An investigation of the neural mechanisms underlying the efficacy of the adjustable gastric band

Neal Forrest

Bachelor of Science

Monash University

Department of Physiology

Melbourne

Australia

2014

Supervisors:

Prof. Brian Oldfield

Dr. Aneta Stefanidis

ERRATA

Page 28, paragraph 3,4: 'LAGB' for 'AGB'

Page 35, paragraph 1: '15mg/ml' for '15mg/kg'. Also '25, 50 and 50mg/kg over 48 hours' for '25, 50 and 50mg/kg'

Page 38 'Figure 10' for 'Figure 9'

Page 45, paragraph 1: 'endings' for 'ending'

Page 48, paragraph 1: 'the' for 'these' and 'had' for 'will have'

Page 49, paragraph 2: '2.3.2' for '2.7.3.2'

Page 51, paragraph 2, replace 'will be' with 'were' and paragraph 3 'concluding section...in progress beyond 2011' with 'concluding section that will finalise this research project'.

Page 58, Figure 14.2 & Page 59, paragraph 3: '6ug/kg' for '6ug/ml/kg'

Page 60, paragraph 1: Delete 'sampled'

Page 63, heading: 'Denervate' for 'De-nervate'

Page 65 Figure 17.1, 17.2 and 17.3 'Time post inflation (Days)' for 'Time post inflation'.

Page 69, Figure 19.1: 'P>0.05' for 'P<0.05'

Page 79, Figure 22.1: 'IGTT' for 'IPTT'

Page 87, paragraph 1 & Page 100, paragraph 3: 'fos' for 'Fos'

Page 91, paragraph 2: Include 'capsaicin (10% of vehicle solution)'

Page 102, paragraph 3: 'nocioreceptors' for 'nociceptors'

ADDENDUM

Page 11, Add at the end of paragraph 2: 'It should be made clear that ghrelin can act directly in the CNS to control nutrient and glucose homeostasis, as well as acting on vagal afferents.'

Page 37, paragraph 3 line 1 should conclude with: '...a whole mount stomach section is visualised through calretinin staining.'

Page 52: Comment: The GTT and ITT injections were given intraperitoneally. The blood was sampled at 0, 15, 30, 60 and 120 minute intervals for both GTT and ITT testing, with an extra sample at 180mins in ITT.

Page 44 section 2.7.2.3 include: "The statistical analysis of GTT and ITT results was an unpaired T-Test used for each of the tests. There was also a one-way ANOVA completed for each of the AUC graphs produced from this data."

Page 47: Comment: This section refers purely to capsaicin testing of the AGB model, which is why it is separated from the sham vehicle experiments done later in the project being compared to this treatment plan.

Page 62: Comment: The images are a representative stomach wall from each treatment group, demonstrating the physical change shown by the food intake test from that group.

Page 67: Comment: These DEXA images reference a singular animal from each group and not the group averages as a whole which has been given in the accompanied text.

Page 72: Comment: The changes in tissue mass cannot be compared between different rat cohorts due to differences in time, location and exact feeding patterns, as well as natural variability.

Page 90: Include at the end of the first line: '(Bregma levels _ to _)'

Page 91: Comment: The topical capsaicin treatment had no effect on the CCK experiments due to the small amount of ablated nerve endings located around the stomach wall. This is why the CCK given could still elicit some effect on eating habits.

Declaration

I hereby declare that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis and that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university. I also declare this thesis is less than 50,000 words in length exclusive of tables, references and appendices.

I would like to acknowledge that I was assisted with some parts of the results. Dr. Aneta Stefanidis undertook the initial cohort of systemic AGB surgeries, and assisted with the post mortem dissection of fat pad masses in the long term high fat cohort. The counts of fos activated neurons following systemic capsaicin treatment were generated by Dr. Juliana Kampe.

I would also like to acknowledge the assistance of Ms Erika Ortiz, Dr Aneta Stefanidis and the Monash Animal housing staff in the assistance of animal husbandry and phenotypic analysis throughout this research project.

Contents

Chapter 4: Discussion

Background

In 2010, the International Obesity Taskforce (IOTF) in conjunction with the World Health Organisation (WHO) estimated that there were one billion people worldwide who were overweight, and a further 475 million classified as obese (IASO 2010). In Australia close to a third of the population are obese, while 70% of males and 56.2% of females were classified as overweight or obese in 2012 (Pink 2012). The prevalence of obesity in Australia is steadily rising, with an increase of 8% from 1995 to 2012, along with its attendant comorbidities such as diabetes, hypertension and coronary-heart disease (Schwartz and Moran 1998). These factors have led a push toward possible therapies for the burgeoning obesity epidemic.

It is clear that the debilitating impact of burgeoning obesity is through its related pathologies. Obesity has been ineffectively managed through programs that aim to facilitate changes in lifestyle and dietary attitudes. While laudable, this management has proven spectacularly ineffective. Weight loss involving non-operative management, such as diet, exercise and behaviour modification rarely achieve long term gains, with studies showing an average long term body weight loss of 4% (Farrell et al. 2009; Goodrick et al. 1996). Pharmaceutical treatments, whilst considered a valuable and effective option capable of 5-10% weight loss, are hampered by market withdrawals before achieving long term FDA approval due to serious adverse side effects. These techniques of weight loss cannot compare with the life changing 20-30% weight loss that bariatric surgeries provide (Farrell et al. 2009; Garb et al. 2009).

The introduction of surgical procedures, as an alternative to medication or extreme diet modification have been shown to be the most effective treatment for morbid obesity. Low complication rates and significant sustainable weight loss further increase the numbers of bariatric surgery procedures per year (Maggard et al. 2005). Additionally, the use of surgically induced weight loss is associated with the resolution or improvement of co-morbid diseases for 75% to 100% of patients (Buchwald et al. 2009). Weight loss surgery can range from relatively simple procedures involving the placement of a gastric band, or more complex operations involving major gut reorganisation as is the case with Roux En Y Gastric bypass surgery.

The popularity of Adjustable gastric banding (AGB) as a treatment is based on its reversibility, and effectiveness. It is a safe laprascopically performed procedure that is most commonly performed in Australia and throughout Europe. It is proven to be an effective treatment for morbid obesity, and causes the body to lose 47% of excess weight over a period of 16 years through existing neural and hormonal feedback systems in place controlling hunger and satiety (O'Brien et al. 2013). There is increasing acceptance that this link is the reason for the profound effects on weight loss that bariatric surgeries founded, and this is currently stimulating future research to uncover the neural mechanisms by which adjustable gastric banding elicits action from the periphery.

1.1 Obesity Mortality and Costs

Worldwide, obesity is responsible for 3.4 million deaths annually (WHO 2011). The magnitude of this problem is demonstrated by a recent report by the American Centre for Disease Control and Prevention (CDC), which states that obesity is fast approaching tobacco as the top underlying preventable cause of death in the USA. Obesity is associated with increased risk for co morbidities such as diabetes (accounting for 60% of type 2 cases), hypertension and coronary-heart disease (accounting for 20% of cases). The number of diabetics is projected to increase from 382 million in 2013 to 592 million in 2035 (Aguiree et al. 2013).

This increased rate of obesity results in increased pressure on governmental health programs. It has been estimated that overweight and obesity and their associated illnesses cost the Australian society a total of AUD $58 billion in 2008 (Access Economics 2008), however the greatest costs to society involve the increased rates of comorbidities due to obesity. The largest contributors are the treatments of diabetes and heart conditions, with diabetes resulting in an economic burden of USD 548 billion in 2013 (Aguiree et al. 2013). These treatments are not curative, which only prolongs the increase in medical care costs with the progression of these diseases.

The various pre-emptive plans to reduce obesity and its comorbidities via governmental initiatives are often futile. As has been shown previously, weight loss through diet and exercise leads to a very small percentage of actual long term weight loss. These initiatives are often expensive due to the target audience, and so aim for ‘mild weight loss’ to treat the more serious conditions of diabetes and heart disease, rather than aiming at the underlying condition of obesity. Despite little success, these programs continue to be funded due to the economic benefits that could be generated from sustained modest weight loss amongst obese persons. An American study found that with sustained 10% weight loss among obese individuals, there would be a halving in incidence of heart disease and stroke, an increased life expectancy of 7 months, and reduced medical care costs of an average of $3750 per person (Oster et al. 1999).

1.1.1 Childhood Obesity

Childhood obesity is one of the most serious public health challenges of the 21st century, with a quarter of Australian children are considered overweight with 7% considered obese (Pink 2012). In America over the past 3 decades, the rates of childhood obesity has doubled for children 2 to 5 years of age and tripled for children 6 to 11 years of age, with 16.9% of all American children now considered obese (Ogden Cl 2002). This issue is compounded over time as overweight children are likely to become obese adults, with the risk of adult obesity was at least twice as high for obese children as for non-obese children (Serdula et al. 1993). They are more likely than non-overweight children to develop diabetes and cardiovascular diseases at a younger age, which in turn are associated with a higher chance of premature death and disability.

There are significant social, financial and public perception barriers that have resulted in this drastic increase in childhood obesity. This is in part due to technological change across the globe, lowering the cost of nutrient-poor, calorie-dense foods (Philipson and Posner 1999). This has resulted in a lowering in the price of calories, with cheaper food containing greater quantities of salts and fats making healthy alternatives proportionally more expensive. Additional factors include the distribution and marketing influence of ‘fun’ foods toward children (Harris et al. 2009). In addition, caloric expenditure has been modified by social and economic development in the areas of transport, urban planning and education with children's dietary habits and preferences as well as their physical activity patterns. Increasingly, these influences are promoting unhealthy weight gain leading to a steady rise in the prevalence of childhood obesity.

1.2 The Efficacy of Diet, Exercise and Pharmacological Methods of Weight Loss.

1.2.1 Does dieting and exercise work?

It is clear that the debilitating impact of burgeoning obesity is through its related pathologies. Obesity has been ineffectively managed through programs to facilitate the lifestyle and dietary attitudes. While laudable, this management has proven spectacularly ineffective.

Weight loss involving non-operative management, such as diet, exercise and behaviour modification rarely achieve long term gains (Goodrick et al. 1996). Non-operative management of obesity is associated with an average weight loss of 4% in long term studies greater than 4 years (Anderson et al. 2001; Lantz et al. 2003; Mann et al. 2007).

Community wide weight loss campaigns have commonly tried to implement strategies of better eating and exercise habits. These focussed on population based intervention studies recruited through different community settings, through primary schools as in the "*Romp & Chomp"* campaign of 2004-2008, school aged children and their families in the *"It's Your Move!"* project in 2006-2008, and the general population *"Measure-Up"* campaign targeting community involvement in 2008. While these programs have been shown to increase awareness with some showing a decrease in calorically-dense food intake, they have been unable to promote significant weight loss over the long term. Numerous studies have shown a resumption of weight after these intervention and awareness campaigns (James et al. 2007; O'Hara et al. 2013).

It has been shown in treatment combination studies that subjects who exercise with a diet plan regain an average of 70% of the weight lost within 30 months (Sweeney et al. 1993). This can vary between studies, as shown by Stalonas (1999), who found that 5 years after the treatment plan, subjects had regained all of the weight lost, and gained a further 1.5 pounds in addition to their original starting weight.

1.2.2 Is there a magic obesity pill?

Pharmacological agents used to induce weight loss may reduce appetite or increase satiety, reduce the absorption of nutrients, or increase energy expenditure (Wilding 2009). This leads to the question; which area of energy regulation is most effective? Most drug targets interact with the pathway from central processing areas which then generate peripheral signals, such as the basal metabolic rate in the case of thyroid hormones and dinitrophenol, which successfully cause weight loss but cause serious side effects (Grundlingh et al. 2011). Alternatively, therapeutic targets associated with energy intake, such as phentermine and sibutramine will act on the fluctuating and variable energy intake periods of feeding. These drugs try to delay initiation of meals or snacks by reducing latent hunger, as well as modifying the feedback response from eating by increasing the satiation signalling.

In the brain the initiation, termination and determination of feeding is regulated by various neurotransmitters and neuropeptides which make up an interconnected neural circuit. These are highly controlled, and moderated by the input stimuli such as endocrine factors and peripheral nerves, as shown in Figure 1 (Adan 2013). Most anti-obesity drugs target these areas of regulation, and target monoaminergic cell groups, as is the case with Sibutramine (Williams and Elmquist 2012). This is a serotonin and noradrenaline reuptake inhibitor developed to suppress food intake through increased satiety and increased energy expenditure by stimulating thermogenesis (Nisoli and Carruba 2000). How monoamines such as dopamine, serotonin, and noradrenaline are involved in affecting energy balance is only partly understood (Adan 2013). This is concerning considering that recent FDA approval has been given to new anti-obesity drugs Lorcaserin and Qsymia, both of which target monoaminergic systems.

Over the past 25 years, more than 120 drugs have been investigated for the treatment of obesity. Amongst the weight loss drugs approved for sale there have been several instances of market withdrawal due to serious adverse events. The modification of a highly complex system in the body such as thermogenesis can be dangerous, with sibutramine causing increases in heart rate and blood pressure that lead to hypertension and cardiac arrhythmias (Florentin et al. 2008). Qsymia has similar effects that included paraesthesia, dizziness and insomnia, whilst Lorcaserin produces adverse effects of headache, dizziness and nausea (Smith et al. 2010). The common issue with drug approval is that uncommon but serious adverse effects may become apparent only when a drug is used in larger populations or over a greater period of time than the preapproval trials (Lauer 2012).

This issue of granted and redacted approval is evidenced by Sibutramine, while a mainstay in the treatment of overweight and obesity over a number of years, the increases in blood pressure and apparent cardiovascular toxicity led to the drug being removed from the market (Henderson et al. 2007). The cannabinoid type 1 receptor inverse agonist, Rimonabant, is another compound that leads to sustained, clinically meaningful weight loss. It is associated with improvements in several cardiovascular and metabolic risk factors, including HDL-cholesterol and triglyceride concentrations (Van Gaal et al. 2005). However, it was discontinued as a frontline anti-obesity pharmacotherapy on the basis of adverse psychiatric outcomes (Pi-Sunyer et al. 2006).

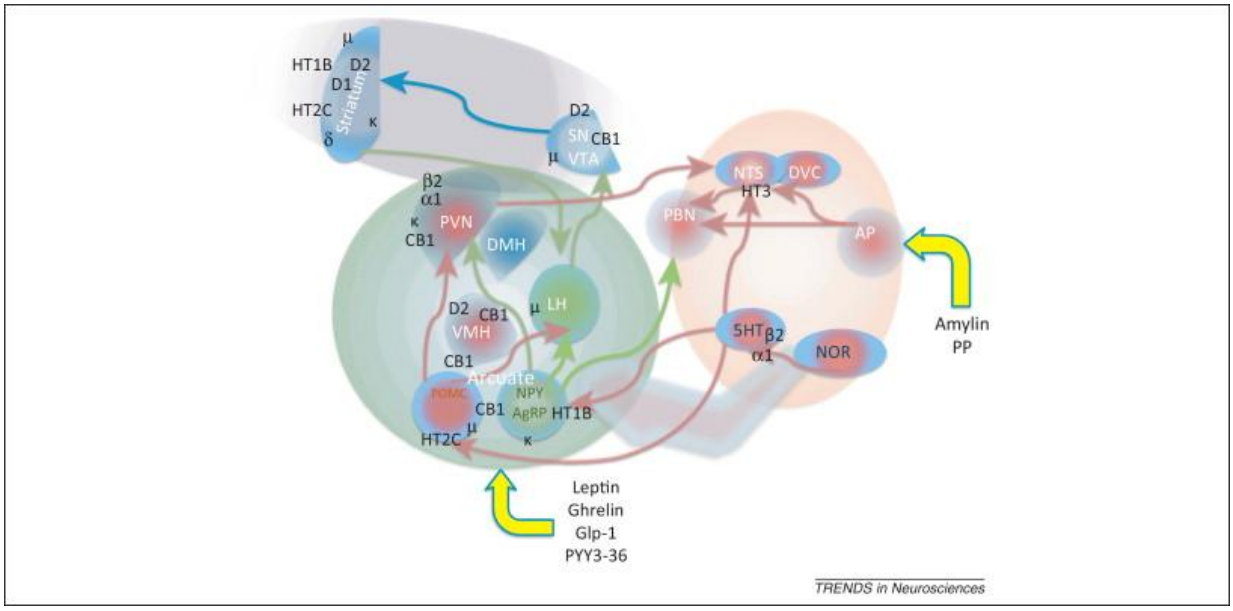


Figure 1: In the brain, the initiation, termination, and choice of meals is controlled by distinct and interconnected neural circuits that use a variety of neurotransmitters and neuropeptides and that receive input on the status of energy balance via endocrine factors and peripheral nerves (Adan 2013).

1.2.3 Bariatric surgery as a treatment of obesity

The only treatment for morbid obesity is bariatric surgery. As defined by the WHO, morbid obesity is a classification given to individuals with a BMI greater than 35 (WHO 2011). Every year, morbidly obese individuals worldwide have around 344,000 surgical procedures (Baltasar et al. 2011). Of those, 220,000 are in the USA and Canada alone. The introduction of surgical procedures as an alternative to medication or extreme diet modification, coupled with its low complication rate and significant sustainable weight loss dramatically increase the numbers of bariatric surgery procedures per year (Maggard et al. 2005). Additionally, the use of surgically induced weight loss is associated with the resolution or improvement of co-morbid diseases for 75% to 100% of patients (Buchwald et al. 2009).

The main methods of bariatric surgery are Roux En Y gastric bypass (RYGB), and Laprascopic Adjustable Gastric Banding (LAGB). RYGB involves extreme and semi-permanent re-organisation of the gastrointestinal tract, compared with the adjustable gastric band which involves the addition of an inflatable cuff on the stomach. Therefore, although there are several methods in combating the obesity epidemic, it is clear that bariatric surgery will provide feasible and sustained weight loss.

1.3 The Regulatory Actions of the Vagus Nerve

1.3.1 The vagus nerve and the stomach

The vagus nerve is an important regulator of the organs contained within the thoracic, and abdominal cavities (Sobocki et al. 2005). It provides an autonomic neurological feedback pathway between the brain and the stomach, so that both afferent and hormonal signals can be used to regulate satiety (Martin and Earle 2011; Sobocki et al. 2005).

The stomach has a vital role in controlling and sensing food ingestion through the various stomach layers which are innervated by sensory vagal and splanchic nerves. Vagal afferents which determine tension and stretch are situated in the external layers of the stomach, while internal layers of mucosa are sensitive to mechanical touch and volume, sensing both gastric distension and nutrient absorption (Schwartz and Moran 1998).

1.3.2 Intraganglionic Laminar Endings

The stretch of the stomach wall is continuously monitored by the vagal afferent mechanoreceptors located in the different layers of stomach tissue, this information is taken to the hindbrain via vagal and spinal sensory nerves. The vagus nerve divides and extends into the stomach wall using many thin projections called intraganglionic laminar endings (IGLE).

Intraganglionic laminar endings are located in the connective tissue capsule of myenteric plexus ganglia, between the longitudinal (outer) and circular (inner) muscle layers (Berthoud and Neuhuber 2000). They therefore respond to muscle tension generated by both passive stretch and active contraction of the muscle layers (Zagrodnyuk, 2001). The IGLEs are appropriate to account for the slowly adapting responses to food intake, as they have been shown to be mechanotransduction sites which generate low threshold firing rates, slowly adapting vagal tension receptors in the stomach. This sustained response is partly the reason for continued satiety after food intake has ceased (Zagorodnyuk et al. 2001).

1.3.3 Central integration of peripheral satiety signals

The vagus nerve is split into sensory afferent systems, which innervate the gastrointestinal tract, pancreas, and liver, and an efferent regulatory system which acts in parallel with the sympathetic nervous system to determine nutrient storage and mobilization (Berthoud 2008b). The hindbrain is the terminal connection point of both the afferent and efferent vagus systems (Shapiro, 1985), with the gastric vagal afferent fibres penetrating the subnucleus gelatinosus, nucleus commissuralis, and medial nucleus of the nucleus of the solitary tract (NTS). Some afferent fibres also penetrate into the area postrema and the dorsal motor nucleus. The pre-ganglionic neurons that project from these areas of the brain then innervate the stomach wall, duodenum, jejunum, and colon.

There is a positive correlation between increased stomach distension, reported levels of satiety, and the level of activation of sub cortical brain regions involved in satiety duration and meal initiation (Berthoud 2008b; Wang et al. 2008). These neurological control systems, involving vagal nerve feedback through appetite neuropeptides and nutrient sensor food mediators, are hypothesised to play an active role in the regulation of food intake.

1.4 Integration of Peripheral Energy Balance Signals Released by the Brain

1.4.1 Gut-brain links

The primary neuroanatomic linking factor in the gut-brain axis is the vagus nerve, which connects the signals elicited by nutrients in the gastrointestinal tract with sites in the central nervous system mediating food intake (Schwartz 2000). Vagal afferents are sensitive to mechanical, chemical, and gut peptide meal-related stimuli. From this, meal-elicited gastrointestinal stimuli in the periphery can activate distinct patterns of c-fos neural activation within caudal brainstem sites, where gut vagal afferents terminate (Berthoud 2008b). Therefore, vagal feedback from the stomach and intestine have an important influence on brainstem activity and ultimately on food intake and metabolism.

1.4.1.1 Regulatory control due to gut derived hormones

Ghrelin is the only identified 'orexigenic' hormone synthesised throughout the gastrointestinal tract, but has the highest production rate in the stomach (Williams and Elmquist 2012). Peripheral production of ghrelin signals for starvation and Growth Hormone (GH) secretion is then relayed to the brain by means of the vagus nerve, which ultimately interacts with the hypothalamus. Ghrelin increases the secretion of growth hormone, food intake and adiposity by stimulating gastric vagal afferents which then acutely activate neurons expressing NPY/AgRP in the arcuate nucleus of the hypothalamus (Nakazato et al. 2001) as well as inhibiting hypothalamic arcuate pre-opiomelanocortin (POMC) neurons (Date et al. 2002b). Recent work suggests that ghrelin may directly counter-regulate leptin and insulin signalling in the hypothalamus, while others have shown that the afferent discharge is suppressed by ghrelin and the anoretic peptide CCK produced in the intestine stimulated it (Date et al. 2002a).

By comparison, nutrient delivery to the gut activates neuroendocrine methods that control digestion and energy intake and utilization with the release of enteroendocrine cells of mediators including a series of anorexigenic hormones glucagon-like peptide-1 (GLP-1), peptide YY (PYY), cholecystokinin (CCK), pancreatic polypeptide (PP), and amylin. These act on the brainstem via a vascular route, or by activation of vagal sensory terminals (Strader and Woods 2005; Swartz et al. 2010; Wynne et al. 2006). The gastrointestinally derived PYY and the pancreas-derived PP elicit their physiological effects by interacting with a specific family of Y receptors located in the brainstem (Batterham et al. 2003). PYY is produced in enteroendocrine cells in the ileum and colon and are secreted after a meal to promote satiety (Ghatei et al. 1983), with studies showing that these signalling mechanisms are influenced by nutrition. Therefore food-withdrawal and diet-induced obesity alter the sensitivity of vagal afferent neurons to stimulation as well as their patterns of expression of receptors and neuropeptide transmitters.

The nucleus of the tractus solitarious (NTS) receives input from the vagus nerve afferents, whereas the area postrema is a target for circulating factors such as amylin and glucagon-like peptide-1 (GLP-1) (Badman and Flier 2005). As shown in figure 2, the arcuate is a major hub for the integration of nutrition information originating from peripheral organs, mediated through circulating hormones and metabolites and/or neural pathways (Nakazato et al. 2001).

Leptin is the dominant long-term feedback signal that regulates the energy reserves of adipose tissue. As shown above in figure 1, leptin can cross the blood brain barrier and binds to specific receptors in the arcuate nucleus controlling appetite (Badman and Flier 2005). This adipose regulatory mechanism enhances the sensitivity of anorexigenic receptors located in the arcuate nucleus, depending on peripheral satiation signals (Batterham et al. 2002). Additionally, studies suggest that the secretion of leptin occurs in the stomach after vagal stimulation (Sobhani et al. 2002). Others have shown the regulation of satiety due to leptin release from the gastric epithelium may be driven through mechanisms of CCK-mediated effects activated by food intake (Bado et al. 1998). This luminal leptin may be involved in vagus-mediated intestinal functions, however the physiological role of gut derived leptin is as yet unclear.

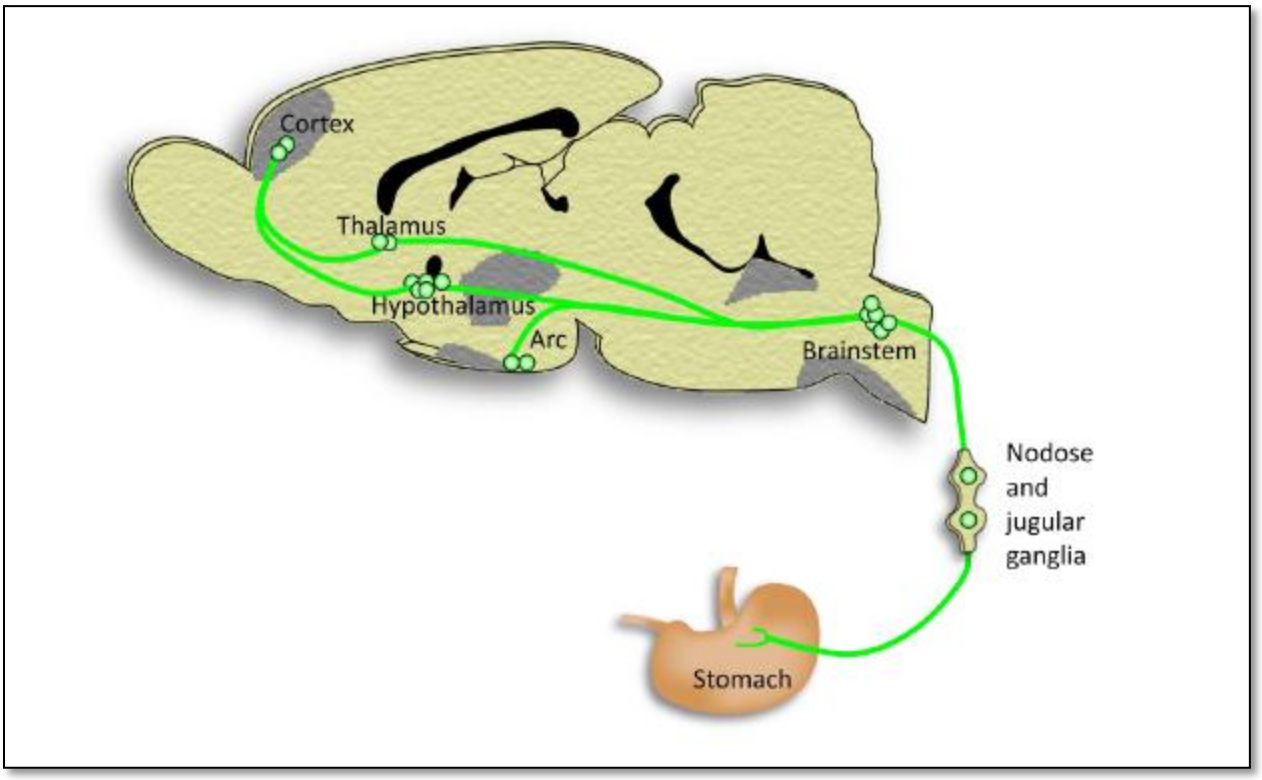


Figure 2: A schematic of the vagus nerve (shown in green), which connects the IGLE afferents on the stomach wall to key food regulatory centres in the brain, intersecting the nodose ganglion (brain shown in a sagittal cross-section). The most active food regulatory centres are the arcuate nucleus (Arc), the hypothalamus, and the brainstem. Adapted from Badman and Flier (2005).

