some of the biological actions of GLP-1 (Asmar M et al 2010).

In contrast to our findings Bose and colleagues found a non-significant decline in total amylin after RYGBP. However, a decline in total amylin in the diet induced weight loss control group suggests that sample collection and processing may have played a part in this un-expected result (Mousumi Bose et al 2010). Others have recently reported a decline after SG (Dimitriadis E et al 2013). De Paula and colleagues showed an increase in amylin secretion that did not reach statistical significance (De Paula et al 2009). However, they do not reveal if this was active or total amylin. Further, Shin and colleagues found an increase in active amylin after RYGBP in rodents (Shin AC et al 2010). The latter findings are in keeping with our study.

6.16 Feeding alters risk-sensitive reward in healthy individuals

The mid brain neural reward pathway is thought to mediate the rewarding aspects food, drugs of addiction and money (Elliott R et al 2003, Volkow ND and Wise RA 2005, Palmiter RD 2007, Platt ML and Huettel SA 2008). Recent evidence from *in vivo* studies confirms the presence of functional receptors to leptin and ghrelin in the reward pathway (Palmiter RD 2007). Metabolic hormones can activate (acyl-ghrelin) or inhibit

(leptin) this reward pathway (Morton GJ et al 2006, Palmiter RD 2007, Lenard NR and Berthoud HR 2008).

The concept that reward perception is subject to homeostatic regulation derives from evidence that food deprivation strongly augments the reward value. One mechanism to explain this effect proposes that metabolic signals such as leptin and insulin tonically inhibit brain reward circuitry and that, by lowering circulating levels of these hormones, energy restriction increases the sensitivity of reward circuits (Fulton et al 2000 and Figlewicz et al 2004). More recently evidence from animal studies and functional magnetic imaging has suggested that primary re-inforcers such as food (Beaver JD et al 2006 and Batterham RL et al 2007) and secondary re-inforcers such as psycho-active drugs (Volkow ND and Wise RA 2005) and monetary rewards (Ernst et al 2004 and Matthews et al 2004) are all thought to mediate their rewarding effects through the reward pathway.

Animals take risks when foraging for food. Risk-sensitive foraging theory states that this risk is dependent on the animal's baseline energy state, the energetic benefit of the food reward and the risks involved in achieving this energetic benefit (Caraco T et al 1980, Joseph M et al 1988, JM McNamara, AI Houston 1992). The metabolic reference point is often taken in ecology as the intake required for survival. The baseline risk will depend upon baseline energy reserves, and energy requirements (Kacelnik A and Bateson M 1996). In other words animals that are energy-replete after a meal, do not need to indulge in risky behaviour around predators, and can do so without the danger of falling below a metabolic target. Risk-sensitive foraging theory describes an integration of risk and food reward in ecology (JM Mcnamara and AI Houston 1992). It is known that activity in the reward pathway is related to presentation of conditioned stimuli linked to natural rewards in animals (Berridge 1994). A comparison between an individual's decision making and animals that make risk-sensitive foraging decisions point to; like animals that have sufficient energy for the day, humans are risk averse when they face potential monetary gains and risk prone when the choice involves potential monetary loss, as when an animal faces inadequate energetic benefit (Lee D 2005). Further, there is some evidence to point towards similarities between monetary and sugary fluid rewards in humans. A uniform pattern of risk sensitive decision making was seen in both humans and non-human primates (Hayden BY and Platt ML 2009). Recent fMRI studies have highlighted the link between risk-sensitive reward and the reward pathway. A study with a simple gambling paradigm did show total winnings correlated with hemodynamic response in the reward pathway (Elliott et al, 2000), other fMRI studies have also adopted this risk-sensitive reward paradigm (Ernst et al 2004

and Matthews et al 2004) and confirm increased activity in the reward pathway during selection of the high-reward/risk option than during selection of low-reward/ risk option. A recent human study to compare and assess choices made for sugary fluid rewards and monetary gains on gambling tasks, revealed a consistent pattern of decision making for both food and monetary rewards (Hayden BY and Platt ML 2009). Here a uniform pattern of risk sensitive decision making was seen in both humans and non-human primates (Hayden BY and Platt ML 2009). However, metabolic state is not known to play a part in economic theories on decision making in humans, this is in contrast to ecological theories on animal foraging behaviour.