1.4.2 Integration of peripheral signals by the hypothalamus

The coordinated regulation of energy intake and expenditure takes place in the hypothalamus, which integrates both sensory inputs and blood borne peripheral signals. Signals reflecting energy stores (i.e. leptin and insulin), recent nutritional stores (i.e. glucose and fatty acids), and other parameters are integrated in the CNS, particularly in the arcuate nucleus (Hahn et al. 1998). As shown in figure 3, the arcuate nucleus houses two key neuronal populations that co-express the orexigenic peptides neuropeptide Y (NPY) and agouti related protein (AgRP), and the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (Allen et al. 1983; Broberger et al. 1998; Parton et al. 2007). There is a wide array of literature on these peptides, with strong evidence that these cell types regulate energy homeostasis .

Orexigenic and anorectic signals of hypothalamic origin include interactions between these central signals and the metabolic signals originated in the periphery. These peripheral hormones are critical for normal regulation of food intake and body weight. An important peripheral signal essential for normal energy homeostasis is leptin. Central mediation of food intake is controlled in part by the functional interaction of ghrelin and leptin (Shintani et al. 2001). Leptin attenuates the action of ghrelin on NPY neurons, and regulation of ghrelin's effect on hypothalamic neurons, particularly NPY/AgRP neurons, may be one of the important mechanisms of leptin signalling in the hypothalamus (Sahu 2004; Shintani et al. 2001). This central mediation of hormones is also seen with AgRP neurons, which can directly inhibit the POMC neurons, as well as blocking melanocortin agonist action at melanocortin receptors. AgRP neurons are responsive to nutritional signals including adipose, gut and pancreatic hormones, brain-derived energy balance-associated neurotransmitters and neuropepetides, and dietary nutrients.

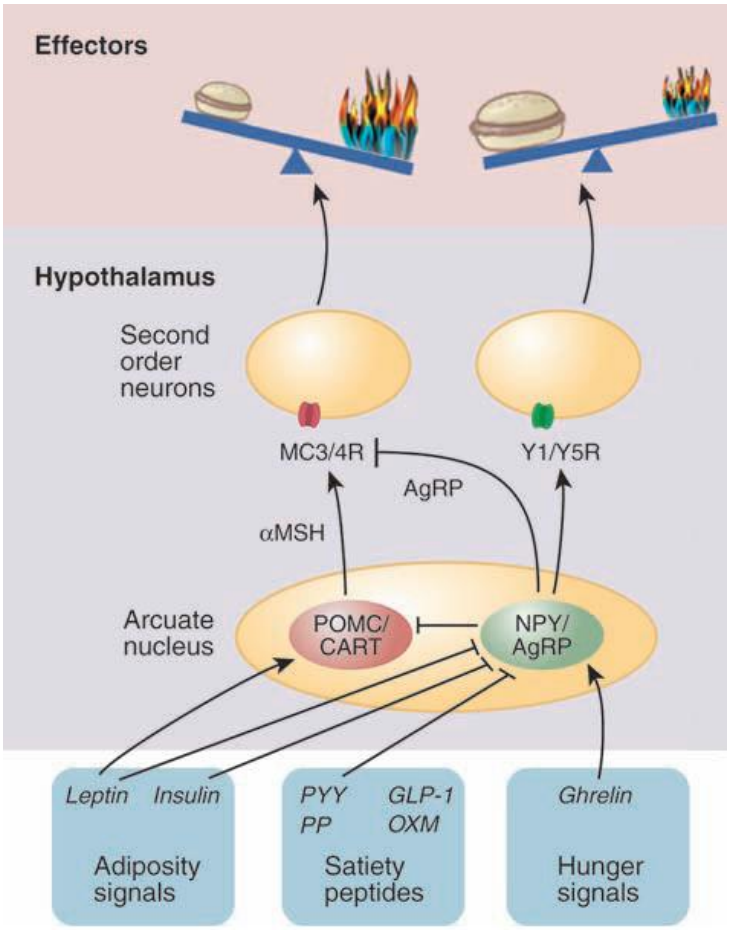


Figure 3: Simplified representation of potential action of gut peptides on the hypothalamus. Primary neurons in the arcuate nucleus contain multiple peptide neuromodulators. Appetite-inhibiting neurons (red) contain pro-opiomelanocortin (POMC) peptides such as a melanocyte-stimulating hormone (αMSH), which acts on melanocortin receptors (MC3R and MC4R). Appetite-stimulating neurons in the arcuate nucleus (green) contain neuropeptide Y (NPY), and agouti-related peptide (AgRP), Adapted from (Badman and Flier 2005).

1.5 Bariatric Surgery-Understanding the types, their effects and outcomes of use

1.5.1 Roux En Y Bypass

Gastric bypass surgery is a significant reorganisation of the gastrointestinal system, with the aim of reducing the ability of the body to absorb nutrients from the food. The RYGB, as shown below (Figure 4), involves a significant change in the way that the food is consumed and processed. After the remodelling of the gastrointestinal tract, the distal stomach, duodenum, and a proportion of the jejunum are removed, promoting malapsorption (Demaria and Jamal 2005). This is achieved through the remodelling of the stomach to form a small proximal gastric pouch. The jejunum is divided into two, with the distal tube attached to this gastric pouch stomach, creating a Roux (alimentary) limb (Farrell et al. 2009). The proximal bowel is the biliopancreatic limb and is connected to this alimentary limb 75-150cm below the gastrojejunostomy.

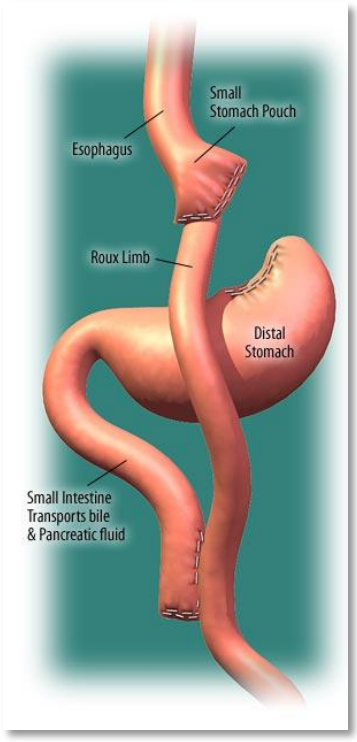


Figure 4: Schematic representation of Roux-en-Y Gastric Bypass surgery. Taken from http://thediabetesclub.com/wp-content/uploads/2011/02/roux-en-y2.jpg

Patients who have had an RYGB must change their diet as a result of the modified ability of their digestion system. As a result of this they are required to eat small, frequent meals of high protein and low carbohydrate content. Long term vitamin and calcium supplements are required, and in order to control this, periodic blood testing is required to quickly identify possible vitamin and mineral deficiencies (Farrell et al. 2009). The most frequently reported complication during the perioperative period are wound infection, gastrointestinal tract hemorrhage, and bowel obstruction. Postoperative conditions also involve incisional hernia and stomal stenosis (Podnos et al. 2003). While some of these may be due to poor planning of food intake, many are due to surgical problems.

There are highly variable reported rates of success of RYGB, with studies reporting on differences of up to 20% in the major complication rates requiring secondary surgical interventions (Blachar et al. 2002; Khoursheed et al. 2011; Perugini Ra and et al. 2003; Schauer 2003; Scozzari et al. 2014; Stroh et al. 2014). It has also been proven in one study that complication rates for RYGB are higher for men (6.3%) than women (5.3%) due to leakage and insufficiency of gastrojejunal anastomosis, sepsis and peritonitis (Stroh et al. 2014). The RYGB operation is a technical and demanding operation, with complication rates decreasing only after surgeons gain significant experience (Ballantyne et al. 2005). The variability shown in complication rates was considered to be a result of surgeon experience, with some studies finding little difference in laparascopic or robotic procedures (Perugini Ra and et al. 2003; Scozzari et al. 2014).

Bariatric procedures have been effective in treating type 2 diabetes, with RYGB being an effective option for those with a BMI of greater than 35 and T2DM co-morbidity (Rao et al. 2014). This has lead to an increase in those who undertake bariatric procedures who are outside the usual bariatric procedural guidelines in order to treat for diabetes. Initial studies into this effect have shown that it can be seen within weeks of surgery, which furthers the research that show the regaining of normoglycaemic control after RYGB is not primarily due to the extensive weight loss resulting from the bariatric procedure (Mistry et al. 2009; Patriti et al. 2005). The current literature has varied between 40-85% complete remission rates of T2DM after RYGB, however most demonstrate that over 90% of patients experience at least partial remission (de Sa et al. 2011; Huang et al. 2011; Lee et al. 2011).

1.5.2 Laparoscopic Adjustable Gastric Band

LAGB was first described in 1993, and has been historically described as a restrictive control. As depicted in the schematic below (Figure 5), modern improvements involve the use of an inflatable cuff device fitted 1cm below the gastroesophageal junction (Belachew et al. 1994), with studies showing that the placement of the AGB directly impacts on its efficacy (Thornton et al. 2009). Through the use of externalised port and tubing to the band, the cuff is inflated using saline injections which forms a virtual pouch which sits just beneath the gastroesophageal fat pad above the oesophageal junction (Ceelen et al. 2003; Klaiber et al. 2000). Attachment and removal set the LAGB apart from other bariatric surgeries, due to its less technically demanding attachment and possible reversing operations (Bowne et al. 2006). Galvani (2006) additionally states that LAGB is not only simpler, but far less invasive and safer to use when compared to RYGB.

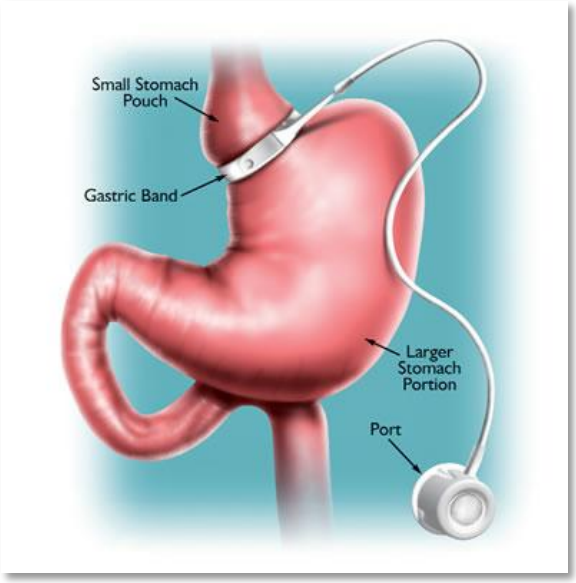


Figure 5: Schematic representation of Laparoscopic Adjustable Gastric Banding. Taken from http://www.utmedicalcenter.org/imagesLive/Users/Departments/Bariatrics/twlsc_surgeryopt-2.jpg

The evidence that LAGB is not purely a restrictive procedure comes from controlled studies involving hospitalized patients who had their satiety levels assessed following an overnight fast (Dixon et al. 2005). Patients with the optimal inflation of their LAGB experienced significantly greater fasting and post-prandial satiety levels than those without inflation. Importantly, those with bands inflated demonstrated less hunger before and increased satiety afterward (Dixon et al. 2005). It is unclear as to the role played by hormonal actions, as glucose, insulin, ghrelin and leptin levels do not appear to alter significantly between individuals with inflated and uninflated bands. The LAGB subjects did show higher ghrelin, and lower glucose, insulin and leptin levels compared with controls though. This data suggests that there are some mechanisms in place beyond those effects produced by food restriction. This crossover study has developed further understanding of the nature of the interaction between the stomach and the brain in regards to inducing satiety as either a neural or humoral effect, furthering (Dixon et al. 2005).

In the search for an effective weight loss therapy, many are dissuaded by surgery, and focus on intensive non surgical weight loss programs such as pharmacotherapy, very low energy diets and lifestyle change. In a random control trial, it was found that bariatric surgery treatment had a mean 10 year weight loss of 63.4% EWL (14.1kg), while those in the non surgical groups had a weight loss of 0.4kg (O'Brien et al. 2013). This clearly shows that, over a long period of time, bariatric surgery is the most effective way to lose weight.

The percentage of excess weight loss over the course of time differs between LAGB and RYGB. Where the RYGB has larger immediate effects over the first two years (67 vs. 42; 67 vs. 53), the percentage of excess weight lost is not statistically significant between the two surgeries for years 3, 4, 5, 6 or 7 years (O'Brien 2006). While this might promote RYGB as the more effective treatment, the use of LAGB achieves the same level of excess weight loss over a 10 year time period (Galvani et al. 2006). A comparison between LAGB and pharmacotherapy, O'Brien (2005) has purported that LAGB is far more effective in weight reduction, and resolving metabolic derangements than any currently available pharmacological treatments.

Like any surgical procedure, LAGB does carry a risk, with the most frequently reported postoperative complications being band slippage or erosion (Galvani et al. 2006). However, mortality rates suggest that LAGB operations are safer than RYGB, with mortality figures projected to be 0.1% and 0.5% respectively (Buchwald et al. 2004; Burton et al. 2011a; O'Brien 2006). It has been shown that patients with RYGB develop significantly more postoperative complications than patients with purely restrictive gastric bypass, or a sleeve gastrectomy. This has lead to the LAGB becoming a popular technique for weight loss, as it produces the same level of effectiveness as other more significant bariatric surgeries but does not result in the typical malapsorptive issues or surgical complication rates that would otherwise be factored into alternative treatment options.

1.5.3 Vertical Sleeve Gastrectomy

The development of bariatric surgery is evolving rapidly, with the primary aim of achieving a safe and effective procedure for the treatment of morbid obesity. As a result, the varied surgical techniques are updated with new methods and procedural guidelines. Malapsorptive and restrictive surgeries such as vertical sleeve gastrectomy (VSG) are becoming more prevalent as a preparatory initial surgery for the super obese (Frezza 2007). This procedure is designed to reduce the size of the stomach and its distension resulting in a physically decreased appetite, without a significant loss in function. As shown below in Figure 6, the VSG procedure is performed by stapling and excising a large portion of the stomach from the pylorus to the angle of His, which creates a gastric tube containing only 40 to 50cc of volume.

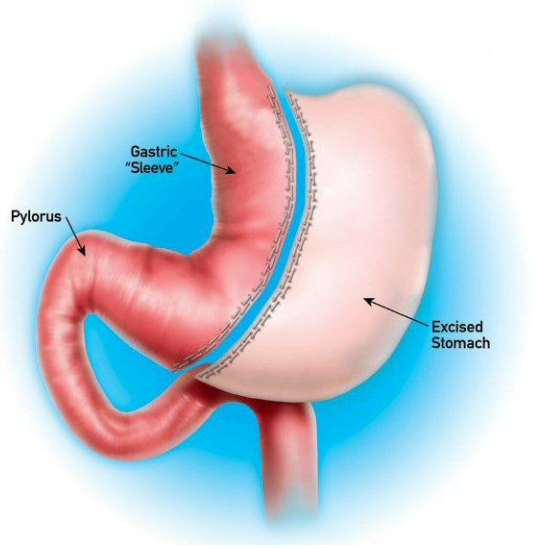


Figure 6: The Vertical Sleeve Gastrectomy showing the creation of a gastric tube or sleeve. Taken from: http://www.ethicon.com/obesity/bariatric-and-metabolic-surgery

The relative safety of performing VSG surgery has lead to its widespread use as a first-stage procedural management for morbidly and super-obese people (Gagner and Rogula 2003). The perioperative variables comparing bariatric surgeries which modify the GI tract show that VSG surgery has a significantly shorter operative time due to its technical simplicity. High risk patients are prepared for more invasive weight loss techniques using VSG due to its low complication rate (7.4%) compared with alternative GI tract surgery such as RYGB (22.8%) (Lee et al. 2007). This has founded new insight into weight loss in those who are at extremely high risk (BMI >60) as VSG may be used as a preparatory surgery for the patient to then be eligible for a safer secondary and more efficacious operation such as RYGB or LAGB (Papailiou et al. 2010).

Secondary procedures such as LAGB performed after VSG surgery has been shown to be the least invasive option, resulted in the shortest operative times, the lowest conversion rate, the shortest hospital stay and the lowest morbidity in a high-risk cohort of super-obese patients (Frezza et al. 2008). The mean weight losses of solely VSG surgery have been less than those reported after various other gastric restriction procedures, and the implications of “super-obese” patients undergoing this treatment is that their weight may normalise, with one study finding all weight was regained after 2 years post-surgery (Powers et al. 1997).

The use of VSG has been shown to have a low complication rate and therefore be an effective treatment for morbid obesity, however the complications that can arise are significant. The major complications include gastric leaking and post operative bleeding, both of which require secondary surgical revision (Dapri et al. 2007; Serra et al. 2007). Other knowns issues are the development of gastroesophageal reflux disease (GERD)(Laffin et al. 2013), appearance of stricture (Dapri et al. 2009), dilation of the gastric sleeve (Langer et al. 2006), and insufficient weight loss (Noel et al. 2014). Blood loss can be the most significant issue, occurring from the staple line during the excision of the fundus and body of the stomach, requiring revisional operative theatre (Dapri et al. 2009). The leak of the VSG is located along the stomach sleeve resection line from the antrum to the gastroesophageal junction, where there is the highest internal pressure (Baltasar et al. 2007; Serra et al. 2007). Studies have shown this to be a difficult problem to treat, requiring stomach endoscopic stents and a long hospital stay (Eisendrath et al. 2007).

Despite the relatively safe surgical technique, the irreversible nature of the VSG operation often causes major issues with these complications, resulting in greater mean operative room time and post surgical care time for VSG when compared with LAGB (Varela 2011). For these reasons, despite its good initial results, further studies must be done into the long term effects of VSG before we can conclude that it is suitable as a single step procedure for morbid obesity (Frezza 2007; Puzziferri et al. 2014).

1.6 Hormonal Control of Food Intake Pre-Surgery and Post-Surgery

1.6.1 Managing obesity with bariatric surgery

In order to fully understand the mechanisms by which surgical approaches improve the levels of weight loss, we must investigate the hormonal responses of the body when put under these conditions. As stated previously, the Roux En Y Gastric Bypass (RYGB) causes changes in hormones that mediate homeostatic shifts in food intake. This is a significant difference compared with LAGB which appears, from human studies, to have little effect on the levels of GI hormones.

The major humoral mechanisms thought to contribute to the control of body weight are leptin and insulin levels. The leptin levels of LAGB individuals are commonly much higher than those with RYGB, with a study by Korner (2006) showing that RYGB individuals had similar levels of leptin to lean individuals. However, this could be resulting from the immediate fat loss following RYGB operations, in comparison to LAGB individuals who have a slower decline in fat loss. Additionally, the removal of large sections of the stomach that have been demonstrated to produce leptin may explain why LAGB patients have greater quantities than RYGP, and may explain the bands effectiveness (Bado et al. 1998).

Insulin levels have also been shown to decrease following both RYGB and LAGB, although greater loss was found in RYGB studies. When this is corrected with a homeostatic model of detection analytical test (HOMA), there is a similar decrease in both bariatric operations when the sensitivity of insulin, and weight lost is included in this calculation (Wing et al. 1994).

Sensory signals from the GI tract are also important, and can be divided into humoral and neural effects. This can be seen in figure 3 above. CCK, an important mediator of satiety acts on the vagal afferent endings to exert its effects. In addition, the neural PYY and GLP-1 derived factor L cells enhance the insulin secretion, thus increasing the glucose tolerance. In addition to these effects, GLP-1 inhibits gastric emptying and possibly improves insulin sensitivity (Drucker 2007). The L-cells located in the ileum and colon are responsible for GLP-1 and PYY secretions which in turn may be important in the mediation of the anoretic effects of bariatric operations.

Korner et al. (2006) found that the hormonal differences between RYGB and LAGB patients after eating are significant. This included 2 to 4 fold increases in postprandial (after eating) concentrations of PYY in the RYGB groups, promoting earlier satiety and meal termination seen in RYGB subjects. There was no significant change in PYY in the LAGB group (Korner et al. 2007). These hormonal changes cannot account for the increased weight loss seen in RYGB individuals compared with the LAGB individuals, as the large increase in PYY levels 6 months after operation do not reflect greater weight loss (Galvani et al. 2006). There are inconsistencies in the literature examining the changes in GLP-1 and PYY, as humans studies have shown there are no clear changes after LAGB, however our lab has shown meal related changes in rats following AGB surgery (Kampe et al. 2012).

The sole orexigenic hormone released from the GI tract is ghrelin released from the stomach. The suppression of ghrelin has been shown to be blunted in LAGB patients to a greater extent than those treated with RYGB, meaning that there is less reception to terminate a meal under LAGB control (Korner 2007). Normal fasting ghrelin levels in RYGB individuals have been shown to decrease significantly post operation, with studies showing either absent or inconsistent fluctuations of ghrelin levels over normal meal periods (Cummings et al. 2002; Leonetti et al. 2003). Ghrelin levels have also been shown to fall after VSG possibly due to the removal of large portions of the stomach, however studies have shown that the effects of VSG are not elicited by changes in ghrelin levels (Chambers et al. 2013).

Date et al. (2002) demonstrated that ghrelin actions through the vagus nerve produce a potent orexigenic response. Ghrelin receptors located on vagal afferent neurons are bound with ghrelin synthesised from the stomach. This then activates neuropeptide Y (NPY) producing neurons located in the arcuate nucleus, an area of the brain primarily involved with the control of food suppression and the central regulation of feeding (Nakazato et al. 2001). Where CCK stimulates the firing of the vagal afferent nerve, ghrelin will act to suppress the firing, initiating hunger (Wren et al. 2000). Furthermore, it has been found that while ghrelin acts to stimulate the expression of NPY, it will also block the feeding restriction put in place via leptin (Stanley et al.).

There has been no significant understanding developed to explain the effectiveness of the humoral contribution of the LAGB in patient cohorts (Korner et al. 2009). As stated previously, data from a rodent model of the AGB has shown an elevation in the satiety hormones GLP-1 and PYY with the band inflated on the background of a standard caloric intake (Kampe et al. 2012). Several studies have noted that the activation seen in neuronal populations in the nucleus of the solitary tract (NTS) seen after band inflation are consistent with the release of GLP-1 and PYY or other gut hormones shown to cause activation of these neurons (Cummings and Overduin 2007b; Habegger et al. 2013; Kampe et al. 2009; Kampe et al. 2012). However, the activation of these neuronal populations are also consistent with the recruitment of mechanosensitive vagal fibres which terminate in this region, which raises the possibility that the effectiveness of the LAGB is as a result of both humoral and vagal regulation (Kampe et al. 2012).

\

1.7 Remission of Co-Morbidities following Gastrointestinal Surgery

1.7.1 Bariatric surgery and diabetes

The link between bariatric surgery and remission of obesogenic co-morbidities such as type 2 diabetes mellitus (T2DM) is undeniable. At birth, the natural lifetime risk of developing diabetes is one in three. When factoring in obesity, at age 18 the difference in the chance of developing diabetes with a normal healthy BMI of 18 to <25, as compared with an obese category of 30 to <35 has a chance difference of 40%, up from 20% to 60% (Narayan et al. 2007).

Meta-analysis of 22,094 bariatric surgeries verify that after RYGB, there are at least 84% of patients who experience complete remission of this disease and return to normoglycaemic levels without medication (Buchwald et al. 2004; Schauer 2003). This raises new possibilities for bariatric surgery as a surgical therapy for T2DM, even in those who are overweight with BMI less than 35 (Cohen 2006). Particularly important to this response is that the resolution of T2DM occurs days after surgery, before substantial weight loss has occurred (Pories 1995).

As a growing interest in treatment for T2DM, Roux en Y is being considered for those who are less obese, or non-obese. As a non-obese individual does not experience the excess weight loss of RYGB bariatric surgery, the remission is shown to be weight independent and is due to an increase in glycaemic control (Cohen 2006). However, RYGB surgery is a serious modification to the GI tract and may not be necessary in individuals who do not require its full body alteration. Recent FDA approval of gastric banding procedures for purely diabetic treatment for individuals with a BMI range of 30 to 40 has shown that the low risk adjustable gastric banding is a greater possibility for widespread diabetes treatment.

1.7.2 What is the hormonal interaction responsible for the effect on type 2 diabetes?

Current research suggests that an improvement in glycemic control occurs with calorie restriction and resulting weight loss, with an improvement in regulatory control of hepatic glucose production (Jackness et al. 2013). The improvement of glycaemic control has been shown to respond to the lower caloric intake, as metabolic control can deteriorate if caloric intake is increased after reduction of BMI (Kelley et al. 1993). This effect is seen in RYGB patients experiencing improved glycemic control within the first 2-3 weeks after the procedure and before most of the weight loss occurs, leading to the hypothesis that there are factors in addition to weight loss that are involved (Cummings 2009; Moo and Rubino 2008).

The aim of bariatric surgery is to provide an intestinal shortcut, increasing the delivery of food toward the lower bowel and reducing nutrient absorption. This results in improved glucose homeostasis due to an increased secretion of incretins GLP-1 and gastric inhibitory peptide (GIP) (Drucker 2007; Laferrère et al. 2008). Several studies have shown an increase in the meal-stimulated incretin levels that are naturally blunted after T2DM will increase after bariatric surgery (Jackness et al. 2013). In parallel with the increased levels of GLP-1 and GIP, the effect of incretins on insulin secretion that is normally impaired in patients with T2DM is markedly increased to levels similar to that of matched controls without T2DM, 1 month after RYGB (Laferrère et al. 2008).

This mechanism of GLP-1 secretion transmitted through a neural pathway is kept intact in LAGB operations, and is disrupted in RYGB surgery. The disruption via nutrient diversion away from the duodenum in RYGB, leads to a theoretical inactivation of L cells. This inactivation would be expected to promote lower GLP-1 levels, however this does not occur and meal-stimulated GLP-1 and PYY secretion are increased following RYGB surgery (Laferrere 2007). In contrast, AGB keeps this neural pathway intact and achieves similar results of diabetic remission, allowing for the preservation of original regulatory mechanisms unlike the more aggressive RYGB surgery (Korner 2007).

Therefore, the possibility that there are differing mechanisms by which T2DM is reduced following RYGB and AGB operations could be conducive to new research. The understanding of the mechanisms in place to regulate glucose and insulin through homeostatic gut activity is therefore important in the development of RYGB and AGB research models.

In conclusion, the hormonal changes of RYGB individuals are greater than those from LAGB individuals, with increased changes relating to energy homeostasis. These modifications result in greater weight loss though gastric bypass than banding operations. Long term changes in hormone levels from the perspective of banding alone shows a steady decrease in insulin levels, as well as leptin levels, promoting greater glucose homeostasis and weight loss (Hanusch-Enserer et al. 2004). The effects of weight loss in plasma ghrelin concentrations after LAGB procedures show little change (Hanusch-Enserer et al. 2004; Leonetti et al. 2003). The fasting insulin concentrations correlate negatively with ghrelin concentrations, however the sensitivity of the insulin is unrelated. This is supported as previous studies have concluded that rising ghrelin levels lead to a reduction in insulin levels (Tassone et al. 2003). While these studies have differing results on the levels of ghrelin after gastric banding, they do show that the actions of ghrelin have different mechanisms of generating weight loss.

1.8 How can the mechanisms underlying LAGB be better defined - Animal Models of Gastric Banding Surgery?

Animal models used for human science must be investigated so that they will correctly interpret human responses. A number of models of the gastric band have been employed with varying success (Belachew et al. 2004; Miranda et al 2009; Monteiro et al. 2006). Without exception these have failed to incorporate the major feature of the human band, which is its adjustability. In addition they have universally ignored e.g. (Monteiro et al. 2006) one of the major differences between the human and rodent stomach, that is, the amotile fore stomach that exists in the latter. This is an expandable epithelium reservoir, which forms the fore stomach of the rat. The lower section of the rat stomach is a glandular stomach similar to a human. The removal of the fore stomach shown in Figure 8, leaving only the glandular stomach of the rat has been shown to produce a rodent model capable of producing results by effectively replicating the human LAGB through both weight loss and metabolic parameters (Kampe et al. 2009).

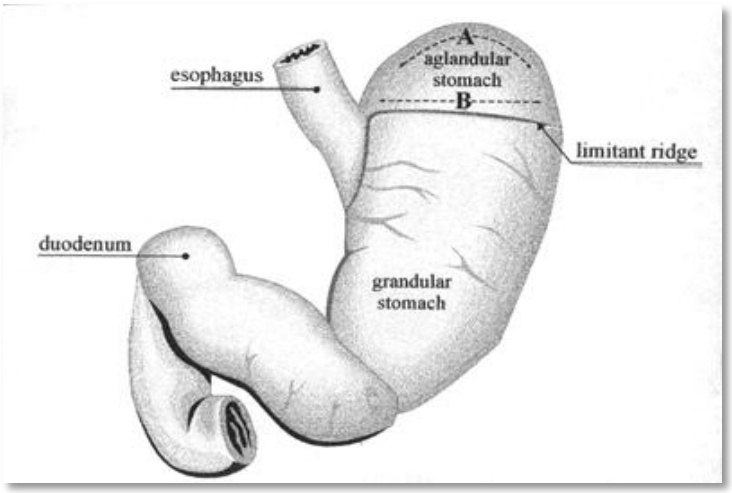


Figure 7: The rat stomach showing the epithelial (aglandular) fore stomach, and the human-like glandular section. Taken from: http://www.scielo.cl/fbpe/img/rca/v20n1/Fig21.gif

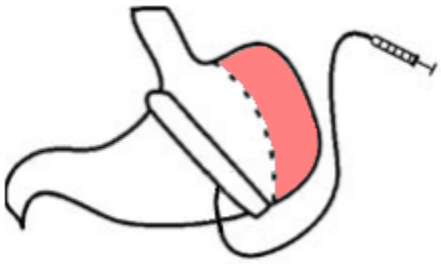


Figure 8: Procedure showing the fore stomach removal and location of the adjustable gastric band in relation to the fore stomach (Source: Kampe et al. 2009).

1.9 The Adjustable Gastric Band and Neural Activation: A weight loss study

Bariatric operations interact with the regulation of the autonomic nervous systems including the brain-gut axis, of which the vagus nerve is an important regulator. There is developed support for the role of vagal afferents in food intake regulation, and it is clear that an increase in vagal stimulation seen after food intake is due to hormonal interactions, as well as stimulation due to gastric distension (Schwartz, 1993). Therefore, we need to understand the relative contribution of these two components, recognising that both stretch and humoral stimuli may be mediated by neural pathways. This is the rationale underlying the purpose of the present study.

The primary aim of this project is to define the peripheral and central mechanisms involved in AGB surgery, with a view to understand the nature of their interaction which controls satiety. This is integral if there are to be therapeutic approaches to weight loss based on these principles of neural and humoral interactions of the AGB.

Chapter 2
Materials and Methods

2.1 Animals

Section one of this study consisted of 71 male Sprague-Dawley rats (approximately 12 weeks of age, initial weight 200-220g). Section two will consist of 64 lean, and 63 obese (approximately 14 weeks of age, final weight 550-750g) male Sprague-Dawley Rats were obtained from either the Animal Resource Centre (Canning Vale, W.A., Australia) or Monash Animal Services (Monash University, Melbourne, Australia). Rats were initially group housed, and later individually housed in plastic 'Wiretainer' cages in a temperature (22±1°C) and humidity (50±10%) controlled environment on a 12/12 hour light/dark cycle (lights on 0600h). Rats had *ad libitum* access to either standard laboratory chow (9% kcal from lipids) or high fat diet (45% kcal from lipids, SF04-001, Specialty Feeds, Perth, WA) and water, unless specified. All experimental procedures were approved by the Monash University, Monash Animal Research Platform (MARP)(Ethics No. 2010/101) and performed in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 7th Edition 2004*.

2.2 Drug Preparation

Capsaicin (>98% grade; Sigma, St. Louis, MO) was mixed in a sterile vehicle consisting of Tween 80% (10%), ethanol (10%), and 0.9% NaCl (80%). Capsaicin was prepared as three intraperitoneal injections of 25, 50 and 50mg/kg over 48 hours. It does not readily dissolve in the vehicle solution, and must be sonicated prior to being injected.

Atropine Sulfate was injected as a standard 0.2mL per rat of dosage 15mg/ml (90% grade; Sigma, St. Louis, MO) and sulfated cholecystokinin octapeptide intraperitoneal injection of dosage 6µg/kg (CCK-8, Peptides International. Louisville, KY) were dissolved in sterile 0.9% NaCl.

2.3 Elimination of Sensory Unmyelinated C-Fibres using Capsaicin

2.3.1 Procedure for injection of capsaicin

2.3.1.1 Preparation for injection

Rats were divided into vehicle (n=14) and capsaicin (n=9) treatment groups. Rats were treated consecutively with increasing doses of capsaicin or vehicle across a three day period. On each of the three days, rats were anaesthetized using 5% isoflurane (Isorrane, Baxter Healthcare, NSW, Australia) in oxygen in an induction chamber, and then maintained on 3.5% isoflurane via a nose cone. Upon loss of both pedal and corneal reflexes, rats were administered an intraperitoneal (IP) injection of Metacam (0.2mg/kg, Boehringer Ingelheim®, Australia) and IP Atropine Sulfate (15mg/ml). Ten minutes later, rats were injected (i.p.) with either vehicle as a control, or a dose of capsaicin (25, 50 and 50mg/kg, 98% grade, Sigma).

2.3.1.2 Ventilatory support during capsaicin treatment

Once the capsaicin treatment was administered, most of the rats altered their breathing rate and volume. Many of the rats exhibit respiratory arrest within 10 minutes of the initial capsaicin treatment. Assistance by manually palpating the chest or positive pressure ventilation (3mL at 85 strokes per minute) induced the resumption of spontaneous breathing. Artificial ventilation was achieved using a Ugo Basile Rat Ventilator (model number: 7025) as shown in Figure 9 below. Positive pressure ventilation was rarely necessary (<10%) with the subsequent higher doses of capsaicin.

Rats were maintained under anaesthesia for 15 minutes following the resumption of spontaneous breathing, and then allowed to recover on a heat pad.

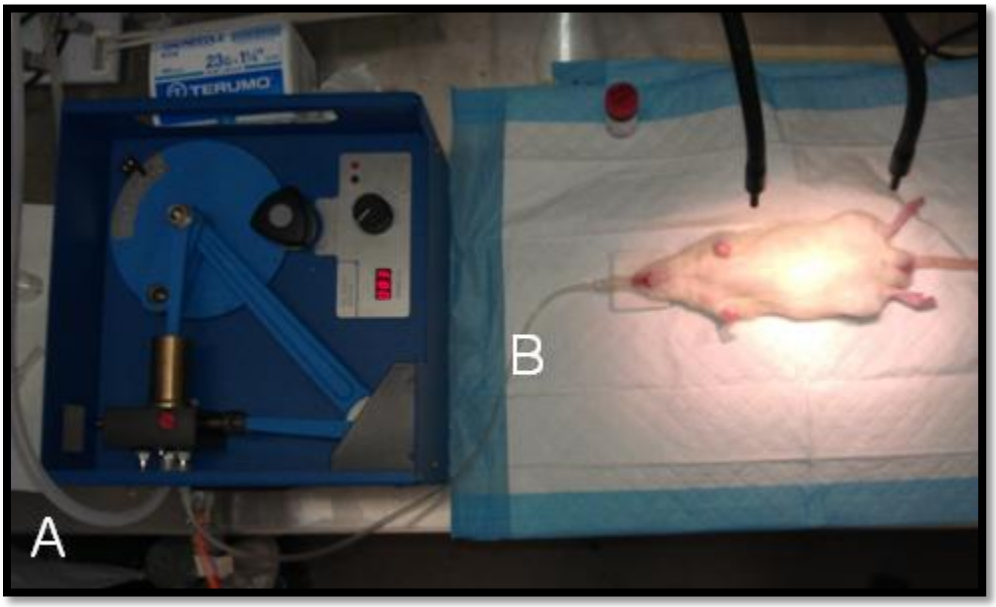


Figure 9: The ventilatory support apparatus, showing anaesthetic being delivered into the ventilator at 'A', and being delivered pressurised into the rodents lungs via nostrils at 'B'

2.3.2 Effectiveness testing of capsaicin treatment using cholecystokinin

Two weeks following the last capsaicin injection, a bio-assay was performed by challenging animals for the known food intake suppressive effects of exogenous CCK. CCK elicits its anorexigenic effects by directly acting on sensory (capsaicin sensitive) vagal afferents in the stomach, and can therefore be used as a direct measure of sensory vagal ablation. Rats had food removed one hour prior to lights out (1700h). The following day, between 0900 and 1000h, rats received an IP injection (1mL/kg) of either CCK-8 (6µg/kg) or saline. Following the IP injection, rats were given immediate access to a pre-weighed amount of food, and food intake and spillage were measured over the ensuing 30 minutes. Rats were then allowed ad libitum access to both food and water for at least the next 48h, before another overnight fast was imposed.

2.3.3 Whole mount dissection of capsaicin treated stomach

The secondary confirmation of sensory afferent ablation in the muscle wall of the stomach was achieved using a whole mount sectioning method. Histological validation of the lesion of the sensory endings of vagal afferents directed to the stomach was derived from immunocytochemical labeling of calretinin-positive intraganglionic laminar endings (IGLEs) in whole mount preparations of capsaicin and vehicle treated animals.

2.3.3.1 Preparation

In order to histologically validate the extent of the capsaicin induced vagal ablation, a whole mount stomach section visualised ablation. Rats were given an overdose of Lethobarb (Virbac, NSW, Australia), the stomach was excised and opened along its greater curvature, which formed a butterfly-like shape (shown below in Figure 9). The opened stomach was rinsed thoroughly with PBS (PBS; 0.9% NaCl in 0.01 M sodium phosphate buffer (PBS), pH 7.2), and pinned mucosal side down onto balsa wood.

The stomach tissue and balsa wood was completely immersed in a fixative solution containing acetic acid (1%), PFA (2%), and Picric acid (0.2%) in PBS overnight. The following day, the tissue was cleaned with an organic solvent solution containing dimethyl sulfoxide (50%) in PBS for ten minutes, repeated three times. The fixed stomach tissue was then stored in PBS containing 0.1% sodium azide until the whole mount dissection was performed.

2.3.3.2 Whole mount dissection

Sampling of the opened stomach was achieved by removing a square centimetre portion of the fixed stomach tissue (see figure 9) The tissue specimen was selected from the central glandular section away from the sling muscle (Figure 11), and care was taken to select a region which represents where the gastric band is normally fitted. The sample was then transferred to a rubber dish containing PBS (0.1% sodium azide) and, serosa side down, the mucosa was first carefully removed using fine forceps and fine spring scissors.

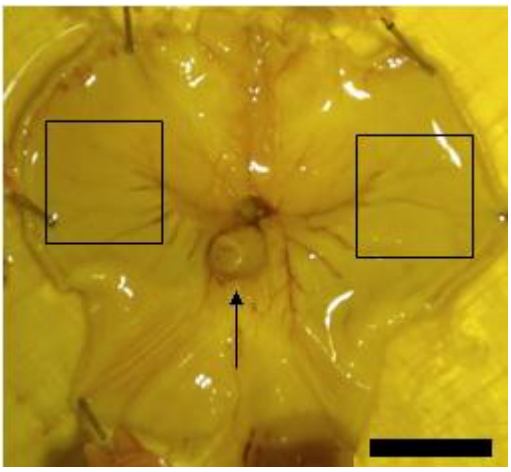


Figure 9: The butterflied stomach immersed in fixative solution. Shown is the esophagus (centre), with vascular supply spreading outward. The esophagus is indicated by an arrow, and tissue samples were taken from the area of either square. Scale bar: 1cm.

Care was taken to remove the mucosa and all underlying connective tissue. The underlying circular muscle layer was gently removed by peeling each strip away from the underlying longitudinal muscle layer, as shown in Figure 12. In order to avoid tearing the muscle layers, it was necessary for the direction of the peeling to be parallel to the circular muscle fibres and that the angle between the direction of pull and the horizontally placed piece of tissue is as small as possible. The myenteric plexus and longitudinal muscle is the final layer of tissue, which incorporates vagal sensory endings.

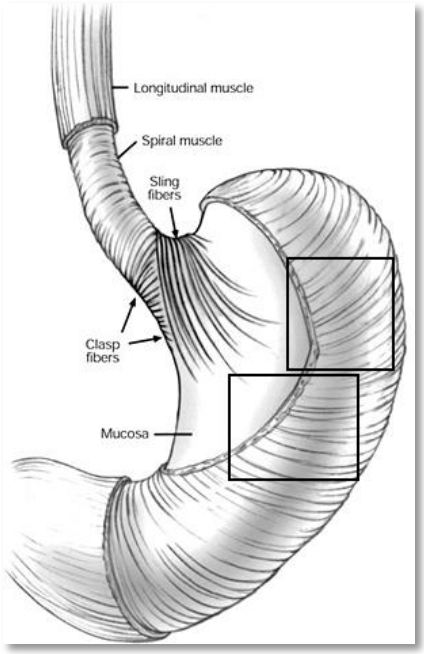


Figure 11: The longitudinal muscle layer of the stomach has been cut away to show the opposing sling and clasp muscle fibres. Each square denotes a possible location of sampled tissue being dissected.

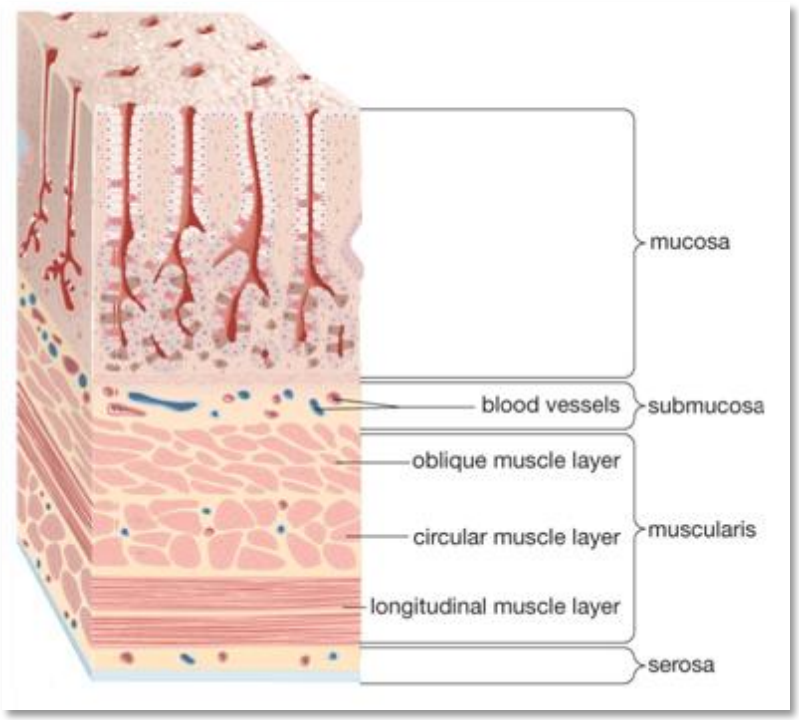


Figure 12: Cross-section of the stomach wall of the rodent. Visible are the mucosa, submucosa, and muscularis layers that are removed.

2.3.3.3 Immunohistochemical protocol for fluorescent calretinin

The free floating whole mount sections were washed twice for 15 minutes in PB (0.1M phosphate buffer (PB), pH 7.0). The sections were then incubated in 0.3% Triton X-100 and 10% normal horse serum (NHS) in PB for 30 minutes. In order to localise calretinin, sections were incubated in primary antisera diluted in 0.1M PB containing 0.3% Triton X-100 and 1% NHS raised against calretinin (1:2000, rabbit, Swant, Switzerland) overnight at room temperature.

The following day, sections were washed twice for 15 minutes in 0.1M PB with 1% NHS before a 90 minute incubation in a donkey anti-rabbit secondary antibody conjugated to Alexa 594 (1:400) (Invitrogen, USA) diluted in 0.1M PB. Sections were then washed twice in 0.1M PB for 10 minutes and later mounted onto microscope slides with fluorescent mounting medium (DAKO, VWR International, USA).

2.4 Neural Activation Following Banding of Denervated Rodents

In order to determine the impact of capsaicin treatment (sensory vagal ablation) on changes in neural activation (measured by expression of Fos protein) in the brain following inflation of the AGB, a separate group of rats were treated with capsaicin or vehicle, prior to undergoing gastric band surgery. The treatment paradigm for these animals was the same as those described above (see section 2.3.1), however following the assessment of the effectiveness of the capsaicin treatment (2.3.2) using CCK, animals were fitted with bands or sham operated (see below 2.4.3).

2.4.1 Treatment groups

A total of 12 rats were included in this series of experiments. This experiment involved pretreating animals with either vehicle (n=8) or capsaicin (n=4). All capsaicin treated rats were fitted with AGBs, however vehicle treated rats were divided into two groups, either fitted with AGBs (n=4) or sham operated (n=4).

2.4.2 Preparation for surgery

Following an overnight fast, rats were anaesthetized using 5% isoflurane in oxygen in an induction chamber. Once the pedal and corneal reflexes had been lost, the anaesthesia was decreased to 2%, maintained throughout the procedure. Rats were shaved at the abdominal region, and a midline incision was made. The stomach was gently separated from the surrounding connective tissue and externalised using blunt forceps.

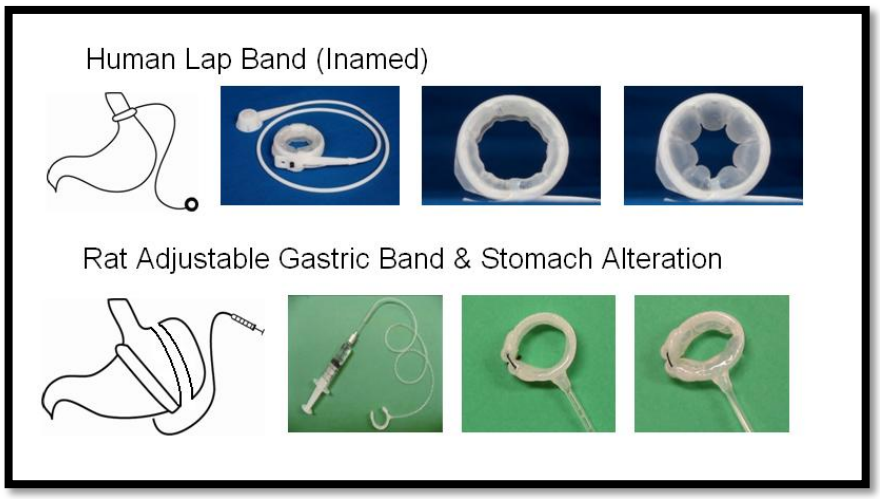


Figure 13: Schematic representation of the human LAGB and rodent AGB, showing placement at the gastroesophageal junction. These images show how the increasing volume of saline in the band reduces the lumen size.

2.4.3 Surgical procedure: fitting the adjustable gastric band

Initially, the forestomach was resected by carefully cutting along the gastro-esophageal junction, indicated by the dividing ridge, shown in Figure 13. This was then sutured closed with six interrupted sutures using absorbable suture (4/0 taper polydioxanone (PDS), Dynek, SA, Australia). The removal of the forestomach is necessary to circumvent the accumulation of food and obstruction in the relatively non-motile squamous portion of the stomach that may arise subsequent to band inflation. Subsequent to removing the forestomach, the adjustable band (vascular occluder, OC16, In Vivo Tech, USA) was placed around the glandular stomach, below the insertion of the oesophagus. This is located immediately distal to the gastro-oesophageal junction. Additional superficial sutures (4/0 taper silk, Dynek, SA, Australia) were placed at three independent sites on the upper glandular stomach to secure and prevent the band from slipping. Running stitches were used to close the muscular and skin incisions. The band tubing was externalized between the scapulae, and secured through an accessible port that allowed inflation in the conscious animal. Sham operated rats underwent the same procedure, however, instead of fitting the band, rats had the same sutures placed but no band was fitted.

2.4.4 Band inflation

On the day of the experiment, food was removed from all rats at the beginning of the light phase. Four hours later, bands were inflated, while sham operated rats were handled in the same way for the same amount of time.

2.4.5 Perfusion and tissue collection

Ninety minutes after handling and band inflation, rats were deeply anaesthetized with Lethobarb (Virbac, Australia) and perfused transcardially with PBS followed by 4% paraformaldehyde in 0.1M PB. Brains were removed and stored overnight in the same fixative.

The following day, the brains were transferred to a solution containing 30% sucrose in PB for 48hrs. Coronal sections (35µm) were then cut on a cryostat (Leica, Australia). The sections were stored in cryoprotectant (50% v/v PB, 20% v/v glycerol, 30% v/v ethylene glycol) in well plates at -20 degrees for later processing.

2.4.6 Immunohistochemistry

Several sections through the hypothalamus and brainstem from each rat were then exposed to an immunohistochemical procedure to visualise Fos protein expression. The free-floating sections were washed three times for ten minutes in 0.1M PBS before they were incubated in 0.3% Triton X-100 and 10% Normal Horse Serum (NHS) in PB for 30 minutes. The sections were then incubated overnight with an anti-fos protein antibody raised in rabbit (1:8000) (Santa Cruz Biotechnology, USA) diluted in 0.1M PB containing 0.3% Triton X-100 and 1% NHS.

The following day, the sections were washed three times for ten minutes in 0.1M PB with 1% NHS before a 90 minute incubation in a biotinylated swine anti-rabbit antibody (1:200) (Vector Laboratories, USA) diluted in 0.1M PB. Three 10-minute washes in PB were followed by a 45-minute incubation in ABC-streptavidin-horseradish peroxidise (1:200) (Vector Elite Kit, Vector Laboratories) diluted in 0.1M PB. Sections were again washed twice in 0.1M PB and then incubated for 10 minutes in 0.01% Diaminobenzidine (DAB) and 0.02% nickel and 0.02% cobalt chloride in 0.1M PB.

Hydrogen Peroxide (0.005%) was added and sections incubated for a further 6 minutes. Sections were then transferred to 0.1M PB before being mounted onto gelatine coated slides. The slides were allowed to dry overnight, and subsequently dehydrated through a series of ranked ethanol dilutions (30%, 50%, 70%, 100%, and Xylene) before DPX mountant (VWR International, USA) and a coverslip was placed over the sections.

2.4.7 Visualisations and quantification of Fos positive nuclei

Transcription of Fos-protein, encoded by the immediate early gene c-fos, occurs immediately after neural activation. Thus, Fos-positive nuclei were visualised using a Zeiss Light Microscope (Carl Zeiss MicroImaging GmBH, Germany) as a marker of neural activation. The extent of labelling of Fos-positive neurons was quantified in specific brain regions in the brainstem. Counts of labelled neurons through the rostro-caudal extent of the nucleus of the solitary tract and distinctions were made between medial and lateral divisions of the parabrachial nucleus were made by an observer blind to the treatment of the groups according to the boundaries defined in Paxinos and Watson (Paxinos and Watson 2006). For the nucleus of the solitary tract (NTS), distinctions were made between rostral (−12.00 to −13.00mm from bregma), mid (−13.00 to −14.00 from bregma) and caudal (−14.00 to −14.60mm from bregma) regions. The PBN bregma levels were outlined to be between -9.16mm to -9.85mm, with the LHA designated as the region between -2.3mm to -3.3mm caudal to bregma.

2.4.8 Analysis

A one way ANOVA was used to assess Fos-positive nuclei of each treatment group, these included the capsaicin and band treated, and vehicle band and non-banded. This was done at all four independent brainstem levels and overall in the brainstem. Results are expressed as means ± SEM, and P values <0.05 were considered significant.

2.5 Establishing a Method for the Topical Application of Capsaicin onto the Stomach

Topically applied capsaicin will ablate vagal sensory afferent ending located in the stomach wall. This will allow for the study of all neuronal feedback in the rodent, without the nerve feedback of the stomach wall. This will narrow the possible responses to band application made only by other organs in the previous global application method. Additionally, it will provide support for the concept of intraganglionic laminar endings controlling food intake.

2.5.1 Application of capsaicin

The topical application of capsaicin involves the isolation of the rat stomach, followed by the placement of capsaicin soaked gauze matting for the ablation of exterior IGLE, and vagus nerve afferents. The rodent is prepared in the same way as the banding procedure (2.4.2), with the stomach externalised. Parafilm is used to cup the stomach, separating the stomach with the open incision, with gauze matting behind the parafilm to stop capsaicin solution from entering the body of the rodent.

The topical application of capsaicin onto the stomach wall is achieved using gauze matting. The gauze is wrapped carefully around the stomach in individual layers, with a set amount of capsaicin to be applied to each gauze layer. This enables the stomach to be evenly and continuously bathed in capsaicin solution for the set period of time.

The capsaicin is made up in a 5% solution, with each rat receiving a total of 1mg of capsaicin over a 30 minute application. The stomach is then rinsed and gently placed back into the intraperitoneal cavity.

Ventilatory support is unnecessary due to ablation being confined to the stomach wall. The rodents had no side effects of fibre ablation in the lung system, and as a result had a better prognosis after treatment.

2.5.2 Post-operative testing

Post operative testing will take place two weeks after the capsaicin operation has been completed. A secondary examination into the IGLE ablation will occur posthumously, with analysis of the stomach wall under microscope.

2.5.2.1 Food intake testing using cholecystokinin

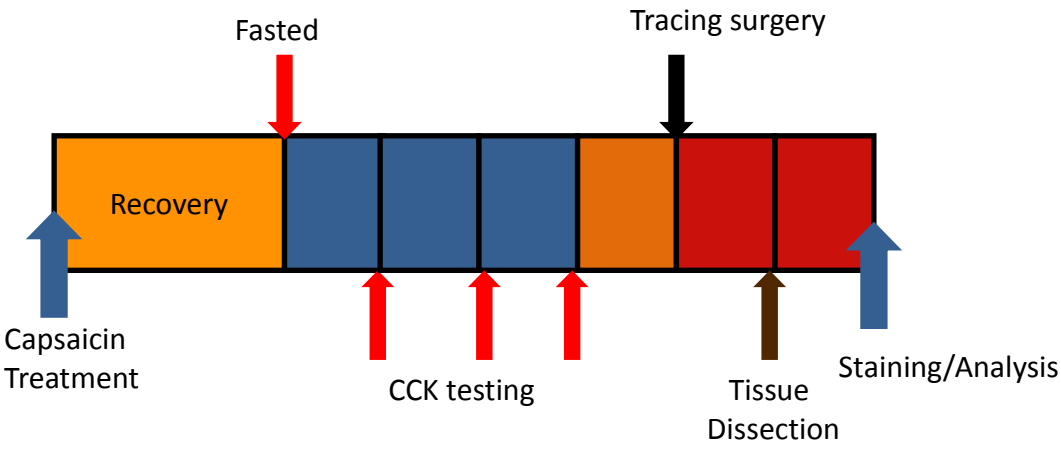
Two weeks following the capsaicin treatment, a bio-assay is performed by challenging animals for the known food intake suppressive effects of exogenous CCK. This test is identical to the CCK test previously performed in 2.3.2. CCK mediates its effects on satiety via the CCK receptor 1 (CCK1R), which is expressed on vagal sensory afferents that innervate the gastrointestinal tract. Peripheral CCK administration will increase vagal afferent firing, as well as neuronal activity in the hindbrain. Following an overnight fast, animals treated with CCK should reduce their 30min food intake.