6.17 Baseline leptin and BMI correlate to risk sensitive reward immediately after a meal in healthy subjects

In our study, BMI and plasma leptin is positively and significantly correlated to the change in monetary risk-sensitive reward from the fasted to fed state. This positive association between baseline energy stores and risk sensitive reward is similar to the risk-sensitive foraging for food seen in animals (Caraco T et al 1980, Lee D 2005). Further, in our subjects this increase in risk-sensitive reward was not to food reward but to monetary rewards. There is a significant positive correlation between circulating plasma leptin and body fat mass. There is also a significant correlation between BMI and body fat percentage. Given this correlation, BMI and leptin did correlate to change in risk averse choices made from the fasted to the fed state. The lipostatic theory on energy homeostasis states that humoral signals generated from body fat stores, act through homeostatic centres to maintain body fat stores (Mayer J 1955). In other words, the higher an individuals body fat stores, the more energy they need to sustain their current energy state. Therefore subjects with a higher percentage body fat and plasma leptin levels, would be expected to be relatively more risk seeking for food reward after the same meal. However, it is interesting that in our subjects this increase in risk-sensitive reward was not to food reward but to monetary rewards. This transfer of effect from food to monetary reward has not been reported before. Subjects in the decision making study were risk averse at baseline. The transfer of effect from one rewarding experience to another is supported by recent evidence suggesting similarities between decisions made for food reward and monetary reward in humans.

However, the positive correlation between leptin and risk-sensitive reward does also raise further questions. The reward pathway is known to have functional leptin receptors that have an inhibitory effect on these neurons (Palmiter RD 2007, Lenard NR and Berthoud HR 2008). A review of *in vivo* studies on leptin and the reward

pathway also point to an inhibitory effect of leptin (Morton GJ et al 2006, Fulton S 2006). In our study, the higher leptin level did not lead to an inhibitory effect on the reward pathway, and make those individual's with higher adiposity less likely to seek further monetary reward, in fact the opposite occurred. Subjects with a higher leptin level displayed a risky approach to reward.

A coherent model to explain the effects of leptin on reward seeking behaviour, at an organism level, will need to take account of the effects of leptin on both the homeostatic pathways governing energy homeostasis, and the reward pathway. The homeostatic pathway and the reward pathway are interconnected and inter-related (Morton GJ et al 2006). The idea that reward perception is subject to homeostatic regulation is now accepted. A lack of availability of food exerts a global stimulatory effect on reward perception, including food reward (Morton GJ et al 2006). However, the opposing scenario, which occurs in obesity, when an individual's abundant of energy store will be expected to lead to the opposite effect and decreased sensitivity, is still debated (Morton GJ et al 2006). Therefore it could be argued that in our subject pool, individuals with a higher body fat mass and plasma leptin would need more energy to sustain their fat mass, and as suggested by the lipostatic theory, they will be more likely to seek further food reward to sustain their energy stores.

There are also other possible explanations for this discrepancy. It is possible that like in obesity and drug addiction, where supra-physiological stimulation of the reward pathway leads to stimulus preferences, a high calorie meal led to the preference of a more rewarding stimulus (Volkow ND and Wise RA 2005). Further, it is also possible that higher centres in the prefrontal cortex that are known to exert executive control over the reward pathway (Lenard NR and Berthoud HR 2008, Figlewicz DP and Benoit SC 2008) , may have played a role in choosing the higher gain monetary incentives on offer, in a predominantly student population.

6.18 Acyl-ghrelin after a meal correlates to risk sensitive reward when satiated in healthy subjects

Feeding significantly altered a subject's risk averse score from the fasted to the fed state and showed a trend towards significance from the fasted to the 1-hour post-meal time point. Subjects became more risk seeking after the meal. Consumption of the meal caused a significant decrease in plasma acyl-ghrelin. In keeping with this there was a significant decrease in subjective hunger score (VAS) from the fasted to the fed state. The mean hunger score at each time point across all visits did show a highly

significant positive correlation to the corresponding mean plasma acyl-ghrelin. There was a significant negative correlation between increase in risky choices from the fasted to the 1-hour post meal stage and the decrease in Δ acyl-ghrelin at the corresponding time point. Results from the decision making study suggests that an individual's metabolic state does influence risk-sensitive monetary reward. The change in an individual's risk-sensitive reward from the fasted to fed state is significantly correlated to his baseline energy stores indexed by the metabolic hormone leptin, further the change in risk sensitive reward at an hour after the meal (when acyl-ghrelin has reached its nadir) is correlated to acyl-ghrelin a hormone that indexes acute nutrient intake.