The effect of CCK on food intake suppression should be diminished following topical application with capsaicin. Due to the effects of topical application, rodents will have intact CCK receptors throughout their GI system and the extended vagus nerve. This test will therefore show the responses to CCK in the rodent without the use of capsaicin-sensitive extrinsic afferent nerve fibres.

2.5.2.2 Calretinin labelling of IGLEs

The secondary confirmation of sensory afferent ablation in the muscle wall of the stomach is achieved using immunocytochemistry. Whole mount stomach sections will be immunolabelled for calretinin, a protein used to mark for vagal sensory endings, as in 2.3.3.3. The IGLEs are located along the longitudinal muscle layer within the myenteric plexus, and analysed using confocal laser scanning light microscopic observations at a high magnification.

2.5.3 Procedural timeline



2.6 Assessing the Role of Vagal Sensory Afferents in AGB Induced Changes in Neural Activity

The induced changes in neural activity following gastric banding has been examined and proved previously. This experiment aims to assess the role of vagal sensory afferents on the stomach wall in mediating these changes shown in neural activity, following acute AGB inflation.

The topical application of capsaicin will allow the study to develop an understanding of the feeding response from AGB, without the sensory feedback of the stomach alone. This inhibition of sensory endings will further demonstrate that the effects of AGB are due to the neurohumoural link from the stomach to the key regulatory hypothalamic areas of the brain. This neural response will examine the application of a band in an acute setting, showing the short term impact of banding on key food regulation areas in fore, mid and hindbrain.

2.6.1 Surgical procedure:

In order to assess the extent of AGB induced changes mediated by the vagus nerve, a group of rats (n=40) will have their vagal sensory afferents ablated using capsaicin (applied topically on the stomach). In addition, these animals will be fitted with an AGB or sham operated. They are recovered over a period of at least seven days. Following this they are lightly fasted overnight to ensure there is no excess food in the stomach, then the next morning they have their bands inflated or sham handled. Rats are killed 90 min following band inflation to test for the presence of elevated expression of Fos protein. The number of Fos positive neurons will be quantified in the brainstem and hypothalamic nuclei.

2.6.1.1 Application of capsaicin

While the stomach is exteriorised, it is bathed in capsaicin as per previous outline 2.5.1.

2.6.1.2 Band application

The stomach is modified, and the band is then applied as outlined previously in section 2.4.3.

2.6.2 Post surgical testing

Testing of both band action and capsaicin ablation are performed after a recovery period finalised by the resumption of normal eating volumes. This is usually two weeks after ablation and banding, where a bioassay of CCK is given testing satiety function. Following this, DEXA scans are performed both before and after the procedures, as described in section 2.3.2.

DEXA scans of the rodents are necessary for proper investigation into the rodent fat masses, if the rodent is still healthy and whether or not the rodent is maintaining its body weight and fat proportions. This ensures that the rodent is still healthy, and there are no extreme modifications being done to the rodent as a result of ill health or surgical modification.

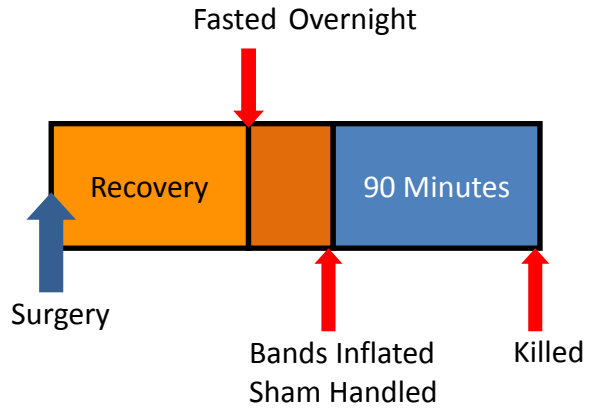
2.6.3 Perfusion

Following their light fast, the bands are inflated or rats are sham handled and they are left alone for 90mins. They are then perfused and fixated as per methods section section 2.4.4 and 2.4.5.

2.6.4 Nickel DAB staining

The brain sections were put through an immunohistochemical process, as previously described in 2.4.6. This process is slightly different, in that a Nickel DAB method was used. This modification to the protocol occurs in the final stages of the stain, where 10mg DAB is combined with 50ml Nickel Sulphate solution. The reaction with DAB is enhanced by using nickel, producing a deep purple/black staining.

2.6.5 Procedural Timeline



2.7 To Determine the Efficacy of the Adjustable Gastric Band with Vagal Afferent De-nervation

Thirty-two male Sprague-dawley rats were put on a high fat diet (SF04-001, 60% kcal from lipids) for 12 weeks. They were then DEXA scanned to determine the increased percentage of body fat mass, and divided into two groups (n=16) for capsaicin treatment. This project methodically works toward investigating the long term changes in body weight influenced by neural effects of the AGB. This is achieved through the use of a high fat AGB model with, and without vagal innervation.

Additional animals will be treated with capsaicin, and subsequent to the confirmation of effectiveness of vagal ablation in a high fat animal, bands are fitted, as described in section 2.4.3. Following band surgery body weight, food intake and body composition will be assessed over the proceeding 6 weeks.

This concluding section will be in progress beyond 2011, and will more closely monitor the changes in weight, and food intake over a long (or 'chronic') period of gastric band usage. These rats include capsaicin treatment, and will push forward with a look into afferent feedback systems developed in high fat rats.

This aim of the final project involves the use of rodents in a more typical clinical setting. This is a more comparative approach, which mirrors the gastric band in its natural human setting. By progressing into this final method, we can provide greater theory on the ability of the band during weight loss, and the neural actions with the band as this occurs.

2.7.1 Surgical procedure:

This is a two part procedure with application of capsaicin first, then after 2 week recovery, band application second.

2.7.1.1 Application of capsaicin

While the stomach is exteriorised, it is bathed in capsaicin as per previous outline 2.5.1.

2.7.1.2 Band application

Special consideration must be taken into account of the capsaicin treated stomach. Exteriorisation of the stomach for 30 minutes, as well as capsaicin ablation causes the stomach to be more sensitive than usual. The stomach needs to be properly cleaned, before modification and removal of the forestomach, so as to ensure internal areas of the stomach are not affected. Additionally, an expansion or inflammation of the stomach due to the toxicity of the capsaicin in noted, and must be taken into account when applying stitches for the positioning of the band.

2.7.2 Post surgical testing

For the rodents recovering from both capsaicin and banding procedures, the recovery can be a few days longer than other treatment groups. This is important to take into account considering the confirmation of ablation tests occur through food intake testing, which can be inconsistent due to the varied levels of recovery.

2.7.2.1 Food intake tests are performed

This is achieved using a bioassay as an assessment of CCK mediated reduction in food intake. The food intake testing occurs two weeks after surgery. Topical ablation of the stomach wall only ablates the vagal afferents located directly on the stomach wall, and leaves intact the other areas of the system throughout the GI tract. This can make the CCK testing variable, due to the interaction with CCK receptors higher up on the system.

2.7.2.2 DEXA scans are performed

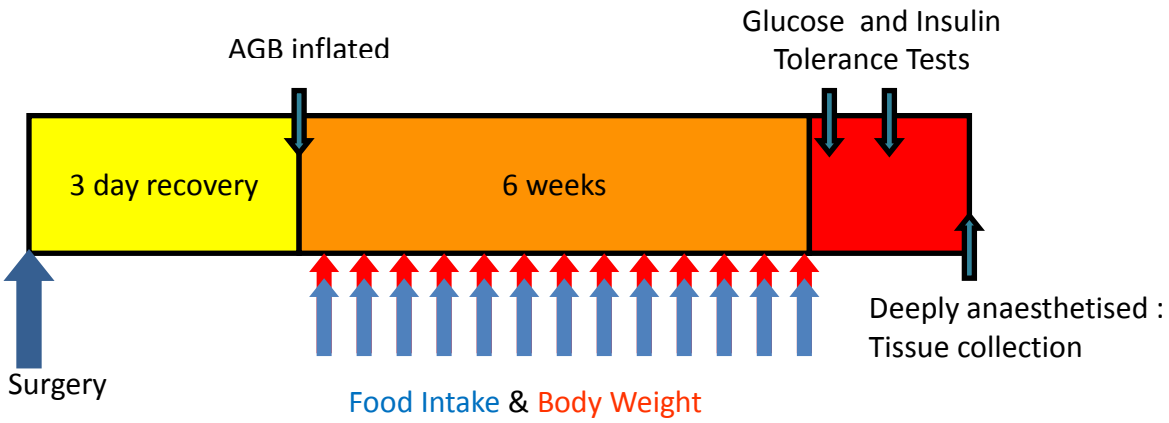
DEXA scans of the rodents are necessary for the proper investigation into rodent fat masses. This ensures that the rodent is still healthy, and there are no extreme modifications being done to the rodent as a result of ill health or surgical modification.

2.7.2.3 GTT/ITT Testing

Glucose tolerance tests are a good measure for the body to overcome glucose loading. This is important for rodents who have been banded and/or ablated to measure if they are able to return to glucose homeostasis. The kidneys are innervated with c-type vagal afferents, so they may be affected by the capsaicin given. This is not a problem with topical application of capsaicin.

Insulin tolerance tests the ability of the rodent to control their blood sugar levels after a drop due to insulin injections. The ability to mobilise glycogen and glucose through the liver is an important regulatory system, and is affected by the capsaicin delivery during global injections, and not through topical application.

2.7.3 Procedural Timeline



Chapter 3
Results

3.1 Evaluation of Sensory Unmyelinated C-Fibres in the Rat by Capsaicin Ablation

This experiment was developed in order to achieve an understanding of the effects of capsaicin on vagal fibres, as well as any functional changes to the rat. Intraperitoneally injected capsaicin causes a systemic ablation of unmyelinated vagal afferents located throughout the body. The level of ablation can be tested using either or both a cholecystokinin bioassay on the rat and post-mortem immunohistochemical techniques on the stomach. A CCK bioassay tests for non-responsive c fibre afferents, confirming their ablation due to the elimination of CCK mediated reduction in food intake.

3.1.1 Evaluation of the extent of capsaicin induced lesions on the vagus nerve

Cholecystokinin is a potent anorexigenic peptide hormone, and when given intraperitoneally (6µg/mL/kg) to lean intact rats (n=4) results in a mean reduction in food intake of approximately 75% (Figure 14.1). CCK induced satiety primarily occurs through the selective agonist located on abdominal vagal afferents which inhibits food intake, as well as vagal fibres originating from the stomach mucosa.

3.1.2 Optimisation of capsaicin treatment

Treatment with capsaicin to eliminate vagal unmyelinated C fibres is well established in previous studies (Berthoud et al. 1997; Blackshaw et al. 2000; Holzer 1998; Martling et al. 1984; McCann et al. 1988; Ritter and Dinh 1988). However, this technique gave sporadic results in terms of quality of recovery and mortality and was modified in the present study to maximise survival rates and improve efficacy, by way of modification to both capsaicin dosages and the introduction of positive pressure ventilation (see methods section 2.3.1.2).

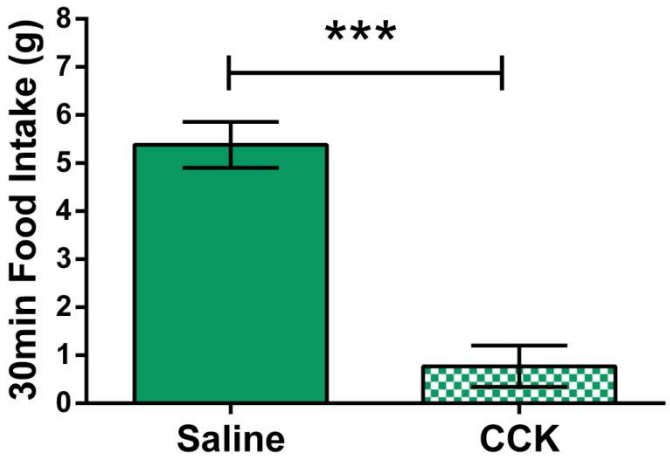


Figure 14.1: Food intake after intraperitoneal injection of Cholecystokinin (6µg/mL/kg) and Saline (0.09% NaOH). Initial testing of mean food consumption of lean rats (n=4) in the 30 minutes immediately after injection with cholecystokinin (6µg/mL/kg). These results are expressed as mean ± SEM. ***P < 0.001, denotes a significant difference in the food intake between saline and cholecystokinin treated rats. Abbreviations: CCK, Cholecystokinin.

3.2 Cholecystokinin Food Intake Testing following Capsaicin Treatment

Food intake using a bioassay of cholecystokinin was used to assess the elimination of unmyelinated c fibre afferents from the vagus nerve. Treatment of rats with capsaicin (125 mg/mL/kg over 3 days) nullifies the effects of CCK which relies on intact vagal sensory fibres to elicit short term anorexic effects, this results in an unchanged food intake regardless of saline or CCK intraperitoneal injection. Rats treated with vehicle solution demonstrate a marked reduction in food intake of 74.6% after CCK injection as compared with saline administration as shown in Figure 14.2. This demonstrates clearly that the rats treated with vehicle injections have an intact vagus nerve feedback system, whilst those treated with capsaicin are unable to respond to the CCK bioassay stimuli due to inactive vagal sensory fibres.

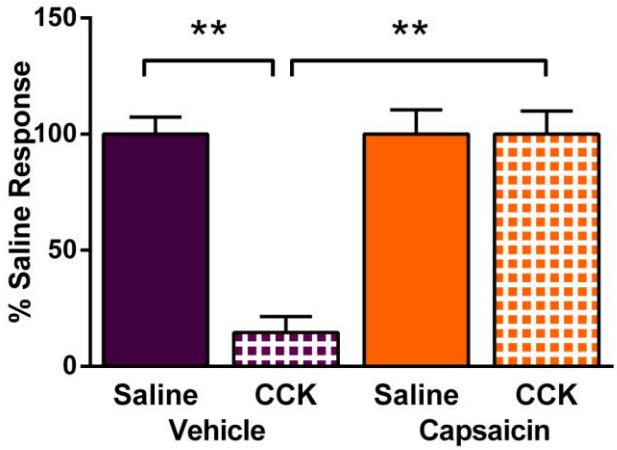


Figure 14.2: The food intake of lean rats (n=13) 30 minutes immediately after injection with cholecystokinin (6µg/mL/kg), following vehicle (n=8) or capsaicin treatment (n=5), shown as a percentage of saline intake. These results are expressed as mean ± SEM. *P<0.05, denotes statistical difference of vehicle treated saline and CCK rats, and Capsaicin treated CCK compared with vehicle treated CCK. Abbreviation: CCK, Cholecystokinin.

3.3 Determining the Efficacy of Capsaicin Ablation on Obese Rats

Obese rats are incorporated in a larger experimental examination into the body weight and homeostatic modifications that adjustable gastric banding may produce in the rat over a long-term period.

This study must confirm the efficacy of the CCK bioassay after vagal sensory ablation in obese rats, especially given the isolated reports of reduced CCK induced anorexia in rats with increasing body weights. Initial testing must determine if a CCK bioassay is appropriate for the assessment of vagal ablation in an obese rat.

A subgroup of obese rats (n=14) were put through capsaicin treatment (see methods 2.2.5) followed by CCK bioassay (6µg/mL/kg) as shown in Figure 14.3. There was successful ablation of the vagal afferents in these high fat fed rats, demonstrated by the lack of CCK mediated reduction in food intake by capsaicin treated (n=6), as compared with the vehicle treated rats (n=7).

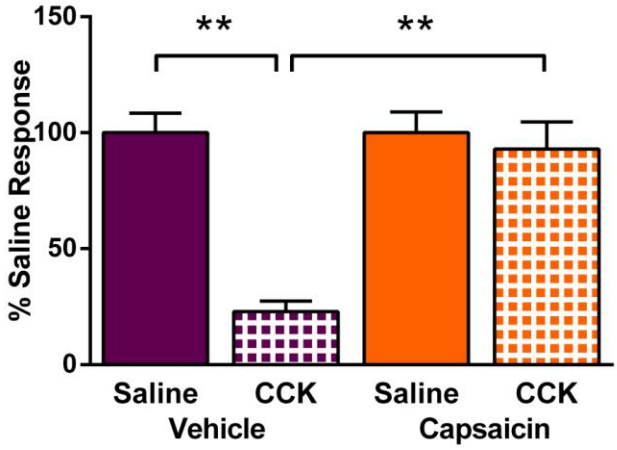


Figure 14.3: Demonstration of the differences in food intake after saline (0.09% NaCl) compared to CCK administration (6µg/ml/kg) in both vehicle and capsaicin treated (125 mg/mL) obese rats (>500g), shown as a percentage of saline intake. Data is mean ± SEM. *P < 0.001, denotes statistically significant difference between Saline and CCK in vehicle rats, and CCK treated Vehicle and Capsaicin rats.

3.4 Histological Analysis of Capsaicin-Mediated Denervation of Sensory Vagal Fibres.

In addition to the "CCK bioassay", an additional approach was used to measure the level of capsaicin denervation of vagal afferents directed to the stomach wall following both its global and local application. Immunolabelling of IGLEs with calretinin, a peptide previously shown to be expressed in these specialised stretch endings was compared in capsaicin and vehicle treated rats. Lean rats were either treated with capsaicin (n= 9) as previously described or with vehicle (n= 14). After the confirmation of ablation using CCK, the rats were killed and stomachs were sampled prepared for histological analyses. They were opened longitudinally (Figure 15) and, in order to visualise IGLEs, the overlying mucosa and circular muscle layers were dissected away leaving the myenteric plexus containing the IGLES.

Whole mount preparations of optimally dissected vehicle treated rat stomach showed clear IGLE localisation with calretinin. These endings had a characteristic "string of pearls" appearance where individual axon swellings or varicosities were interspersed with thinner fibre connections (Figure 16 A).

In rats pre-treated with global or topical capsaicin, whole mounts immunocytochemically labelled with calretinin showed a myenteric plexus completely devoid of IGLEs (Figure 16 C). In order to evaluate whether an adjustable gastric band fitted to the stomach would itself lead to the denervation of sensory endings, stomachs from rats pretreated with vehicle and fitted with a band were examined. These showed no diminution of calretinin immunolabelling of IGLEs (Figure 16 B).

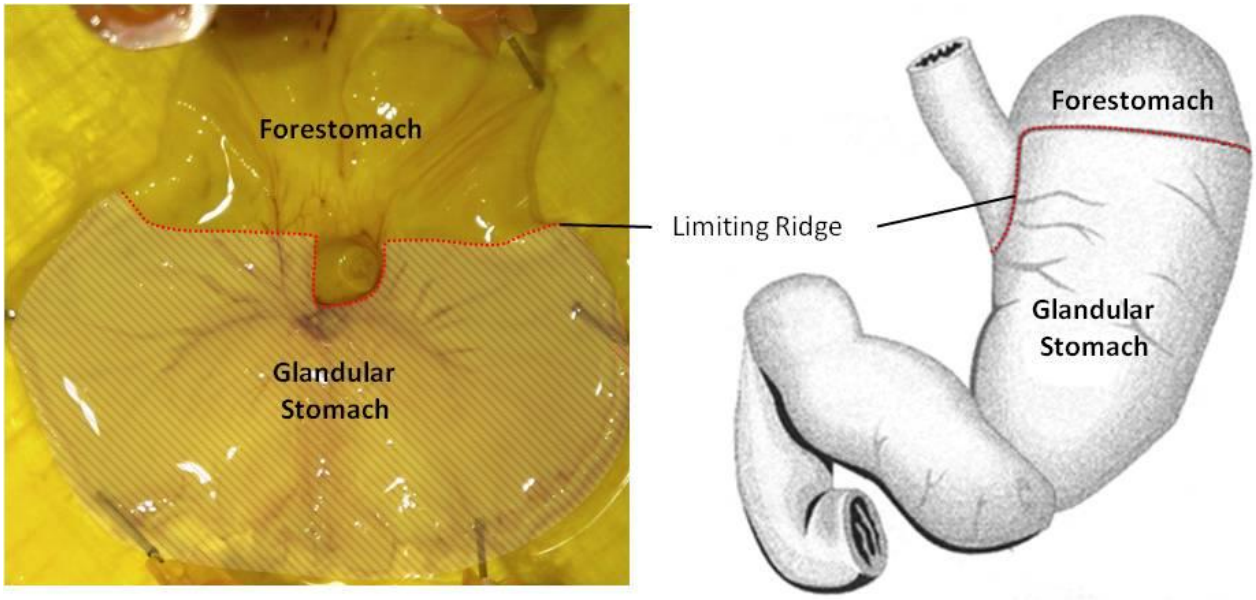
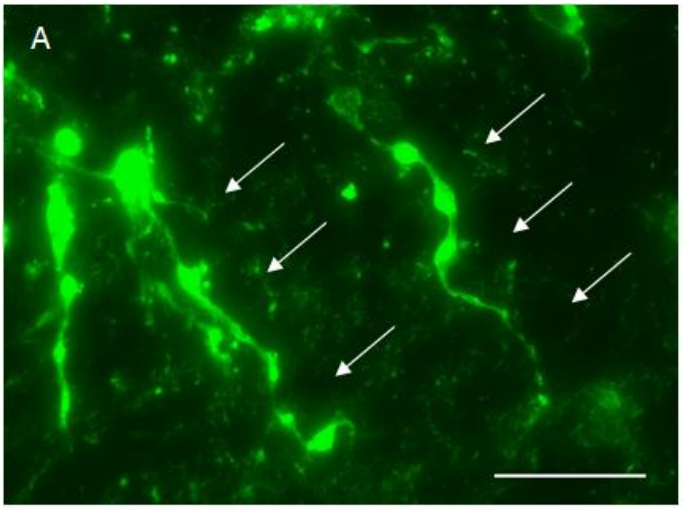
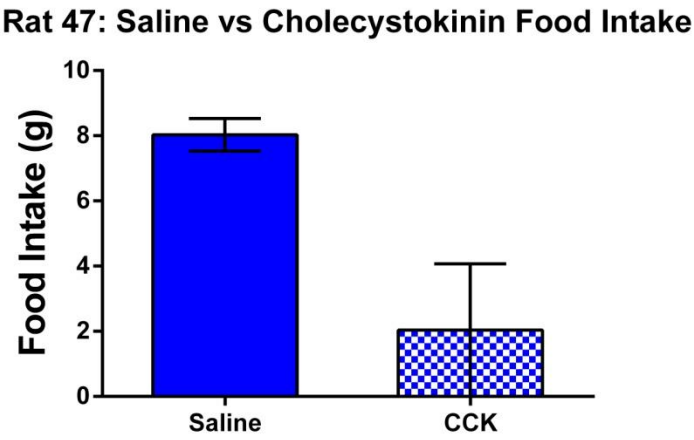
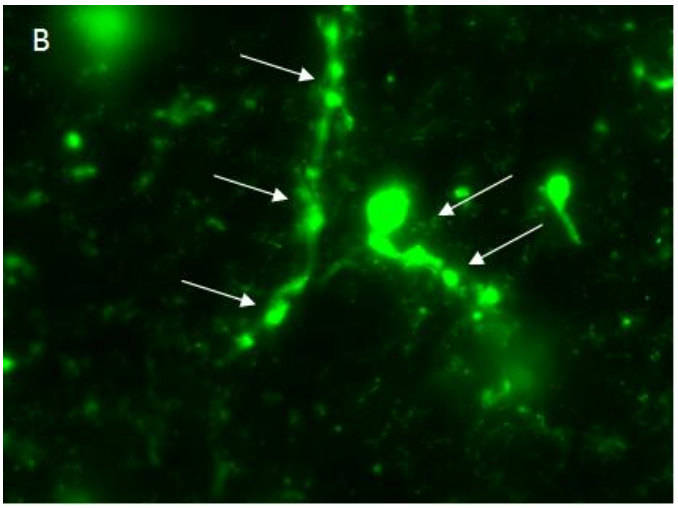
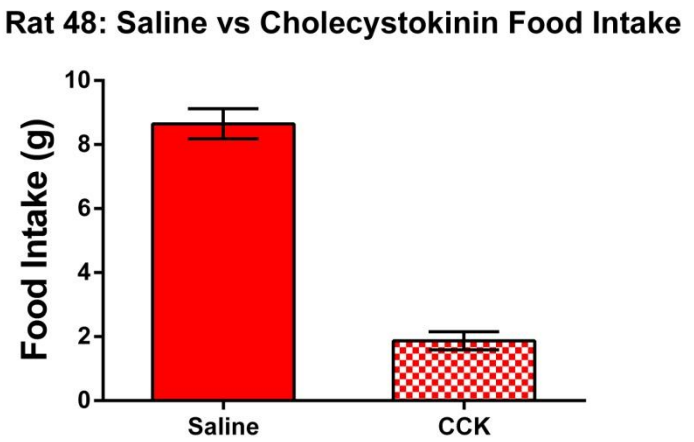


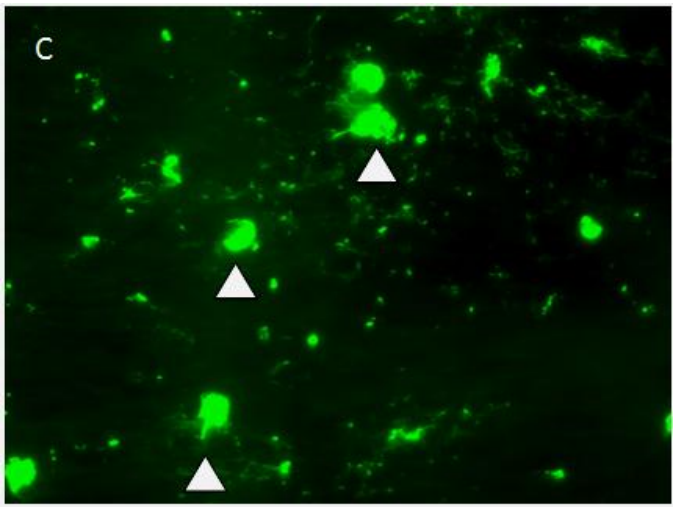
Figure 15: A whole mounted stomach on balsa board, immersed in fixative. The stomach is split in half longitudinally, and reveals the epithelial layer (A) and the glandular stomach wall (B) flattened to allow immunohistochemical investigation of IGLEs. The square denotes the location of sampled tissue being dissected which corresponds closely to the area of the stomach at the gastro-oesophageal junction where the band will be positioned in the rat model of the AGB.











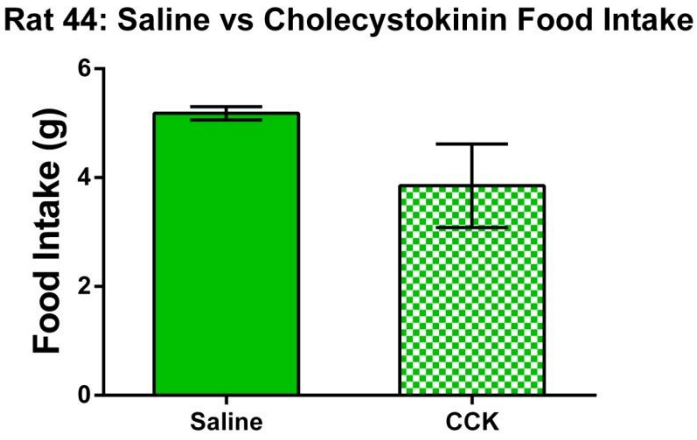


Figure 16: Whole mount preparation of stomach from (A) Vehicle treated sham operated, (B) vehicle treated and AGB rats, and (C) capsaicin treated and AGB. Arrows in A, B and C correspond to calretinin stained varicose IGLEs. Arrow heads show single neuronal cell bodies within the myenteric ganglion. Scale bar 10µm, corresponds to A, B and C. Abbreviations CCK: Cholecystokinin.

Part 1:
The involvement of vagal sensory fibres in mediating the metabolic consequences of the AGB.

Encompassing:

2.3 Elimination of sensory unmyelinated c-fibres using capsaicin

2.5 Establishing a method for the topical application of capsaicin onto the stomach wall.

2.7 Determining the Efficacy of the Adjustable Gastric Band with Vagal Afferent De-nervation

4.1 The Effect of Capsaicin Lesions on Food Intake following AGB Inflation

In order to determine the effects of the adjustable gastric band on metabolic parameters, 25 rats were put through AGB or sham surgery, both with and without intact vagal systems. Initially fed a high fat diet (45% kcal from lipids, *Specialty Feeds*, Methods section 2.1) for 12 weeks, DEXA scanning would ensure they were divided into three weight-equivalent groups (shown in Figure 18). Control rats were treated with vehicle and sham operated (n=8) compared with vehicle treated and AGB operated (n=9), and capsaicin pre-treated and AGB operated (n=8).

After 2 weeks recovery, rats had their bands inflated and metabolic parameters were monitored for the following 4 weeks. Initial band inflation was sub optimal (0.3mL), then increased to maximal inflation from week 2 onward (0.5 to 0.7mL). The treatment groups were weighed 3 times weekly, with daily food intake monitored throughout the assessment period.

There was no significant difference in the cumulative food intake of capsaicin treated AGB rats compared with vehicle treated sham rats at any point in the inflation testing period shown in Figure 17.1. Vehicle AGB rats consistently ate less than sham operated and capsaicin AGB treated animals, effects that were significant from day 14 of the measurement period. This is shown by the cumulative food intake shown as a percentage of control rats in Figure 17.2.

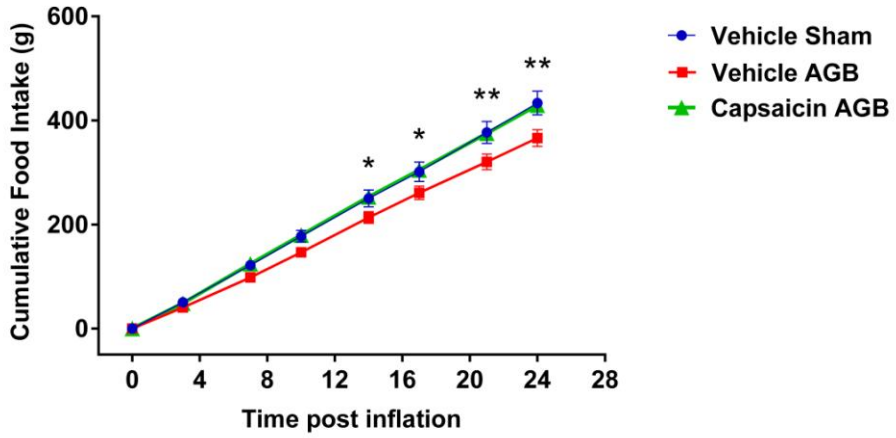


Figure 17.1: The four week cumulative food intake measurement of high fat rats. The treatment options compare the testing of capsaicin AGB rats (n=8), with vehicle treated sham operated rats (n=8) and vehicle AGB (n=9). Results are Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, denotes a significant difference between capsaicin AGB and vehicle sham rats compared with vehicle AGB rats.

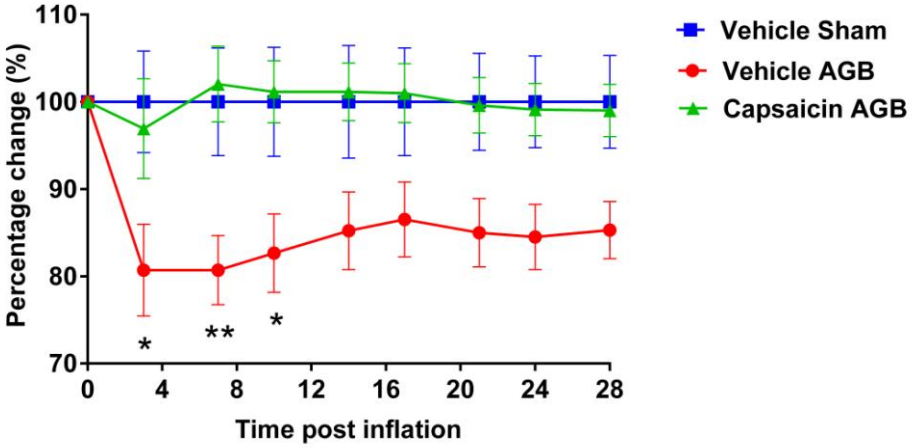


Figure 17.2: The cumulative food intake as a percentage of control. The food intake of the vehicle sham (n=8), vehicle AGB (n=9) and capsaicin AGB (n=8) rats. Results are Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, denotes a significant difference immediately after band inflation between capsaicin AGB and vehicle sham rats compared with vehicle AGB. There was no significant difference between any group after day 10.

4.2 The Effect of Capsaicin Lesions on Weight Change following AGB Inflation

The first (suboptimal) inflation of the band (0.3ml) led to rats with inflated AGBs and vehicle treatment trending to a lower weight gain, as compared with capsaicin treated and AGB fitted rats, and sham operated and vehicle treated rats. The second band inflation (0.5-0.7ml) lead to a significantly reduced body weight gain culminating in a 51.7% reduction in vehicle AGB treatment compared with Vehicle sham and Capsaicin AGB 28 days after the first inflation (P<0.01)(Figure 17.3). There is no statistical difference between vehicle treated sham operated rats as compared with capsaicin treated AGB animals at any time during the measurement period.

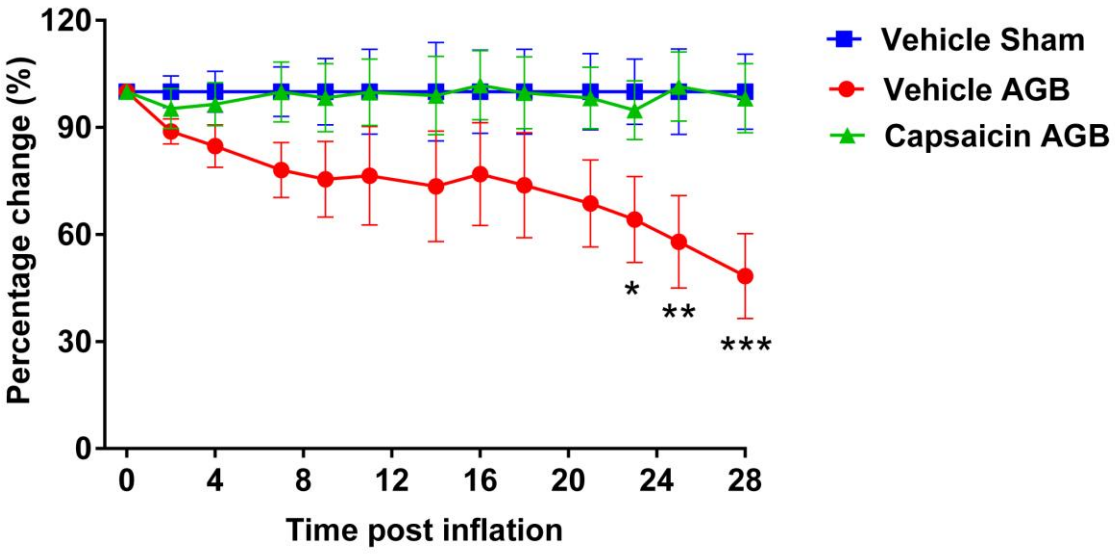


Figure 17.3: The change in weight (in percentage) from baseline control (Vehicle sham, n=11) values comparing Capsaicin-AGB (green line, n=8) and Vehicle AGB (red line, n=6) treatments.

Results are Mean ± SEM. *P<0.05, **P<0.01, denotes a significant difference between Vehicle-AGB rats and both of the alternative treatment options. *P<0.05, **P<0.01, *** P<0.001, denotes a significant difference in the percentage decrease of body weight when comparing the vehicle AGB rats (-48% ±11.9) compared with Capsaicin AGB (98% ±9.7) and vehicle sham (100% ± 10.5) rats.

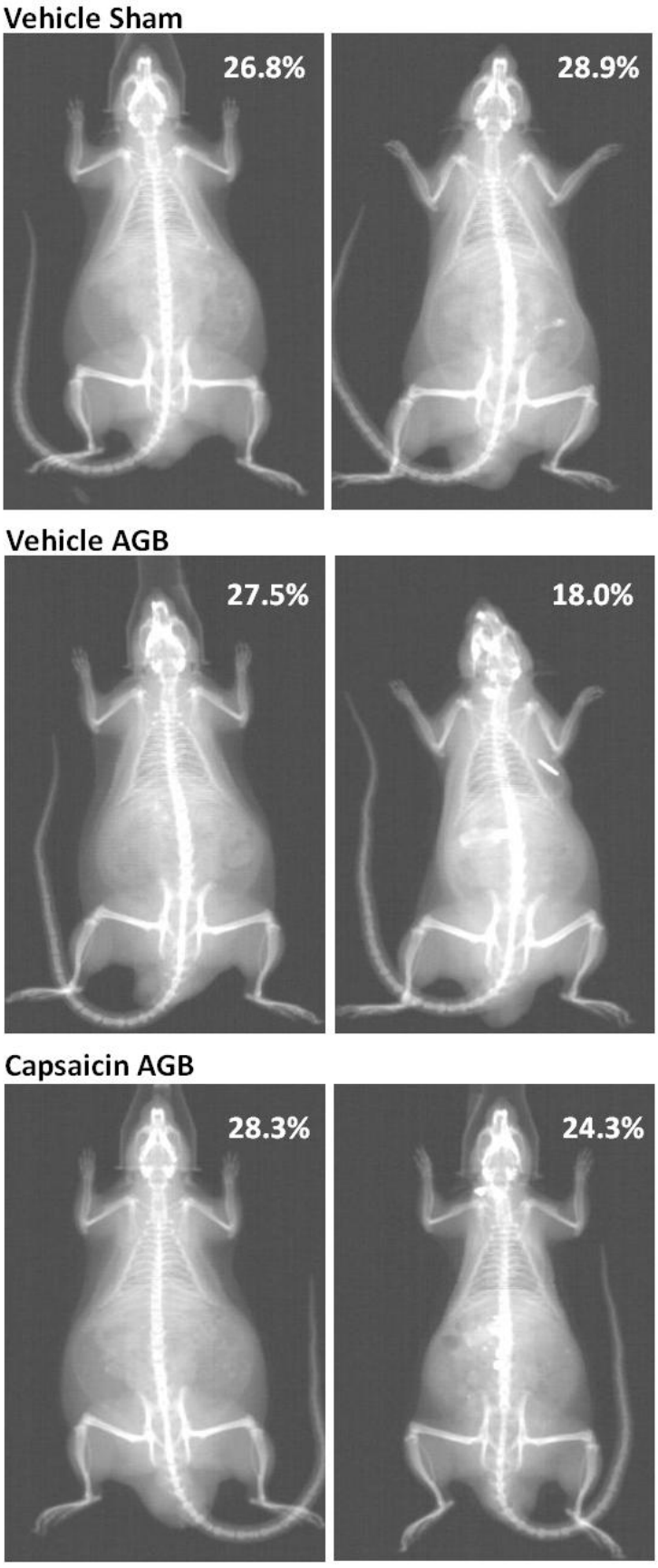


Figure 18: DEXA scans demonstrating the typical changes in fat percentage of a vehicle sham operated rat (+2%) Vehicle AGB rat (-9.5%) and capsaicin AGB rat (-0.6%) as determined by DEXA scanning. These scans were taken after 12 weeks of diet-induced obesity (left), then after 4 weeks of treatment (right).

4.3 Alterations to Glucose and Insulin Homeostasis

In order to assess the alterations in glucose tolerance and insulin sensitivity due to capsaicin or AGB operation, DIO rats were tested throughout treatment by IPGTT and IPITT. These were performed twice, before capsaicin treatment and AGB surgery, and after the 4 week testing period in which bands were inflated.

DIO treatment ran for 12 weeks, culminating with a weight of >500g. The cohort was then divided into three groups to examine the effects of capsaicin treatment on AGB activation, with vehicle sham and AGB, compared with capsaicin AGB groups. These groups were DEXA scanned and divided with equal average weights and put through glucose and insulin tolerance testing. Changes in blood glucose were monitored following an injection of glucose (1g/kg) over 120 min in the case of the IPGTT in Figure 10.1, and an injection of insulin over 180mins for the IPITT in Figure 10.2. After recovery of normal eating habits (2 weeks) the rats had their bands inflated and 4 weeks of monitoring identical to the section 3.1.1 above.

At the conclusion of the band inflation testing period, IPGTT and IPITT were repeated. This would ensure a better understanding of the possible effects from the 4 week band inflation assessment.

4.3.1 Glucose Tolerance Testing

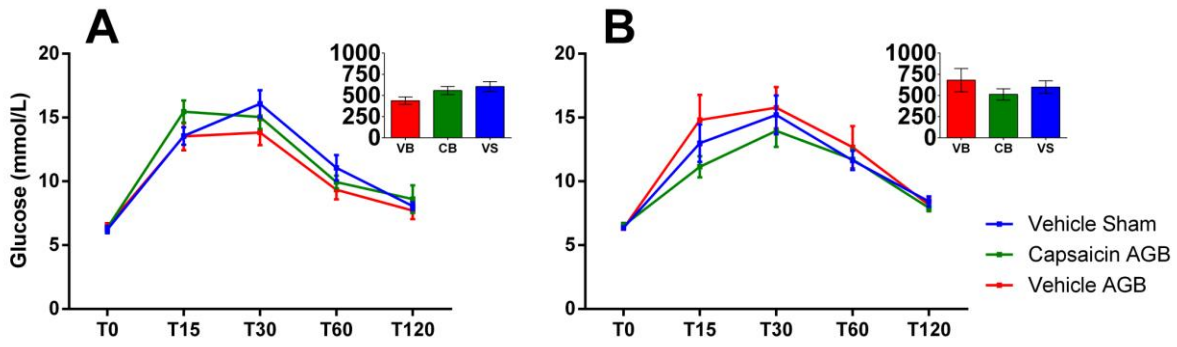


Figure 19.1: Glucose tolerance testing (A) before surgical intervention or treatment including AUC graph and (B) after 4 week band inflation period with AUC graph. Treatment groups include vehicle AGB (Red line, n=8), capsaicin AGB (Green line, n=6) and vehicle sham (n=6).

Data are Mean ± SEM. P<0.05, denotes no significant difference between AGB and Sham animals, nor capsaicin and vehicle treatments in the AUC graph of the pre- or post-AGB inflation period GTT. Abbreviations: *VB* - Vehicle AGB, *CB* - Capsaicin AGB, *VS* - Vehicle sham.

4.3.2 Insulin Tolerance Testing

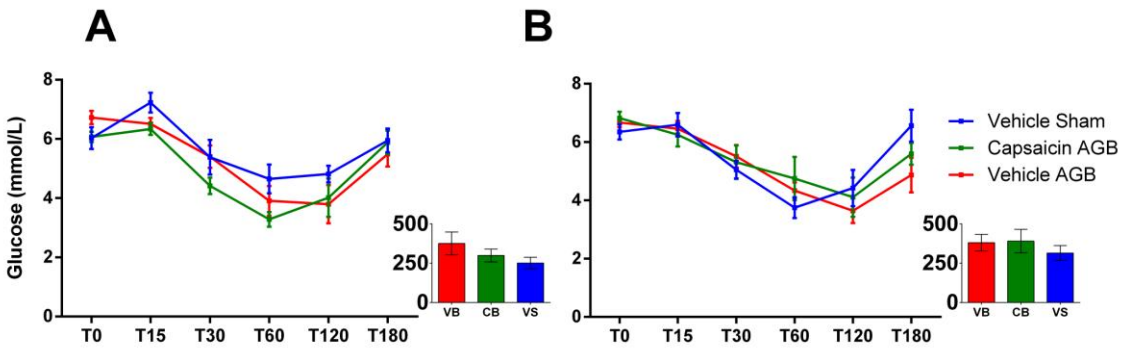


Figure 19.2: Insulin tolerance testing (A) after DIO treatment including AUC graph and (B) after 4 week band inflation period with AUC graph. Treatment groups include vehicle AGB (Red line, n=8), capsaicin AGB (Green line, n=6) and vehicle sham (n=6).

The changes in blood glucose were monitored following an injection of insulin (0.75units/kg) over 180 minutes. Data are Mean ± SEM. Significance of $P<0.05$ was not reached. Abbreviations: *VB* - Vehicle AGB, *CB* - Capsaicin AGB, *VS* - Vehicle sham.

4.4 Changes to Body Composition and Tissue Modification

Dual-Energy X-ray Absorbtiometry (DEXA) scans were used to examine the changes in body composition in soft tissue mass and bone mineral density. Initial measurements were taken after 12 weeks of diet induced obesity feeding, the rats were divided into groups of equal weights for capsaicin AGB (n=8), vehicle sham (n=6) and vehicle AGB (n=11) groups. At the conclusion of the 4 week inflation period, DEXA scans were repeated and final changes to body composition were compared with pre-surgical values.

4.4.1 Changes to fat mass

Tissue mass changes as measured by DEXA demonstrate that the vehicle sham group gained fat mass, with significant positive growth over the 4 week period compared to other treatment options. Vehicle AGB rats demonstrated a significant 41% reduction in percentage change compared to control vehicle sham operation rats, while there was a 56.8% reduction in the Capsaicin AGB rats compared with control. These results are shown in Figure 20.1. There was no significant difference between the AGB groups, however there was a trend for the capsaicin treated rats to a lower fat mass compared to the vehicle.

4.4.2 Changes in lean mass and bone mass

The percentage changes in lean mass from baseline values before the 4 week inflation period demonstrate that vehicle AGB, capsaicin AGB, and vehicle sham treatment plans show no significant differences.

There was no significant difference in the bone mass changes of the treatment options. However, there was a trend of decreased values in AGB operated rats, particularly those treated with capsaicin ($P<0.07$).

4.4.3 Overall tissue mass changes

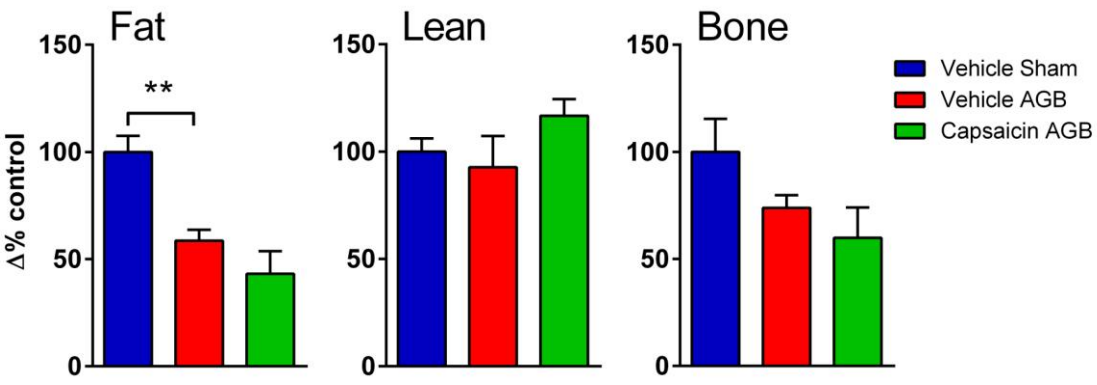


Figure 20.1: Tissue mass changes in Fat, Lean and Bone mass shown as change in percentage of vehicle AGB (red, n=11) and capsaicin AGB (green, n=8), compared to control vehicle sham rats (blue, n=6). Initial values were taken before AGB inflation testing period and compared with scans at the conclusion of the 4 week inflation testing period. Results are Mean ± SEM. **P < 0.01, denotes a significant difference in fat mass between vehicle AGB, and vehicle sham rats (41% reduction).

4.5 AGB Induced Changes in RWAT Tissue Mass

In the comparison of pre-inflation to post-inflation changes in RWAT tissue (Figure 20.2), vehicle band rats had a trend for decreased change when compared to vehicle sham RWAT values (P=0.10, 22.4% reduction). Capsaicin treated AGB fitted rats produce the lowest percentage of RWAT compared to the control weights, however there is no significant difference between this group and the vehicle treatment banded rats.

Specific areas of adipose tissue demonstrated increased growth, with vehicle sham control rats exclusively developing a greater quantity of retroperitoneal adipose tissue over the inflation period (not shown). This was not achieved by the other treatment options.

4.6 AGB Induced Changes in EWAT Mass

Testing the efficacy of the AGB after capsaicin treatment shows that the capsaicin AGB cohort has a decreased amount of epididymal white adipose tissue compared with the vehicle AGB cohort (P<0.01, a reduction of 37.4%). There was no significant difference between the vehicle sham operated group and the vehicle banded group, shown in Figure 20.2.

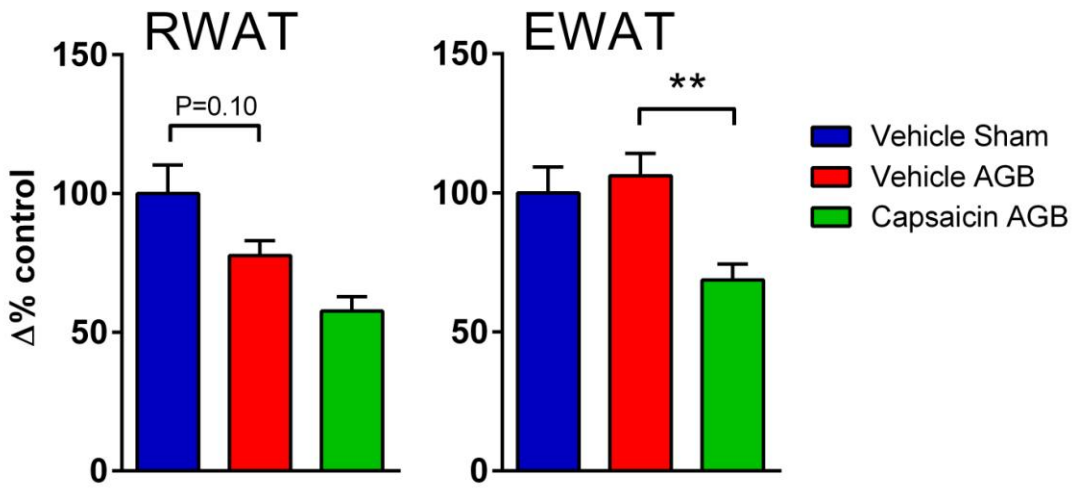


Figure 20.2: White adipose tissue change in RWAT and EWAT as a percentage of control tissue weight change over the AGB inflation testing period in vehicle sham (blue, n=8), vehicle AGB (red, n=8), and Capsaicin AGB (green, n=8) groups. Results are in Mean ±SEM. **P<0.01, denotes a significant difference between the vehicle AGB group and the Capsaicin AGB in EWAT percentage mass.

4.7 The Efficacy of the Adjustable Gastric Band in Systemic Capsaicin Treated DIO Rat

It has been shown previously that rats with gastric bands applied eat less high fat food, and gain less weight than rats who are sham operated (Kampe & Stefanidis, 2011). In order to compare the impact of capsaicin (IP) with and without AGB intervention, metabolic parameters were evaluated in two cohorts consisting of fourteen high fat rats at an average of 500 grams, given a standard capsaicin dose (see Section 2.2.5). This experiment examines the role of the vagus nerve system in regulating weight gain from effects of the gastric AGB inflation.

The cohort of 14 were split into two equally weighted groups after capsaicin treatment, and were then fitted with an AGB (n=7) or sham operated (n=7). After a recovery period of two weeks identified by the resumption of normal eating habits, the rats had their bands inflated and were monitored for a 4 week assessment period. Cohorts had their weight gain measured three times weekly and food intake measured daily. DEXA scanning for body fat mass and percentage tissue change was performed after weight gain and before surgery, as well as after the completion of 4 week band inflation assessment period. Glucose and insulin tolerance testing was performed in the same schedule as DEXA scanning.

4.7.1 Food intake of DIO capsaicin treated rats with or without AGB inflation

The cumulative food intake of DIO systemic capsaicin treated rats indicate that there was an increased intake of high fat pellets by sham operated compared with AGB fitted rats ($P<0.05$). This trend became significant in the final measurements from day 22 onward, shown in Figure 21.1.

The cumulative food intake expressed as a percentage of the control intake demonstrates there is an insignificant difference between these groups daily food intakes. Figure 21.2 shows a trend for decreased food intake in the AGB group compared to sham animals which increases over the measurement period, however this does not reach significance.

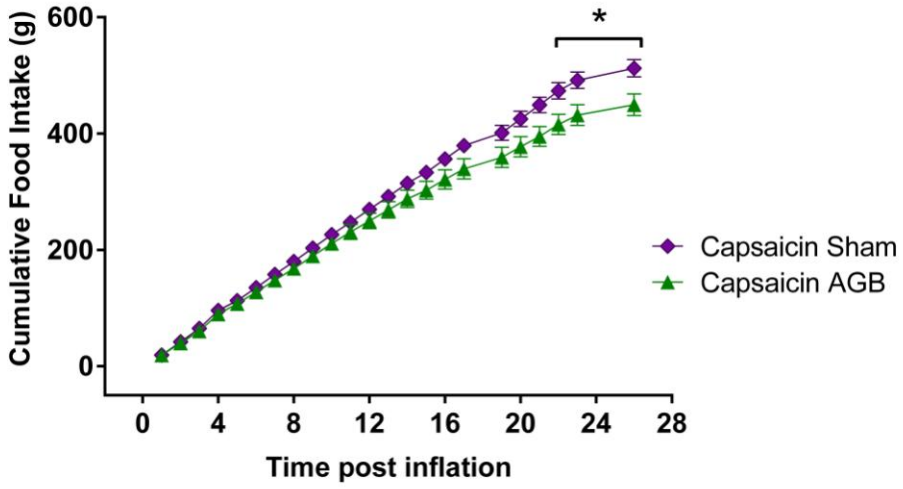


Figure 21.1: The cumulative food intake over 24 days comparing the capsaicin sham (n=7) and capsaicin AGB (n=7) rats. There was no significant difference between the sham or AGB until day 22 of the measurement period. Results are Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, denotes a significant difference from 22 days after band inflation until the completion of the examination.