Other fMRI studies have adopted a risk-sensitive reward paradigm (Ernst et al 2004 and Matthews et al 2004). A study with a simple gambling paradigm did show total winnings correlated with haemodynamic response in the reward pathway (Elliott et al, 2000). In other words the neural substrates, responsive to monetary reinforcement overlap extensively with those responsive to primary reinforcers such as food in animals (Elliott et al 2003).

An individual's approach to risk-sensitive monetary reward and a foraging animal's approach to risk-sensitive food reward are known to share common characteristics (Lee D 2005). Further, as the vast majority of human studies on risk sensitive reward involve monetary rewards, a review of human risk preference and animal food reward, suggests that risk-sensitive reward in humans and animals share common characteristics, both are better predicted by a measure of risk that relates variability of outcomes to expected returns (EU Weber 2004). The reward pathway is able to assay metabolic signals and afferent neural inputs from other regions of the brain, and in turn inform neural pathways to bring about an individual's desired behavioural response (Lenard NR and Berthoud HR 2008). The reward pathway is thought to play a significant role in feeding behaviour in our current calorie abundant environment (Volkow ND and Wise RA 2005, Morton GJ et al 2006, Palmiter RD 2007, Lenard NR and Berthoud HR 2008, Stoeckel LE et al 2008).

In our subject population, a calorie rich meal significantly increased an individual's risk sensitive monetary reward seeking behaviour, from the fasted to the fed state and showed a trend towards significance at an hour after the meal ($p=0.137$). It could be argued that the high calorie meal in a safe environment increased their reward seeking behaviour to gain other, namely monetary reward. In other words one rewarding experience increased their appetite for other rewarding experiences. The "priming"

effect of a small amount of a palatable food on binge eating described as the 'priming' effect is also seen in addiction behaviour, where even a small dose tends to elicit a strong 'craving' and compulsion for further use, hence in our study food may have caused a 'priming' effect (Davis et al 2004) Recent evidence linking addiction behaviour and obesity to the reward pathway (Volkow ND and Wise RA 2005, Engelmann JB 2006, Palmiter RD 2007, Stoeckel LE et al 2008) also lends support to this concept of transfer of effect between food and money. Animal studies have shown a link between food deprivation and relapse of drug seeking behaviour, after a prolonged drug free period (Shalev U et al 2000). This effect was attenuated by leptin infusion, suggesting that food deprivation may augment reinstatement of drug seeking, by its actions on the dopaminergic reward pathway (Shalev U et al 2000).

The temporal profile of acyl-ghrelin in our study, confirms that the plasma acyl-ghrelin level is significantly altered from fasting to an hour after feeding, this change in acyl-ghrelin does also show positive and significant correlation to hunger VAS. The magnitude of this change in plasma acyl-ghrelin is negatively correlated to the change in risk-sensitive reward seeking in our subject population. In other words, an individual with a small change in plasma acyl-ghrelin level became more risk seeking to monetary reward. Acyl-ghrelin is able to promote feeding behaviour by its actions on the homeostatic and reward pathways (Cummings DE 2006). Therefore acyl-ghrelin is able to relay information about energy gains from food intake to the homeostatic centre and regulate feeding behaviour. Ghrelin is also able to promote food intake by acting on the reward pathway (Ghigo E et al 2005, Cummings DE 2006, Palmiter RD 2007), where it is known to have an excitatory effect, on these neurons (Abizaid A et al 2006, Palmiter RD 2007), and the reward pathway is thought to mediate the rewarding aspects of food and financial reward (Volkow ND, and Wise RA 2005, Palmiter RD 2007, Platt ML and Huettel SA 2008). This may explain the transfer of effect from food to money seen in our study. In those individuals with a relatively low suppression of acyl-ghrelin will be expected to promote further food intake by its action on the homeostatic pathway to maintain energy homeostasis, and acyl-ghrelin will also be expected to promote food intake through the reward pathway, in these same individuals. The risky approach to monetary reward, in individuals with low acyl-ghrelin suppression is in agreement with risk-sensitive foraging behaviour in animals, when an animal is not able to meet its daily energetic requirement with the "safe source" of food on offer, it would seek more variable and risk prone food sources **(**A Kacelnik and M Bateson 1996). In our subjects, as with leptin, a transfer of effect from food to monetary reward is seen, again this has not been reported before. However our findings of an increase in risky choices