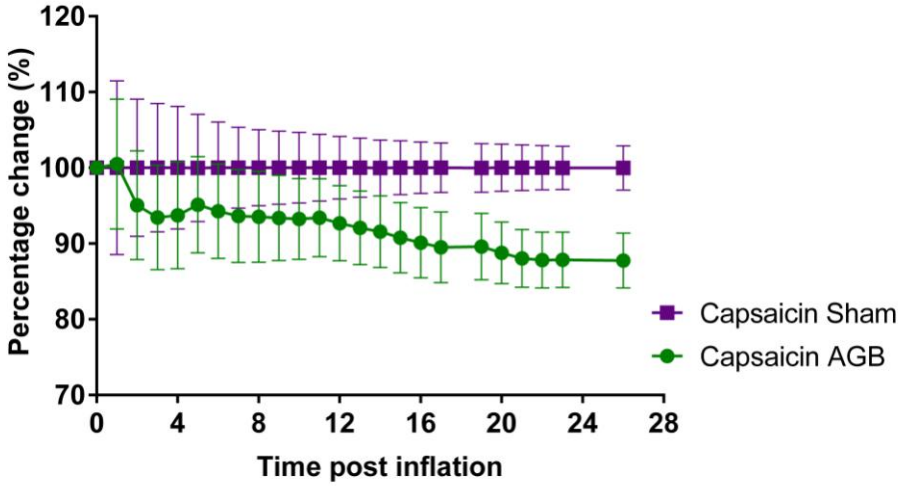


Figure 21.2: The cumulative food intake of high fat rats shown as a percentage of the intake of control rats. The treatment options compare the testing of capsaicin AGB rats (n=7) with capsaicin sham (n=7). Results are Mean ± SEM. There is no significant difference between capsaicin AGB and sham rats throughout the measurement period**.**

4.7.2 Weight change of DIO capsaicin treated rats with or without AGB inflation

The weight changes of DIO rats demonstrate that both the AGB fitted and sham rats have unchanged weight throughout the measurement period. The AGB rats (green line, n=7) fluctuate over time when compared to the control capsaicin sham (purple line, n=7). Figure 21.3 demonstrates that there is no significant difference in weight gain over time. There is a trend for a reduction in weight in the AGB group in the final measurement period, however this is not significantly different with a maximum reduction of 7% at day 20 (P<0.07).

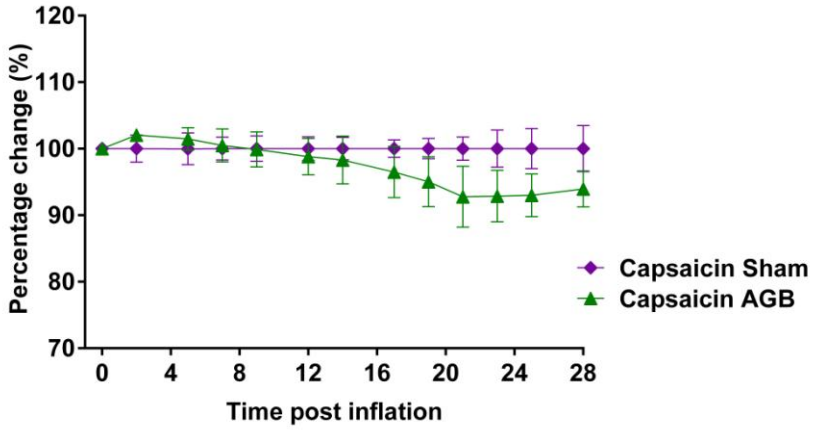


Figure 21.3: Body weight change of AGB fitted rats (green line, n=7) as a percentage of sham operated (purple line, n=7) rats for a period of 28 days, after both are treated with Capsaicin (25, 50, 50mg/ml/kg).

Rats with bands were re-inflated (between 1-2mL/AGB depending on port length) every two weeks, and body weight (g) was measured three times weekly over the entire treatment period. P=0.09, no statistical significance between capsaicin AGB and capsaicin sham animals over the measurement period.

4.8 Investigation into Homeostatic Blood Glucose Regulation after Capsaicin Treatment

In order to assess possible changes occurring in glucose and insulin sensitivity with treatment, IPGTT and IPITT were performed after DIO rats were significantly obese, treated with capsaicin and had the relevant surgery. The bands were inflated and IPITT and IPGTT tests were repeated at the conclusion of this assessment period 4 weeks later. The AGB was incrementally inflated from the first week, to reach maximal inflation in week 2. The bands were reinflated every two weeks to ensure consistency.

4.8.1 Glucose tolerance testing before and after capsaicin treatment

All rats were fasted (food removed at 0600hrs, beginning of the light period) and then injected with glucose 6 hours afterward (1200hrs). Blood sampling was then taken over a period of 2 hours to determine homeostatic response.

Glucose tolerance testing of this cohort (Figure 22.1) indicates that there was no statistical difference between the AGB and sham rats before the 4 week measurement period, indicating both groups were normoglycemic after capsaicin treatment and surgery. The second GTT at the conclusion of the 4 week trial demonstrated that capsaicin AGB animals had a trend for larger increases in glucose concentrations, however this did not react significance ($P<0.08$). AGB fitted animals were able to clear a glucose load just as effectively as sham animals.

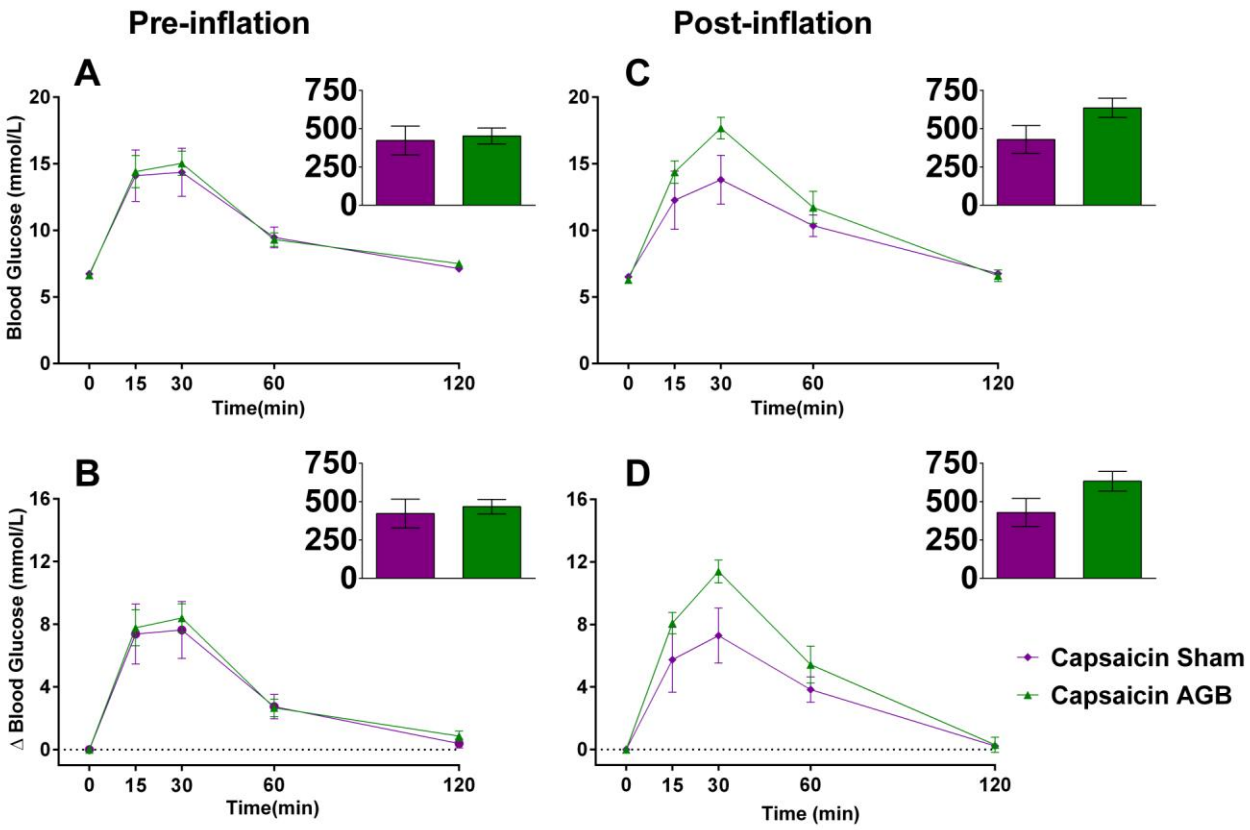


Figure 22.1: The effects of banding or sham operation following capsaicin treatment on glucose tolerance testing of DIO rats (n=14).

(A) Pre inflation IPITT, (B) Percentage change in blood glucose from baseline during Pre-inflation IPITT, (C) Post inflation IPITT and (D) Percentage change in blood glucose from baseline during Post-inflation IPITT. Data are means ± SEM. Abbreviations: *IPGTT,* intraperitoneal glucose tolerance test.

4.8.2 Insulin tolerance testing before and after capsaicin treatment

All rats were fasted (food removed at 0600hrs, the beginning of the light period) and then injected with insulin 6 hours later (1200hrs). Insulin tolerance testing of the capsaicin cohort indicates that there was no statistical difference between the AGB and sham rats both before and after the 4 week measurement period.

These tolerance tests demonstrate that high fat animals who had been systemically treated with capsaicin were able to effectively control their blood glucose and respond to an insulin challenge. The difference in resting glucose values for the pre-inflation rats have been amended in graph B and D of Figure 22.2, which shows the change in blood glucose values. There is an insignificant trend for a faster response in normalising glucose values in the capsaicin AGB rats as compared with sham treated, as shown by the accompanied AUC graph.

Post inflation testing demonstrates that there is no significant difference between either band or sham rats, with similar responses to insulin injection.

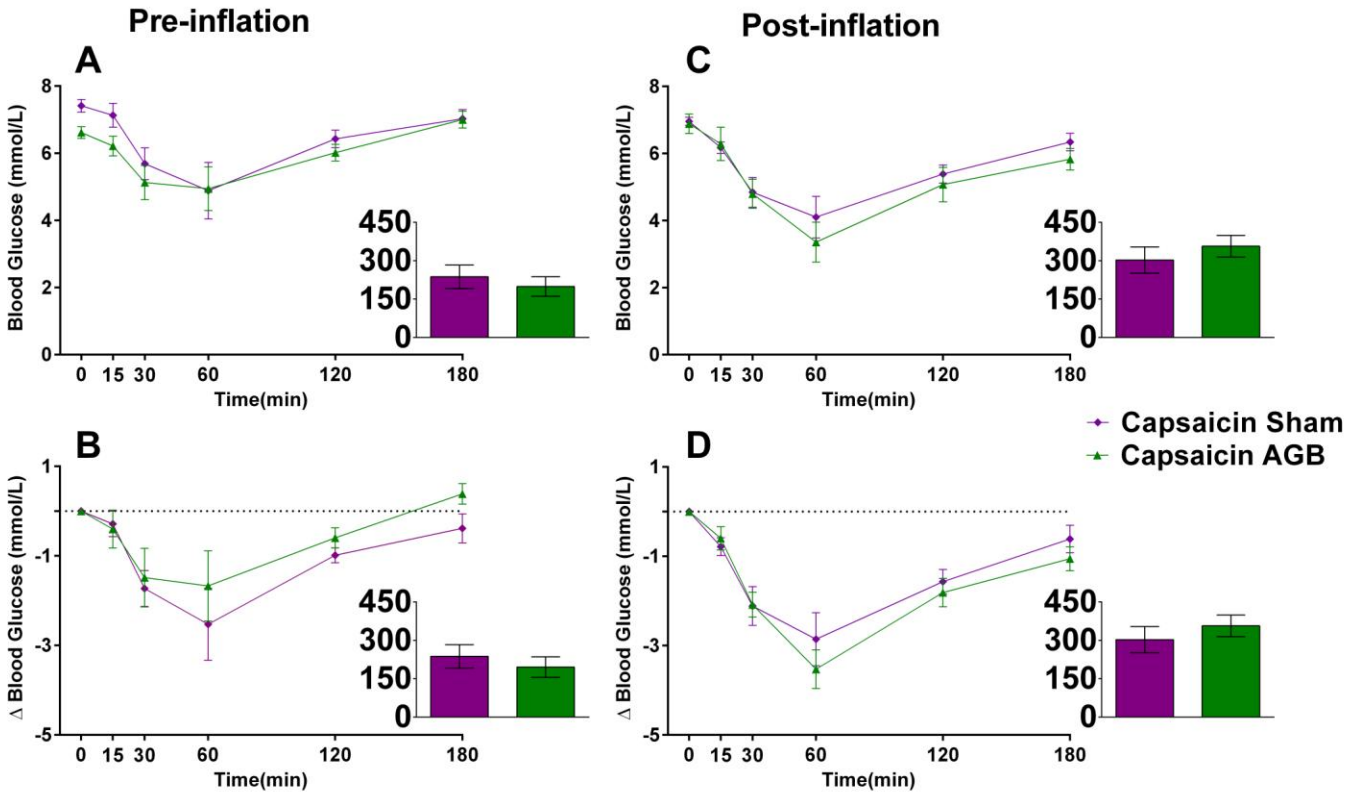


Figure 22.2: Insulin tolerance testing of DIO systemic capsaicin treated rats, either sham (purple, n=7) or AGB (green, n=7) operated.

Changes in blood glucose were monitered following an injection of insulin (0.75units/kg) over 120min. (A) Pre inflation IPITT, (B) Percentage change in blood glucose from baseline during Pre-inflation IPITT, (C) Post inflation IPITT and (D) Percentage change in blood glucose from baseline during Post-inflation IPITT. Data are means ± SEM. Abbreviations: *IPITT,* intraperitoneal insulin tolerance test.

4.9 Adipose Tissue Changes in Capsaicin Treated Rats after AGB Inflation

In order to compare the impact of capsaicin (IP) with and without AGB intervention, metabolic parameters were evaluated in two cohorts of AGB fitted and sham surgery capsaicin treated rats. They were given a standard capsaicin dose (see Section 2.2.5) and had AGB fitted to half as per methods section 2.4.3.

At the conclusion of the 4 week band inflation testing all rats were individually euthanised and DEXA scanned generating the Fat, Lean and Bone mass values (Figure 24). Adipose tissue masses are then removed from the rat and weighed.

Following the inflation testing period, AGB fitted rats show no statistical difference in IWAT or EWAT tissue when compared to the control sham operated rat (Figure 23.1 below). There is a trend for lower mass in the banded animals, as demonstrated by the RWAT tissue values which show a decreased mass in the banded animals, however this did not reach significance (P=0.07).

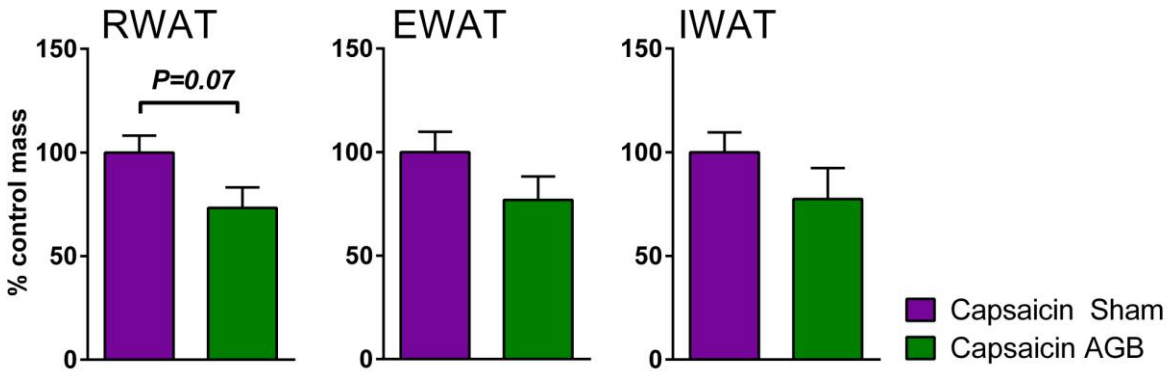


Figure 23.1: DEXA scans of DIO systemic capsaicin treated rats, either Sham operated (Blue line, n=7) or AGB (Red line, n=7). There is no significant difference between the total mass (g) of AGB and sham groups. Results are Mean ±SEM. With P<0.05, there is no significant difference between adipose tissue quantity of AGB and sham rats. Abbreviations: *IWAT*, Inguinal white adipose tissue, *RWAT*, retroperitoneal white adipose tissue, *EWAT,* Epididymal white adipose tissue.

DEXA scans were performed before the 4 week band inflation assessment period, and repeated at its conclusion (Figure 24). Change in mass of the fat, lean and bone values were determined as a percentage of the control capsaicin sham group over the period of this experiment. As shown in Figure 23.2, lean and bone values did not change significantly. There was a trend for decreased fat mass in the capsaicin band group, this did not reach significance (P=0.13) and parallels adipose tissue trends in Figure 23.1.

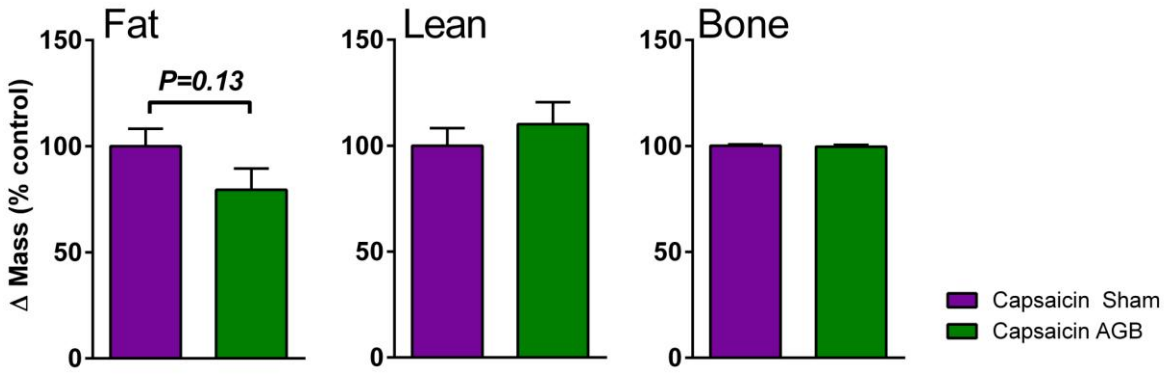


Figure 23.2: The change in fat, lean and bone (mineral density) mass comparing the sham (purple, n=7) and AGB (green, n=7) operated capsaicin treated rats. No significant difference between the lean or bone mass of AGB and sham groups. There is an insignificant trend for AGB rats to have less fat than sham operated (P=0.10). Results are Mean ±SEM. Abbreviations: *Fat,* Fat mass, *Lean,* Lean mass, *Bone*, Bone mineral density.

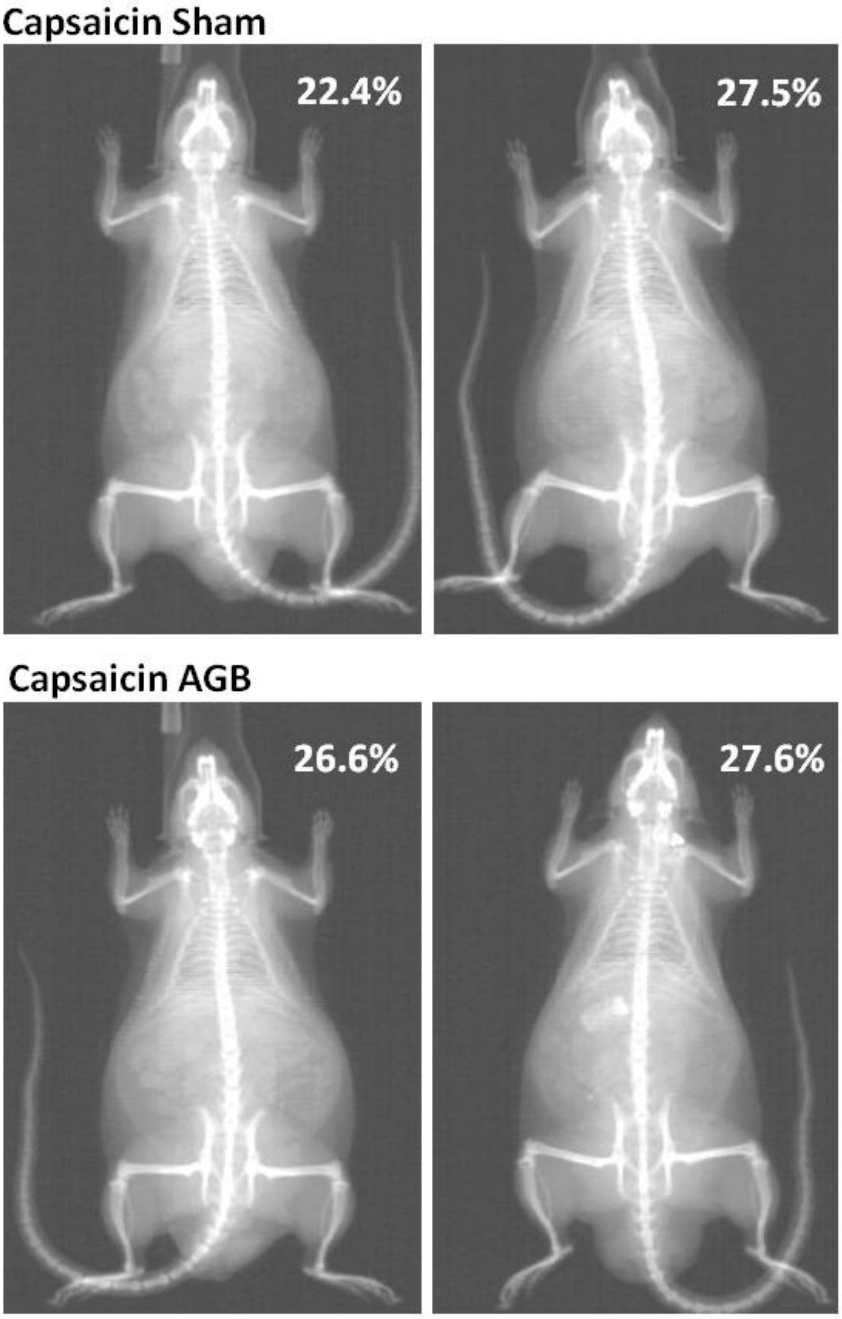


Figure 24: Average rat DEXA scans of systemic capsaicin treated high fat diet animals.

(A) sham operated at beginning of measurement period (left image) and after 4 weeks measurement (right image). (B) before AGB application (left image) and after 4 weeks of AGB inflation, arrow denoting band placement (right image).

Part 2:
The involvement of peripheral and central neural patterns in the maintenance of the effects of the AGB

Encompassing:

2.4 Neural Activation Following Banding of Denervated Rodents

2.6 Assessing the role of vagal sensory afferents in AGB induced changes in neural activity

5.1 Neural Activation Following Banding of Globally Denervated Rats

In order to confirm that the reductions in food intake and body weight observed during AGB inflation were mediated at least in part by a neural mechanism, it is necessary to selectively ablate the sensory component of the vagus. This approach also serves to discriminate between a neural or a hormonal drive of the activation patterns observed in the brainstem of rats with bands inflated (Stefanidis and Kampe., 2011, under review). The efficacy of intraperitoneal capsaicin to ablate vagal fibres was assessed, only those rats with ameliorated CCK responses were included in the capsaicin AGB group.

This ablation allowed for the study of neural activation following band inflation. Rats with effective ablation would have AGB attached, the band would be inflated and 90min later (at the peak of neural activation) the rat would be euthanized. After the brain was treated and stained, brain activation could be counted by way of activated neurons which develop the c-fos protein. Activation areas could then be quantified, the response of band inflation on key hypothamaic nuclei involved various known feeding response areas.

5.2 Brainstem C-Fos Activation after Capsaicin Treatment

The Nucleus of the solitary tract (NTS) is a major section of brainstem with inputs from the vagus nerve, which provides sections of quantifiable c-fos activation (Figure 25.1). In rats pretreated with vehicle and fitted with an AGB, inflation of the AGB resulted in a significant increase in Fos positive neurons in the NTS of banded rats as compared with sham operated controls. This is apparent throughout the rostral and caudal areas of the NTS, with the mid-NTS developing a trend for higher vehicle AGB activation (P=0.06).

Rats with capsaicin treatment and AGB inflation showed no such elevation of brainstem neural activity, consistent with the stimulations of previous parameters measured such as body weight and food intake. The elevation in activated neurons throughout the NTS was shown only in rats with an intact vagus nerve, this increased fos protein activation was completely nullified in rats with inflated bands after capsaicin treatment.

Total NTS combines the rostral, mid and caudal brainstem counts to provide an overall understanding of the activation. There is a large increase in activated nuclei in the vehicle AGB compared to the vehicle sham and capsaicin AGB cohorts.

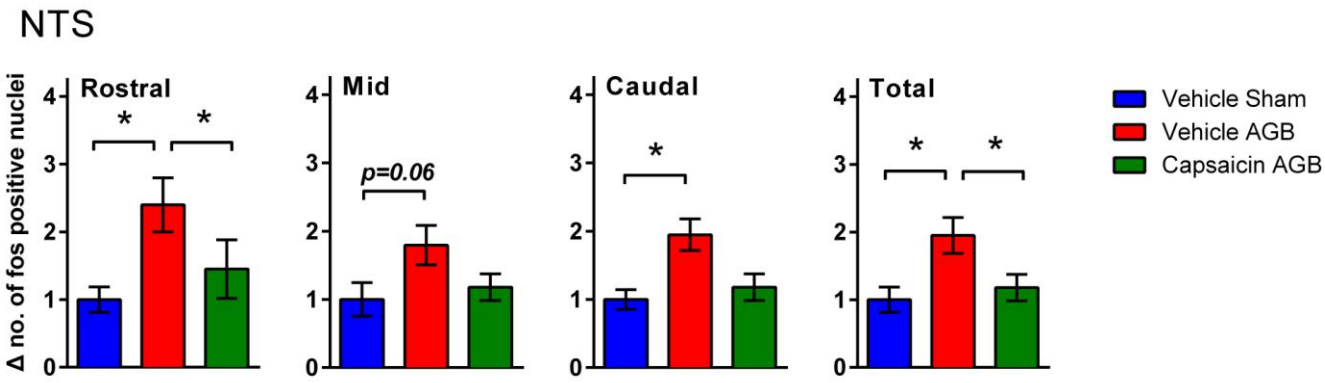


Figure 25.1: The fold change in Fos-positive immuno-reactive nuclei in the NTS of the brainstem following AGB inflation. Comparative across three different treatments including vehicle and sham operated (blue, n=4), vehicle and AGB operated (red, n = 4) and capsaicin treated and AGB operated (green, n=4). Results are expressed as mean ± SEM. *P<0.05, denotes a significant difference between the vehicle AGB group and both the Capsaicin AGB and Vehicle sham rats in the rostral, caudal and total NTS. There was an insignificant trend when comparing the vehicle AGB rats with the Vehicle sham rats in the Mid-NTS (P=0.06).

5.3 Neural Activation of key Hypothalamic Regions of the Brain

The parabrachial nucleus (PBN) located in the brainstem interacts with the vagus nerve and hypothalamus similarly to the NTS. This similarity in function parallels the similarity in results of the c-fos protein stain. The parabrachial nucleus has a marked elevation of fos positive neurons in response to AGB inflation in vehicle treated animals in the medial regions, when compared with control sham operated animals (Figure 25.2). This is most evident in the medial PBN ($P<0.01$), as the lateral PBN did not reach significance between these two treatment cohorts ($P=0.09$). The capsaicin treatment and band inflation has little fos protein elevation in comparison to the vehicle banding, with a significant difference in the lateral and medial PBN counts ($P<0.05$).

There were no differences in the numbers of Fos positive neurons on either side of the brain following AGB inflation and as such counts were made bilaterally.

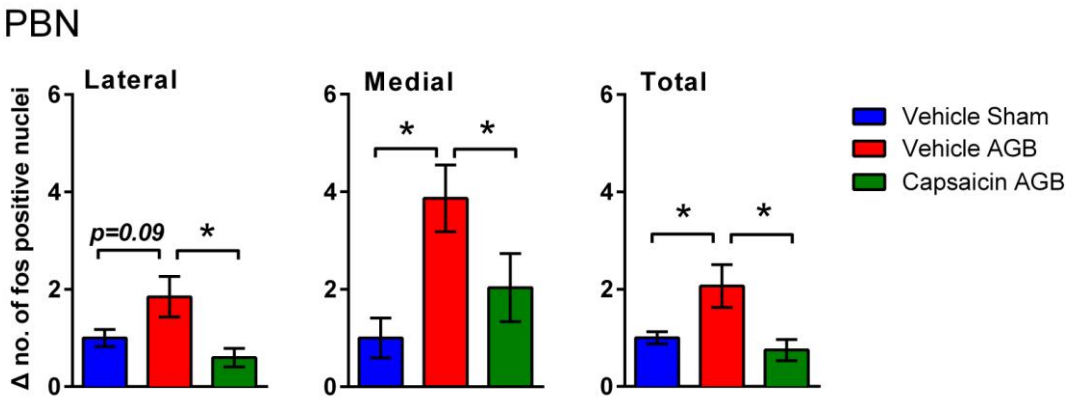


Figure 25.2: Parabrachial Nucleus count expressed in fold change of activated (fos positive) nuclei following acute AGB inflation.

The lateral PBN demonstrates a significantly greater increase in vehicle AGB rats fos positive nuclei as compared to capsaicin AGB (*$P<0.05$), while there is an insignificant trend for the vehicle sham animals ($P=0.09$). The medial section expressed a significant increase in vehicle AGB rats compared with Capsaicin AGB, as well as vehicle sham (*$P<0.05$). Results are expressed as Mean fold change ± SEM. *$P<0.05$ denotes a significant difference between vehicle AGB rats and capsaicin AGB rats in the medial section of the PBN.

In feeding related sites in the hypothalamus including the arcuate nucleus, paraventricular nucleus, perifornical hypothalamus and lateral hypothalamus there was no significant elevation in Fos positive neurons following inflation of the band as compared with sham operated animals (Figure 25.3).

The inflation of the AGB in the arcuate nucleus was significantly reduced in the capsaicin treated cohort in comparison to the vehicle ($P<0.05$). There is no significant elevation in fos protein when comparing the vehicle sham with the AGB, which demonstrates a reduced feedback to the arcuate, due to systemic capsaicin treatment.

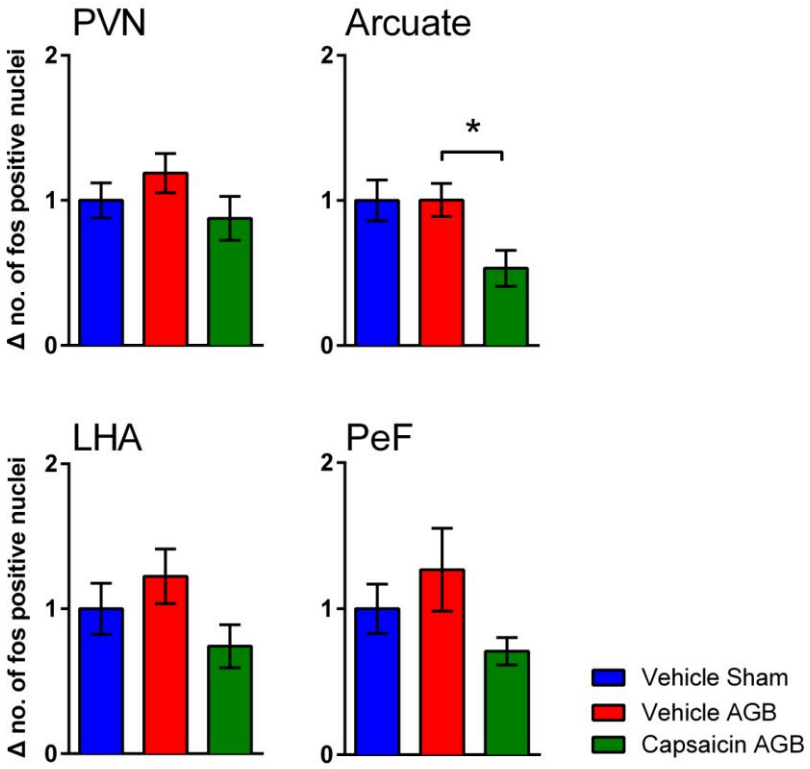


Figure 25.3: the fold change in expression of fos positive nuclei in key hypothalamic feeding centres the PVN, LHA, Arcuate and Perifornical area.

Results are expressed as mean ± SEM. The mid section of brainstem (bregma _ to _) expressed a significant increase in fos positive nuclei from both the vehicle AGB and vehicle sham rats, as compared with the capsaicin AGB animals. Results are expressed as Mean fold change ± SEM. *$P<0.05$ denotes a significant difference between vehicle AGB rats and capsaicin AGB rats in the Arcuate.

Abbreviations: *PVN,* Paraventricular Nucleus, *Arcuate,* Arcuate Nucleus, *LHA,* Lateral Hypothalamic Area, *PeF,* Perifornical Area.

5.4 Evolution of Capsaicin Ablation: A method for the Topical Application of Capsaicin.

Topically applied capsaicin will ablate vagal sensory afferent endings located only in the stomach wall, allowing for the study of neuronal feedback of the AGB with other organs untouched. This will narrow the possible responses to AGB application made only by other organs in the previous global application method. Additionally, it will provide support for the concept of intraganglionic laminar endings controlling food intake.

In order to evaluate the effect of vagal sensory ablation directed solely to the stomach, capsaicin (X mg/mL or 10%) was applied topically to the stomach focusing on the region of the gastro-oesophageal junction subjacent to the position of the band. After a recovery period (2 weeks) the rats were tested with CCK to determine the extent of vagal sensory denervation. As shown in Figure 26, there was an incomplete reversal of the CCK response in capsaicin treated rats, with food intake still being regulated by the injection of CCK.

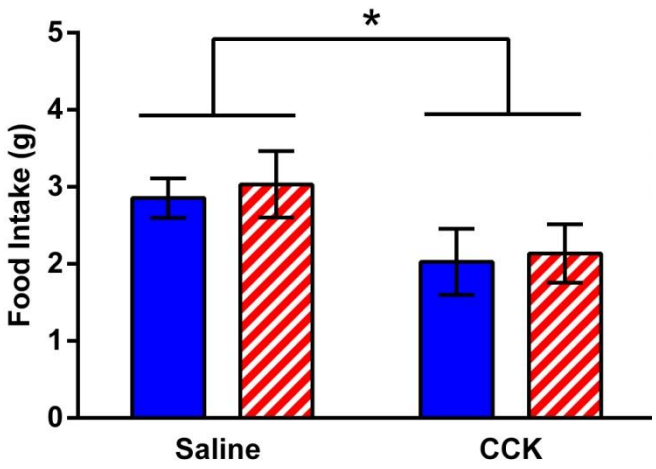


Figure 26: Food intake testing of topically applied capsaicin (blue, n=8) or vehicle (red stripe, n=8) after either Saline or CCK injection. A significant decrease in both groups is shown comparing food intake after IP Saline or IP CCK, * P<0.05. Abbreviations: *CCK* – Cholecystokinin.

5.5 Assessment of IGLE Fibres after Calretinin Labelling of Topical Capsaicin Ablation

Topical application of capsaicin directly to the stomach wall results in hyperstimulation of c-fibre type afferents. An effective treatment of capsaicin results in the inactivation of these nerve terminals, known as intraganglionic laminar endings (IGLEs) located in the stomach wall. The bioassay testing food intake (Figure 26) shows that there is an incomplete reduction in the receptors for the effectiveness of CCK in topical capsaicin ablated rats. Visual assessment of these nerve fibres after topical capsaicin application is achieved using calretinin labelling, which will provide secondary visual evidence of capsaicin ablation.

The stomach wall is surgically reduced to the epithelial layer and stained using a whole-mount calretinin method. This demonstrates the effectiveness of capsaicin, with IGLE endings clearly left intact in vehicle applications as shown in Figure 27.2 below, while capsaicin treatment has removed nerve afferentation.

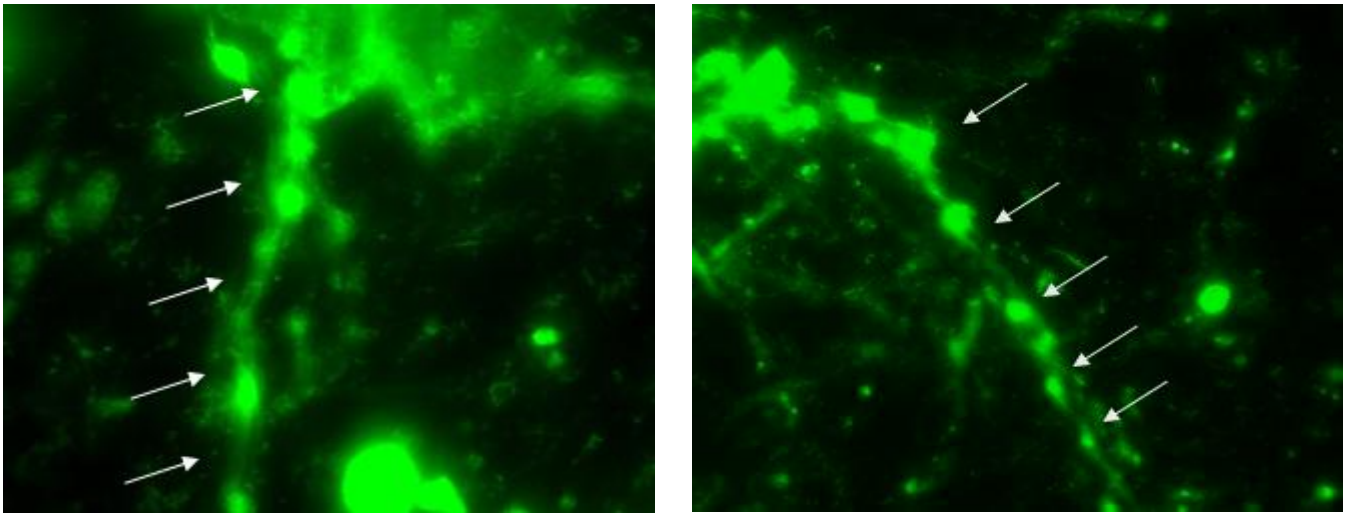


Figure 27.1: The visualisation of the epithelial layer of stomach tissue after the topical application of the vehicle treatment. Stomach was stained using a whole-mount method, using the calretinin protein. Arrows indicate intact IGLE fibres.

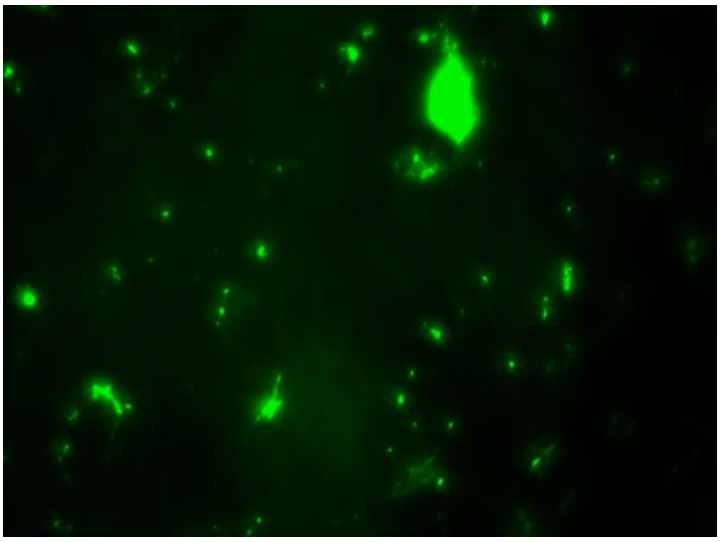


Figure 27.2: The topical application of capsaicin demonstrates clear reduction in IGLE afferent fibres on the epithelial layer. The stomach wall has been stripped of IGLE fibres, demonstrating only random binding of calretinin.

5.6 Assessing the Role of Vagal Sensory Afferents in AGB Induced Changes in Neural Activity after Topical Capsaicin Ablation

The validation of topical capsaicin treatment as an effective eliminator of vagal sensory endings has been finalised by a complete histological analysis. The effects of topical capsaicin treatment has shown no significant increase in brainstem counts made in central integration sites of baroreceptors and chemoreceptors, such as the nucleus of the solitary tract (NTS)(Figure 28.1), and parabrachial nucleus (PBN)(Figure 28.2). These brainstem counts are the primary areas of activation, but demonstrate that there is no significant difference in the activation patterns of rats with or without AGB treatment. Additionally, they do not significantly differ to the control rats treated with saline and sham operated.

As significance was reached in the brainstem counts, this activation pattern may be further examination into more caudal areas of the brainstem and hypothalamus such as the paraventricular nucleus (PVN), Arcuate nucleus and lateral hypothalamic area (LHA). These secondary counts demonstrate that the topical ablation of the stomach wall produces no significantly different neural activation patterns from each treatment group in these areas. This was a similar result to those rats treated with an intraperitoneal systemic injection.

5.6.1 Activation of key areas of the brainstem after topical capsaicin treatment

The Nucleus of the solitary tract (NTS) is a major section of brainstem with inputs from the vagus nerve, which provides sections of quantifiable c-fos activation (Figure 28.1). Fos positive nuclei were counted with specific rules regarding the location of the various key hypothalamic areas. Bregma levels were as follows: Rostral 22-28, Mid 29-32 and Caudal 33-37.

In rats pretreated with vehicle and fitted with an AGB, long term inflation resulted in a significant increase in Fos positive neurons in the NTS of banded rats over any other treatment option. This is apparent throughout the rostral, mid and caudal areas of the NTS.

Rats with capsaicin treatment showed no such elevation of brainstem neural activity, consistent with the results of previous parameters. Neither of the capsaicin treatment options were statistically dissimilar in any area of the brain, with no increase in response from the inflation of the band as compared with sham surgery.

The elevation in activated neurons throughout the NTS was shown only in rats with an intact vagus nerve and an AGB fitted, the vehicle sham rats did not experience elevation in any area of the NTS as compared with the capsaicin treated options.

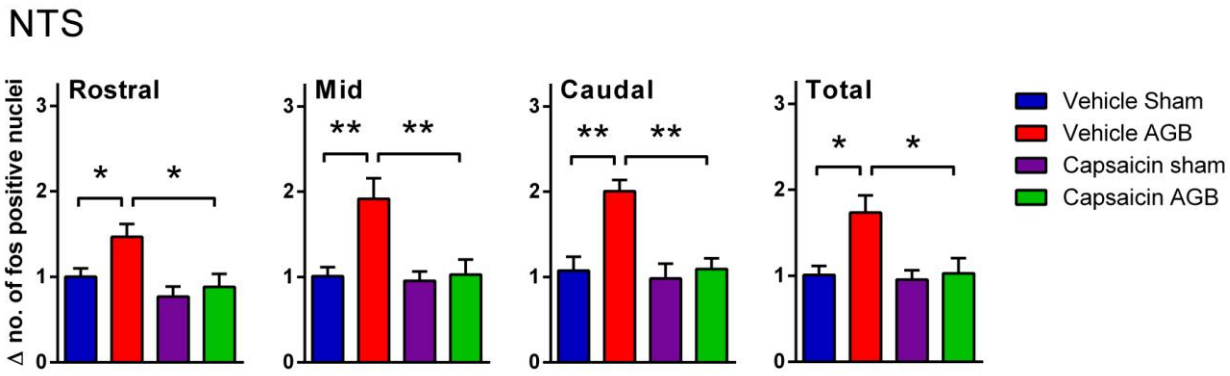


Figure 28.1: The mean number of Fos-positive immuno-reactive nuclei in the Nucleus of the Solitary Tract of the brainstem following AGB inflation. Comparative over four treatments, topical capsaicin application with AGB (green, n=10) or Sham operation (purple, n=5), and Vehicle with AGB (red, n = 9) or sham (blue, n=6). Results are expressed as mean ± SEM. *P<0.05, denotes a significant difference between the vehicle AGB group and both the Capsaicin AGB and Vehicle sham rats in the rostral, mid and caudal NTS.

5.6.2 Activation of key areas of the hypothalamus after topical capsaicin treatment

The parabrachial nucleus (PBN) located in the brainstem interacts with the vagus nerve and is involved in transmission of activation toward the hypothalamus, receiving input from many organs. The topical application of capsaicin will result in reduced brainstem feedback, however it will still retain peripheral feedback systems.

The parabrachial nucleus has a marked elevation of fos positive neurons in response to AGB inflation in vehicle treated animals, this is seen throughout the PBN and is similar to the systemic treatment experiments. When the vehicle AGB increase in neural activation is compared with the capsaicin AGB rat, there is significantly less activation in the capsaicin treatment in the lateral and whole PBN counts. This significance does not extend into the medial region of PBN, however there is still a trend for this pattern (P=0.06)(Figure 28.2).

The vehicle AGB demonstrates significantly more fos positive nuclei than their sham operated counterparts, this is seen in all regions of the PBN. There is no significant difference between the sham treatment options, with a trend for increased fos positive nuclei in the capsaicin sham cohorts compared with the vehicle sham, however this does not reach significance.

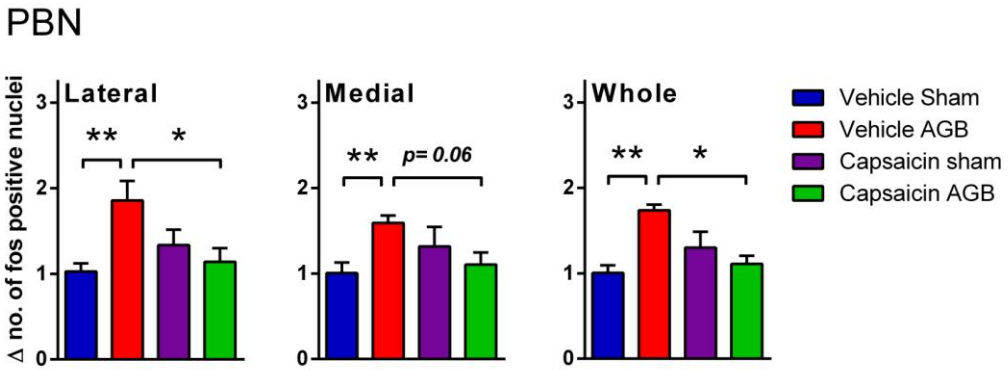


Figure 28.2: The mean number of Fos-positive immuno-reactive nuclei in the Parabrachial Nucleus of the brainstem following AGB inflation. Comparative over four treatments, topical capsaicin application with AGB (green, n=10) or Sham operation (purple, n=5), and Vehicle application with AGB (red, n = 9) or sham (blue, n=6). Results are expressed as mean ± SEM. *P<0.05, denotes a significant difference between the vehicle AGB group and the Capsaicin AGB in the Lateral and Whole PBN. **P<0.01, denotes a significant difference between the vehicle AGB group and the vehicle sham group in the Lateral, Medial and Whole PBN.

The hypothalamic feeding sites including the arcuate nucleus, paraventricular nucleus and lateral hypothalamus are the secondary areas that activation can be found after progressing through the brainstem. Figure 28.3 demonstrates that there is no significant elevation in Fos positive neurons following inflation of the band as compared with sham operated animals in area of these hypothalamic regions.

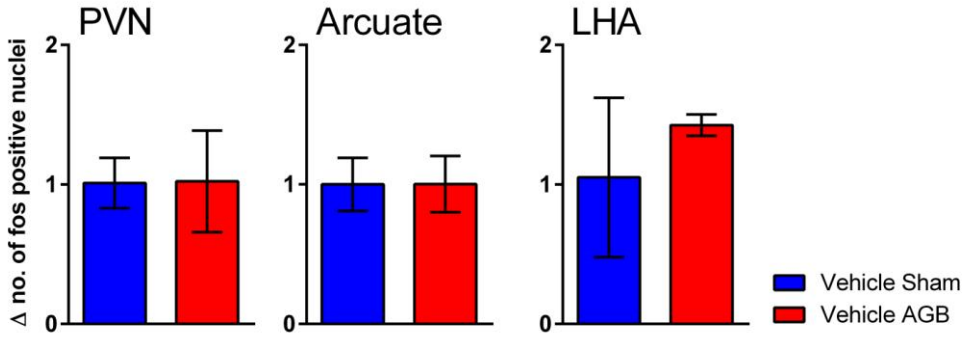


Figure 28.3: The mean number of Fos-positive immuno-reactive nuclei in the Paraventricular nucleus, Arcuate Nucleus and Lateral Hypothalamus following AGB inflation. Comparative over two treatments, topical capsaicin application with AGB (n=10) or Sham operation (n=5). Results are expressed as mean ± SEM. P<0.05 denotes an insignificant difference between the treatment groups.

Abbreviations: *PVN,* Paraventricular Nucleus, *Arcuate,* Arcuate Nucleus, *LHA,* Lateral Hypothalamic Area, *PeF,* Perifornical Area.

Chapter 4

Discussion

The present study demonstrates that the elimination of vagal fibres using systemically administered capsaicin prevents AGB mediated reduction in food intake or weight loss that otherwise intact rats exhibit. Inflation of the AGB caused a 52% decrease in body weight compared to controls after the 28 day treatment period. There was significant loss of overall weight and a diminished food intake after AGB inflation in the vehicle, but not capsaicin treatments. This loss of weight in the vehicle treatment is demonstrated by a significant decrease in retained fat pad mass. This reduction in adiposity, as measured by DEXA, was not reflected in a regional shift in fat distribution. Capsaicin treatment prior to band inflation ameliorated, to some extent, the reduction in adiposity induced by the band. Additionally, the testing of glucose homeostasis showed no verifiable changes to regulation after systemic capsaicin treatment in obese animals.

The efficacy of the capsaicin lesion was validated in a twofold approach; by using a biological assay which involved using CCK, an anorexigenic gut hormone which exerts its effects via vagal sensory afferents (Hayes and Covasa 2006; Holzer et al. 1994; McLaughlin et al. 1985; Raybould 1991). The visualisation of ablation was a secondary histological approach that involved immunolabelling for calretinin, a peptide antigen that binds to the IGLE fibres in the myenteric plexus of the stomach wall.

These results show a significant elevation in Fos protein in neurons in the brainstem after AGB inflation, specifically in the primary site of termination of vagal sensory fibres, the NTS. This activation was reversed in rats fitted with an inflated AGB treated with capsaicin systemically. A similar response is seen in the PBN in rats with an intact vagus nerve, where AGB inflation results in a significant increase in neuronal activation. This resultant activation is removed after systemic capsaicin treatment which does not demonstrate any rise in PBN located c-fos protein after an inflated AGB. Further, in experiments where vagal ablation using capsaicin was specifically targeted to the stomach there was no significant elevation of brainstem activation after AGB inflation. There was significant neural activation in vehicle AGB rats in the NTS and PBN however. The localised capsaicin treatment shown to annul AGB inflation induced effects still had intact systemic cranial nerves.

6.1 Results in the context of previous publications

6.1.1 Affirmation of AGB rodent model

Our model of AGB surgery is proven effective by the shift in body weight of obese rats replicating those results seen both in previous research and patient studies (Stefanidis et al, under review; Habeffer 2013). Obese rats with an AGB fitted to the gastroesophageal junction show a 51.7% reduction in body weight in 4 weeks, replicating human studies which demonstrate a 50-62% reduction (Chapman et al. 2004; O'Brien et al. 2013). This weight loss is reflected in a reduction in fat mass mainly associated with the abdominal compartment, with a trend for decreased RWAT mass. The effectiveness of rat based AGB surgery therefore demonstrates an alignment with currently performed *Lap-band* surgeries on humans.

6.1.2 A greater understanding of the Adjustable Gastric Band

The initial treatment of obesity using the adjustable gastric band focussed on restriction as the reasoning behind its efficacy. This is an inadequate explanation of the complex interplay occurring between the gut and the brain that may underpin the efficacy of this surgical intervention (Burton and Brown 2011). This rodent model of the AGB has shown that both neural and humoral gut-brain conduits are involved in AGB induced satiety (Kampe et al. 2009; Kampe et al. 2012).

Systemic capsaicin treatment produced no significant shift in weight commonly seen after AGB inflation, with food intake increased after capsaicin treatment in AGB inflated rats. This result is consistent with studies that implicate the stretch receptors of the stomach in the efficacy of AGB induced weight loss (Burton et al. 2009). These results are also consistent with the observations of fasted patients who report enhanced satiety in the absence of food entering the stomach (Dixon et al. 2005), thus disproving the theory of the effectiveness of the AGB as a restrictive aid. This research demonstrates that the effectiveness of the AGB in mediating satiety is initiated by the stretch of the stomach wall, and warrants an investigation into these stretch receptors.

6.1.3 The role of the vagus nerve in the mediation of the effects induced by AGB inflation

This study using a rat model demonstrates the importance of vagal mechanisms in producing the satiety effects that are seen after the application of an adjustable gastric band. Previous animal-based studies and observations from the clinic support a central role for the vagus nerve as the major conduit between the stomach and the brain in the mediation of band-induced satiety. Miniaturised bands in rat models have previously demonstrated that the termination points of the vagal sensory fibres activate central neural pathways from the muscular wall of the stomach (Kampe et al. 2012; Habeffer et al. 2013). Additionally, human studies have shown that gastric distension in patients results in activation of similar cortical sites by way of GI innervation (Ladabaum et al. 2001; Wang et al. 2008). These studies suggest that the vagus nerve acts as a major conduit between the stomach and the brain in the mediation of stomach induced satiety brought on by the AGB in both human patients and our rat model.

6.1.4 Intraganglionic laminar endings as a regulatory network

The effects of the band on the stomach wall has been thought to activate the stretch receptors located in the gastro-oesophageal junction. These intraganglionic laminar endings consist of unmyelinated c-fibres that contain the initial nocioreceptors and mechanoreceptors forming the vagus nerve innervation of the stomach wall (Berthoud et al. 1997; Wang et al. 2000). Electrophysiological studies indicate that IGLEs not only function as transduction sites for distension sensitive vagal mechanoreceptors, but also function as tension-sensitive endings that are low threshold and slowly adapting vagal tension receptors (Zagorodnyuk et al. 2001). Therefore, IGLEs are likely candidates for detecting continued compression induced by AGB inflation throughout the day, where this effect could be exacerbated during a meal.

The systemic and topical capsaicin ablation of these sensory vagal afferents in the myenteric plexus enabled a second tier of analysis through the visualisation of IGLE fibres. Calretinin staining of the myenteric plexus demonstrated a clear difference between the characteristic "*arborizations*" or interlinking chains of IGLEs in vehicle rats, with significantly ablated capsaicin treated tissue showing only bare individual neurons.

6.1.5 An understanding of vagal sensory fibres and their role in obesity control

These experiments highlight the centrality of vagal sensory fibres to the mediation of AGB induced metabolic changes. The role of the vagus nerve in controlling eating behaviour has been studied over the last decade, with early studies involving truncal vagotomy reporting large amounts of weight loss post-surgically (Cummings et al. 2004; Kral et al. 1993). This is thought to be due to elimination of the normal responses to orexigenic hormones such as ghrelin (le Roux et al. 2005; Williams et al. 2003). Based on the understanding that the vagus nerve controls satiety and energy homeostasis, an alternative minimally invasive treatment, the so-called "Vagal Blocking for Obesity Control" (VBLOC), has been developed to intermittently block vagal nerve trunks with high frequency and low power electrical signals through a laparoscopically implanted device (Camilleri et al. 2008). These methods are intuitively difficult to reconcile with the current widely read view that activation of the vagal sensory afferents by AGB or removal of this input (ie. By vagotomy or VBLOC) both lead to improved metabolic outcomes. The activation of a nervous system in response to feeding is clearly demonstrated by this thesis, and further elucidates the role of vagal afferentation surrounding this system.