after feeding in the cohort does contradict the decline in risk-sensitive monetary reward seeking behaviour with greater suppression of acyl-ghrelin.

6.19 Leptin and acyl-ghrelin interact to signal energy stores

It was initially thought that leptin regulated ghrelin levels (Barazzoni R et al 2003). Though more recent evidence points towards multiple factors regulating ghrelin secretion; nutrients (carbohydrate and protein suppress ghrelin more than lipid), insulin, intestinal osmolarity, enteric neural signalling and vagal response, have all been shown to suppress ghrelin secretion after a meal (Ghigo E et al 2005, Cummings DE. 2006, Klok MD et al 2007). There is some evidence pointing at insulin communicating information on both short term energy gains and long term energy stores, to ghrelin producing cells in the oxyntic mucosa (Cummings DE, Foster KE 2003, Klok MD et al 2007). It is thought that adiposity related changes in insulin, but not leptin may convey information on long term energy stores to ghrelin producing cells (Foster KE 2003, Cummings DE 2006). Ghrelin producing cells appose the basement membrane, in close proximity to the vascular compartment and are not known to be in direct contact with the gastric lumen (Cummings DE 2006). Therefore ghrelin producing cells are more likely to respond to blood borne signals and less likely to respond to luminal contents. It is now thought that leptin and ghrelin act in parallel on the homeostatic and reward pathways (Cummings DE 2006, Cummings DE, Foster KE 2003, Klok MD et al 2007). They are metabolic counterparts with opposing actions. Ghrelin is an evolutionarily conserved protein, conveying information on intestinal energy stores, in lower non vertebrate organisms (Cummings DE, Foster KE 2003).

6.20 Energy stores and feeding alter reward behaviour

The decision making study suggests that an individual's metabolic state does influence his monetary decisions. The risk-sensitive monetary decisions were influenced by both long-term metabolic signals indexing energy stores, and short-term metabolic signals that index energy gains. This is not surprising, given that the homeostatic mechanisms that regulate body energy stores also influence reward pathways (Morton GJ et al 2006). At the neurobiological level, our results suggest an overlap between food and monetary reward. This has significant implications for all decisions that incorporate risk and monetary reward. The implications of the results from the decision making study are wide ranging, given that all individual's make assessment of risk and reward in many aspects of our daily lives, from crossing the road to placing a bet at the grand-national. An individual's body mass index and his nutritional intake could alter

behavioural patterns in the financial services industry; as long term energy stores will influence risk sensitive reward seeking, should all who take risks to attain monetary reward be encouraged to adhere to a ideal body weight. Further, after a meal, as the energetic value of the meal in relation to an individual's energetic requirement will influence risk sensitive reward. Should these individuals to be satiated at time of risk taking? It could be argued that all aspects of human behaviour does assess risk and reward, perhaps we should all take note of our hunger when it comes to pursuing any task that involves an assessment of risk and reward.

My bariatric study and decision making study have both identified correlation between acyl-ghrelin, the active gut hormone and appetite, and risk sensitive reward seeking behaviour. I propose to undertake further work utilising acyl-ghrelin infusions in healthy volunteers to induce risky choices in the risk reward paradigm. Further, studying post-operative RYGBP and SG subjects with the disparate changes in acyl-ghrelin will enable me to compare and contrast risk sensitive reward seeking behaviour in these patients after surgery and correlate that to acyl-ghrelin changes after RYGBP and SG.

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