6.1.6 Assessment of capsaicin ablation using food intake

The use of capsaicin to ablate c-fibre type afferents has long been proven effective, with this study confirming the additional ablative effects of capsaicin on IGLE fibres. Capsaicin has been administered intraperitoneally as a systemic treatment for the lesioning of all unmyelinated c-fibres in the rat model, as well as topically so as to isolate the ablation of capsaicin to the area of the stomach associated with the AGB.

The elimination of vagal sensory fibres after capsaicin treatment was affirmed by an effective bioassay using a cholecystokinin mediated reduction of food intake. CCK induced satiety primarily occurs as a selective agonist for abdominal vagal afferents, particularly the unmyelinated c-fibres that support IGLEs, in decreasing proximal gastric motility and therefore gastric emptying (Raybould and Taché 1989). The anorexigenic effect of CCK is well documented, and the reduction of its induced effect can be correlated with the effectiveness of the capsaicin ablation. When administered, CCK provides a robust evaluation of the ablation of capsaicin-sensitive vagal afferent neurons.

The introduction of topical ablation would isolate the stomach wall, leaving a myriad of vagus nerve afferentation intact. This method of topical application resulted in inconclusive bioassay testing, as dorsal vagal complex neurons can be excited by CCK acting at other sites throughout the GI tract. Many of the subdiaphramatic vagal sensory fibres are therefore found to be left intact and receptive to CCK after topical treatment, as compared with our previous systemic treatment model which completely eliminated CCK-induced satiety response. This persistence of myenteric neurons to produce a feedback response is aligned with research examining the neural response to CCK-induced increases of hindbrain Fos immunoreactivity, where even after repeated systemic capsaicin treatments and vagectomy surgeries, the administration of CCK would still be effective (Sayegh and Ritter 2000). A second possibility is that high doses of CCK may activate capsaicin insensitive spinal sensory neurons, which in turn may activate the dorsal vagal complex via the spinosolitary pathway (Sayegh and Ritter 2000).

It is important to note that with extensive vagal sensory lesions, as gauged by this approach, there is a complete elimination of the elevated Fos labelling in the brainstem (Figure 3) but only a partial attenuation of AGB induced weight loss. The failure to completely eliminate AGB induced weight loss with capsaicin highlights the likely involvement of hormones acting directly on the brain.

6.1.7 The role of gut-derived hormones in the mediation of AGB-induced satiety

Interest in pharmacological treatment for obesity has increased exponentially over recent years, particularly in the aim of developing a drug that acts in the brain to elicit the same effects as bariatric surgery. Satiation derived from GI tract feedback occurs via two principal routes; afferentation feedback from distension of the stomach muscle, and the release of GI peptides in response to the presence of luminal nutrients (Page et al. 2012). We have demonstrated the effects of the nervous system in response to stomach distension through nerve afferentation, however, there may be a place for a neurohormonal peptide response which acts through nervous tissue.

The search for the stimulus behind the actions of the AGB has focussed on the regulation by vagus nerve feedback, however the involvement of humoral candidates may not be completely eliminated. Various bariatric surgeries have contrasting results in circulating hormone levels, with GLP-1 shown to be static over the LAGB timeline compared with RYGB patients that see a significant increase (Korner et al. 2007). There is also a suggestion that postprandial elevation of PYY is greater after LAGB than presurgical increases but these remain substantially lower compared to post-RYGB values (Korner et al. 2009). As such, the humoral component of human bariatric surgeries have not been fully elucidated.

It is possible that satiety and weight loss may be mediated by the actions of the band through the release of GLP-1 and / or PYY, with an increase found in the rodent model of AGB after a standard caloric meal is given as a bolus on the background of an inflated band (Kampe et al. 2012). The release of GLP-1 in the small intestine has been shown to increase satiation, stimulate insulin release, suppress glucagon secretion and influence the mechanisms controlling gastric emptying (Schirra and Göke 2005). The resulting increase in gut transit time would be consistent with reductions in food intake and body weight, and there is evidence that gut hormones including PYY are effective in reducing gastric emptying in man (Batterham et al. 2003; Le Roux 2006) and rodents (Batterham et al. 2002; Suzuki et al. 2005).

The search for a single mechanism of action for the AGB has focussed on either neural afferentation or humoral candidates, however these are not mutually exclusive. Peptide hormones released from the GI tract can signal satiety directly to the CNS, and strong functional evidence exists to show that vagal afferent innervation is the link transporting these chemical cues into the brainstem and higher brain centres (Page et al. 2012). The release of hormones as drivers of the effectiveness of the AGB cannot be discounted by the elimination of the effects shown after systemic and topical vagal ablation, as the local release of these agents is likely to act via vagal nerve endings (Cummings and Overduin 2007a). This is true for both GLP-1 and PYY which are derived from L cells in the proximal duodenum and act via receptors on vagal endings. The placement of the band on the proximal duodenum opens the possibility that there is a site of release of these hormones in the proximal small intestine.

The mechanism of action of the AGB may also be a direct hormonal effect on the receptors in the brain stem. It is possible that there is local release from the stomach under the influence of the band but evidence for substantial local gastric production of PYY and GLP-1 is scarce (Taylor 1985). In this respect, Ritter and colleagues have shown that intravenous infusion of GLP-1 caused a reduction of food intake that was independent of an intact vagus nerve demonstrated by capsaicin treatment or vagotomy (Zhang and Ritter 2012). It is acknowledged by these authors that this anorexic effect of “endocrine” GLP-1 on brainstem receptors may operate in concert with the paracrine actions of GLP-1 on receptors in local vagal endings (Zhang and Ritter 2012).

The chemosensory pathway from the gut may act to improve sensitivity of the system in the brain and periphery based on nutrient loading, leading to a preference of anorexigenic hormones during gut regulation by central control. There are several studies that demonstrate the involvement of afferent pathways that respond to nutrient levels (Holzer et al. 1994; Raybould 1991; Raybould 2002), while Grill and colleagues show an increase in the effectiveness of anorexigenic leptin after gastric distension, which acts on brain stem receptors to mediate a reduction in food intake (Huo et al. 2007).

The distension of the stomach itself has been shown to reduce gastric emptying, a process necessarily involving capsaicin – sensitive vagal sensory fibres (Bozkurt et al. 1999), as well as involving satiety hormones PYY (Suzuki et al. 2005). While these data may support a role for reduced rates of gastric emptying in the effectiveness of the AGB there is no evidence that this occurs after the AGB surgery in humans. In fact the opposite has been shown where gastric emptying has been categorically eliminated as a contributing factor to band induced weight loss.(Burton et al. 2011b; Jong et al. 2009).

In conclusion, despite the lack of human LAGB-induced hormone changes, studies involving rat models demonstrate that the release of hormones as stimuli for the effectiveness of the band cannot be discounted (Cummings and Overduin 2007b). The elimination of body weight caused by the band after vagal ablation shown in this study demonstrates that the local release of these agents may act via vagal nerve endings. Peptide hormones released from the GI tract can signal satiety directly to the CNS, and strong functional evidence exists to show that vagal afferent innervation is the link transporting these chemical cues into the brainstem and higher brain centres (Page et al. 2012). Within these brain regions contain gustatory, olfactory and textural inputs, thought to integrate with past experiences in controlling feeding behaviour (Broberger and Hökfelt 2001; Marciani et al. 2006).

6.1.8 Neurological activation caused by AGB inflation

Diet-induced obese rats demonstrated a significant decrease in food intake 14 days following AGB inflation. This is similar to other studies based on balloon distension methods, whereby gastric distension associated with ingestion of a meal in patients results in activation of cortical sites that increase satiety (Mönnikes et al. 2003; Wang et al. 2008). The inclusion of capsaicin treatment causes a significant attenuation of c-fos expression in these models of gastric distension, due to the inhibition of axonal transport of peptides (Hayes and Covasa 2006; Martinez 1995; Martínez et al. 2006; Mönnikes et al. 2003). The conduit link between the stomach and neurological mechanisms involved in mediating AGB activation are therefore of great importance.

The current study demonstrated that the inflation of the AGB causes an increase in the levels of neural activation. The assessment of the NTS and PBN show that with an inflated band, there is increased neurological activity. This increase in Fos protein was evident in the NTS of the brainstem, with significance reached at the most rostral portions of the nucleus. The hindbrain is the terminal connection point of both the afferent and efferent vagus systems, with the gastric vagal afferent fibres penetrating the subnucleus gelatinosus, nucleus commissuralis, and nucleus of the solitary tract (Shapiro and Miselis 1985). Several animal studies have demonstrated an increase in neural activation following gastric distension arising from gastric balloon inflation, as well as band compression which both recruit similar nerve endings (Sabbatini et al. 2004; Willing and Berthoud 1997).

Treatment with capsaicin resulted in decreased activity in the brainstem in the NTS and PBN with an inflated band. This demonstrates that the actions of the band in mediating satiety relies on vagal sensory fibres throughout the rat to transmit information from the stomach wall to these key hypothalamic areas. In line with the current findings, Stefanidis and Kampe (2012) found a significant elevation in the levels of Fos protein in the NTS, a region of the brainstem known to receive vagal afferents from the stomach (Rinaman and Schwartz 2004), to be involved in the mediation of food intake (Rinaman et al. 1998), the transfer of satiety signals from the gut (Woods et al. 1998), and the integration of neural activity arising from meal-related stimuli (Emond et al. 2001). These neurological control systems, involving vagal nerve feedback through appetite neuropeptides and nutrient sensor food mediators, are hypothesised to be the predominant control in LAGB surgery.

6.2 Limitations of the experimental model

6.2.1 Modifications to the delivery of capsaicin treatment

The methodology required some revisions to be made in both capsaicin dosages and the introduction of positive pressure ventilation (see methods section 2.3.1.2). Early studies involving the application of capsaicin directly onto the vagus nerve have reported effects on cardiovascular and respiratory function (Jancsó and Such 1983), however these effects have been generally alleviated by gentle palpation of the chest (Berthoud et al. 1997; Berthoud and Neuhuber 2000; Jagger et al. 1997). An alteration from chest palpation via external chest compression to mechanical airway ventilation resulted in greater than 90% recovery. Capsaicin administration of 20, 50 and 75mg/mL/kg lead to increased mucous secretion in the airways under anaesthesia, and with the inclusion of intraperitoneal atropine (3mg) reduced tracheobronchial mucous flow. Information derived from US based researchers (R.C. Ritter, personal communication) lead to the modification of capsaicin dosage rates to reduce the final dosage (25, 50, 50mg/kg), benefitting survival rates through an increased initial dosage.

6.2.2 Addressing the impact of surgery

There are some demonstrable effects of both the banding and capsaicin treatments on the effectiveness of food intake testing using an IP injection of CCK. Open surgery necessary for the modification to the stomach results in adhesions and scar tissue generation, which may cause a reduction in the effect on vehicle treated and banded animals. Additionally, capsaicin treatment causes a noticeable increase in adhesions throughout the abdominal cavity, which can result in greater difficulty in AGB surgery. These treatments may cause physical separation of the CCK injection from areas of the GI tract due to the increased regeneration in peritoneal tissue and mesentery connective tissue after the surgical stress caused on the system.

6.2.3 The changes induced in adiposity and eating habits by capsaicin

Capsaicin treatment causes system wide ablation to the afferent network of several homeostatic systems in place in the rat. After treatment they would routinely lose up to 15% of their original weight due to changes in eating habits. Once the resumption of normal eating habits were established, they would then be surgically operated on for the attachment of the AGB. As a result of this treatment regimen those rats with a greater surgery time would have lower than average weights, and required several weeks of weight gain before the band inflation measurement period could begin. The initial 12 week period of weight gain can be partially compromised, and the regrowth of excess weight may be a confounding factor. This was controlled for in the second group of capsaicin treated rats, where there was a comparison made between treatments of capsaicin sham and capsaicin AGB. It was demonstrated that there was little difference between the two groups in terms of weight loss, demonstrating that the secondary AGB surgery does not cause excessive stress. Additionally, there was no significant difference in the baseline food intake of either group, further demonstrating that AGB placement without inflation has no effect.

6.2.4 The effects of obesity on Capsaicin and CCK treatment techniques

The release of satiety hormones such as CCK after a meal will delay gastric emptying, and has been shown to alter centrally controlled gastroduodenal motor function, and its associated feedback regulation in man and animals. The vagal afferents and cholecystokinin signalling pathway have long been known to play important roles in intestinally induced satiety (Smith et al. 1985), with CCK concentrations found to be higher in specific areas of the hypothalamus of obese rats (McLaughlin et al. 1985). This in conjunction with feeding studies which demonstrated an inhibition of gastric emptying and gut motility after fat intake (Azpiroz, 1985; Heddle, 1988). As a result, the effectiveness of capsaicin treatment and CCK injections must be tested using the high fat model. Diet induced obese animals are integral to the long-term study on weight loss associated with the AGB in this thesis. They are given a sustained imbalance in nutritional value compared with their energy expenditure, which leads to the accumulation of massive amounts of adipose tissue.

Obese animals have decreased endogenous levels and increased meal sizes due to a decrease in sensitivity to satiety factors (Covasa and Ritter 1998; McLaughlin and Baile 1980; 1981; McLaughlin et al. 1985). They eat a larger meal when compared to lean rats, with a longer duration and a faster rate, which was thought to possibly produce erroneous values in a 30 minute food intake testing scenario after a CCK injection. However, it was found that the meal size of obese animals were decreased by CCK in the same values as in the lean animals. The CCK is administered by weight resulting in greater injection sizes for the obese animals, and may have counteracted the problems associated with obese testing. These differences in daily feeding intakes of obese and lean rats may be due to differences in gastrointestinal activity, evident by the functional adaptations due to high-fat diets in reducing sensitivity to some peptide satiety signals (Covasa and Ritter 1998). Certain aspects of the feeding behaviour of DIO rats may be affected differently than in lean animals because of forced differences in ad lib feeding pattern, modifying GI function due to hyperphagia and/or differences in their baseline metabolism (Boomhower et al. 2013).

6.2.5 The effects of capsaicin and AGB treatment plans on glucose and insulin tolerance testing

The gastro-intestinal tract is richly innervated with peripheral primary afferents, with important glucose sensing organs such as the liver and pancreas innervated with vagal afferents (Berthoud 2008a; Nonogaki 2000). Therefore, alteration to the regulation of this system could affect glucose balance via multiple mechanisms, such as changes in pancreatic insulin release, hepatic glucose output, and/or peripheral glucose utilization.

Studies into the regulatory control of vagal afferent fibres in glucose homeostasis have been largely incongruent. The afferent Aδ and C- fibres are involved in the control of glucose homeostasis, and after capsaicin ablation some studies can demonstrate an improved glucose tolerance in mice. This is thought to be due to an increased insulin response after glucose injection from increased insulin sensitivity (Karlsson 1994; van de Wall et al. 2005). However, others have shown that afferent sensory nerves are not implicated in the adrenergic control of insulin (Guillot et al. 1996), with neonatal capsaicin studies showing a diminished insulin response to IV glucose infusion (van de Wall et al. 2005).

Peripheral glucose-sensitive receptors exist in the liver, and many studies demonstrate the importance of functioning vagal afferents for the glucohomeostatic control (Karlsson 1994; Niijima 1983; Okazaki et al. 1993; Thorens and Larsen 2004). In comparison with others who can demonstrate conflicting data showing that a functioning vagus nerve is not necessary for a complete counter regulatory hormone response to moderate hypoglycaemia (Cardin et al. 2001; Jackson et al. 2000), nor hyperglycemia (van de Wall et al. 2005). This is aligned with our study, which demonstrates that systemic ablation of the vagus nerve does not result in significant change to glucose control. Additionally, rats with AGB fitted show no change in speed of normalisation after glucose nor insulin challenges.

It has been shown that capsaicin affects glucose metabolism by directly inhibiting the vagally-mediated release of insulin from pancreatic β cells, however in vitro experiments on isolated islets exposed to capsaicin show no effect on B-cell function (Karlsson 1994). This was demonstrated by our study, as in the systemic injection of capsaicin there was no significant elevation in insulin response to a glucose challenge, nor was there a change in the response time.

It is thought that the delivery of glucose and insulin as an intraperitoneal injection may be an inefficient method, especially in obese rats. The delivery of glucose or insulin by intraperitoneal injection is subject to considerable variation in injection placement, leading to a variance of the location of the injected volume. These problems are exacerbated by the increased fat mass in the obese model, along with the increased injection volumes that are dependent on weight. This problem may be resolved using oral glucose gavages that administers an equivalent dosage and rate.

Concluding Remarks

The data derived from these studies is in keeping with previous work obtained from animal models developed in this laboratory. These demonstrate that inflation of the band leads to a significant reduction in metabolic parameters including body weight, fat mass and food intake. Additionally, this demonstrates clearly a central role of vagal sensory fibres in the mediation of band induced weight loss.

The impact of the band on glucose regulation in the animal model, similarly in the current clinical situation, remains unresolved. In particular, whether any positive changes in glucose regulation are primary or secondary to band induced weight loss.

6.3 The necessity of the central control of the vagus

Ablation of vagal sensory fibres with the vagal sensory specific neurotoxin capsaicin shows that the positive metabolic effects of the band can be reversed by the elimination of vagal sensory input to the brain. This is underlined by the fact that neural activation in the medullary NTS resulting from inflation of the AGB is prevented in the capsaicin treatment animals.

These data also show the likely involvement of specialised sensory endings in the stomach, the so called intraganglionic laminar endings. These have shown to be eliminated in those rats resulting in an absence of the characteristic neurological activation patterns usually seen after the inflation of the AGB.

These results support the notion of a vagal sensory conduit between the stomach and the brain, necessary for the positive AGB outcomes. However, they do not preclude an involvement of gut derived hormones such as PYY and GLP-1, which we know are released in response to band activation and which may act via vagal sensory endings in the gut.

6.4 Future Directions

6.4.1 Mutagenesis model of mouse-based AGB surgery

In order to discriminate between the stretch mediated or hormonal mediated effects via the vagus, and their relative contribution to the banding effect, it may be possible to control for both the actions of gut derived peptides and their receptors. There are several routes by which satiety hormones released from the gut may influence satiety centers in the brain. They are broadly split into either the hypothalamus and brainstem, or action involving a vagal sensory intermediary such as the afferent endings in the gut wall or vagal sensory cell bodies in the nodose ganglion. Due to this, the best approach for this work is through a mouse mutagenesis model where these options can be effectively eliminated.

This study will involve the application of an adjustable band in a mouse model, with preliminary experiments demonstrating this approach is indeed feasible and we can recapitulate the weight loss demonstrated in the rat.

6.4.2 Hormone sensitization of neurological mediation

The band operates in part to sensitize the brain to anorectic hormones such as leptin, CCK, GLP-1 and amylin. These will require individual experiments where such hormones are introduced systemically with or without band activation or inflation. Using sub-effective levels of gut-derived anorexigenic agents and assessing food intake will determine whether band inflation sensitises the brain to the weight reducing effects of these hormones. It is hypothesised that the neural activation due to these hormones is seen in the same region of the NTS after AGB inflation.

6.4.3 Glucose metabolism and its interaction with the AGB

It is not clear whether the metabolic effects associated with the AGB can induce acute changes in glucose regulation independent of the effects resulting from chronic weight loss and reduced food intake. In order to discriminate if the changes apparent during testing are mediated by the band or are due to the effects of weight loss, then the use of oral glucose administration is more appropriate during glucose tolerance testing. This will circumvent any confounds of body weight, body composition and energy expenditure and evaluate the effects of the band directly on glucose regulation.

6.5 The Future of the Adjustable Gastric Band

For many reasons which may be functional and clinical, but are more likely to be political and economic, the gastric band is losing market share worldwide. This may relate to something as simple as reduced subsidies for post-operative care. In view of this, potential to enhance the effect the impact of the band may be derived from adjunct therapies from combining pharmacological with the band which will enhance the reductions in appetite and/or increase energy expenditure to promote greater weight loss.

Bibliography

Access Economics. The Cost of Obesity. Canberra: 2008.

Adan RAH. Mechanisms underlying current and future anti-obesity drugs. *Trends in neurosciences* 36: 133-140, 2013.

Aguiree F, Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, Hirst M, Hwang C, Magliano D, and Patterson C. IDF Diabetes Atlas. 2013.

Allen Y, Adrian T, Allen J, Tatemoto K, Crow T, Bloom S, and Polak J. Neuropeptide Y distribution in the rat brain. *Science (New York, NY)* 221: 877, 1983.

Anderson JW, Konz EC, Frederich RC, and Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. *Am J Clin Nutr* 74: 579-584, 2001.

Badman MK, and Flier JS. The Gut and Energy Balance: Visceral Allies in the Obesity Wars. *Science* 307: 1909-1914, 2005.

Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau J-P, Bortoluzzi M-N, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, and Lewin MJM. The stomach is a source of leptin. *Nature* 394: 790-793, 1998.

Ballantyne G, Ewing D, Capella R, Capella J, Davis D, Schmidt H, Wasielewski A, and Davies R. The Learning Curve Measured by Operating Times for Laparoscopic and Open Gastric Bypass: Roles of Surgeon's Experience, Institutional Experience, Body Mass Index and Fellowship Training. *Obesity Surgery* 15: 172-182, 2005.

Baltasar A, Bou R, Bengochea M, Serra C, and Cipagauta L. Use of a Roux limb to correct esophagogastric junction fistulas after sleeve gastrectomy. *Obes Surg* 17: 1408-1410, 2007.

Baltasar A, Perez N, Serra C, Bou R, Bengochea M, and Borrás F. Weight Loss Reporting: Predicted Body Mass Index After Bariatric Surgery. *Obesity Surgery* 21: 367-372, 2011.

Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, and Bloom SR. Inhibition of Food Intake in Obese Subjects by Peptide YY3–36. *New England Journal of Medicine* 349: 941-948, 2003.

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, and Bloom SR. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature* 418: 650-654, 2002.

Belachew M, Legrand MJ, Defechereux TH, Burtheret MP, and Jacquet N. Laparoscopic adjustable silicone gastric banding in the treatment of morbid obesity. *Surgical Endoscopy* 8: 1354-1356, 1994.

Berthoud H-R. The vagus nerve, food intake and obesity. *Regulatory Peptides* 149: 15-25, 2008a.

Berthoud H-R, Patterson LM, Willing AE, Mueller K, and Neuhuber WL. Capsaicin-resistant vagal afferent fibers in the rat gastrointestinal tract: anatomical identification and functional integrity. *Brain Research* 746: 195-206, 1997.

Berthoud HR. Vagal and hormonal gut-brain communication: from satiation to satisfaction. *Neurogastroenterol Motil* 1: 64-72, 2008b.

Berthoud HR, and Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci* 85: 1-17, 2000.

Blachar A, Federle MP, Pealer KM, Ikramuddin S, and Schauer PR. Gastrointestinal Complications of Laparoscopic Roux-en-Y Gastric Bypass Surgery: Clinical and Imaging Findings1. *Radiology* 223: 625-632, 2002.

Blackshaw LA, Page AJ, and Partosoedarso ER. Acute effects of capsaicin on gastrointestinal vagal afferents. *Neuroscience* 96: 407-416, 2000.

Boomhower SR, Rasmussen EB, and Doherty TS. Impulsive-choice patterns for food in genetically lean and obese Zucker rats. *Behavioural Brain Research* 241: 214-221, 2013.

Bowne WB, Julliard K, Castro AE, Shah P, Morgenthal CB, and Ferzli GS. Laparoscopic Gastric Bypass Is Superior to Adjustable Gastric Band in Super Morbidly Obese Patients: A Prospective, Comparative Analysis. *Arch Surg* 141: 683-689, 2006.

Bozkurt A, Oktar BK, Kurtel H, Alican I, Coskun T, and Yegen BC. Capsaicin-sensitive vagal fibres and 5-HT3-, gastrin releasing peptide- and cholecystokinin A-receptors are involved in distension-induced inhibition of gastric emptying in the rat. *Regulatory peptides* 83: 81-86, 1999.

Broberger C, and Hökfelt T. Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiology & Behavior* 74: 669-682, 2001.

Broberger C, Johansen J, Johansson C, Schalling M, and Hökfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proceedings of the National Academy of Sciences* 95: 15043-15048, 1998.

Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, and Schoelles K. Bariatric Surgery. *JAMA: The Journal of the American Medical Association* 292: 1724-1737, 2004.

Buchwald H, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, and Sledge I. Weight and Type 2 Diabetes after Bariatric Surgery: Systematic Review and Meta-analysis. *The American journal of medicine* 122: 248-256.e245, 2009.

Burton P, Brown W, Laurie C, Lee M, Korin A, Anderson M, Hebbard G, and O'Brien P. Outcomes, Satiety, and Adverse Upper Gastrointestinal Symptoms Following Laparoscopic Adjustable Gastric Banding. *Obesity Surgery* 21: 574-581, 2011a.

Burton PR, Brown W, Laurie C, Richards M, Afkari S, Yap K, Korin A, Hebbard G, and O'Brien PE. The effect of laparoscopic adjustable gastric bands on esophageal motility and the gastroesophageal junction: analysis using high-resolution video manometry. *Obesity surgery* 19: 905-914, 2009.

Burton PR, and Brown WA. The mechanism of weight loss with laparoscopic adjustable gastric banding: induction of satiety not restriction. *Int J Obes* 35: 144, 2011.

Burton PR, Yap K, Brown WA, Laurie C, O'Donnell M, Hebbard G, Kalff V, and O'Brien PE. Changes in satiety, supra- and infraband transit, and gastric emptying following laparoscopic adjustable gastric banding: a prospective follow-up study. *Obesity surgery* 21: 217-223, 2011b.

Camilleri M, Toouli J, Herrera MF, Kulseng B, Kow L, Pantoja JP, Marvik R, Johnsen G, Billington CJ, Moody FG, Knudson MB, Tweden KS, Vollmer M, Wilson RR, and Anvari M. Intra-abdominal vagal blocking (VBLOC therapy): Clinical results with a new implantable medical device. *Surgery* 143: 723-731, 2008.

Cardin S, Jackson PA, Edgerton DS, Neal DW, Coffey CS, and Cherrington AD. Effect of Vagal Cooling on the Counterregulatory Response to Hypoglycemia Induced by a Low Dose of Insulin in the Conscious Dog. *Diabetes* 50: 558-564, 2001.

Ceelen W, Walder J, Cardon A, Van Renterghem K, Hesse U, El Malt M, and Pattyn P. Surgical treatment of severe obesity with a low-pressure adjustable gastric band: experimental data and clinical results in 625 patients. *Ann Surg* 237: 10-16, 2003.

Chambers AP, Kirchner H, Wilson-Perez HE, Willency JA, Hale JE, Gaylinn BD, Thorner MO, Pfluger PT, Gutierrez JA, Tschop MH, Sandoval DA, and Seeley RJ. The effects of vertical sleeve gastrectomy in rodents are ghrelin independent. *Gastroenterology* 144: 50-52, 2013.

Chapman AE, Kiroff G, Game P, Foster B, O'Brien P, Ham J, and Maddern GJ. Laparoscopic adjustable gastric banding in the treatment of obesity: A systematic literature review. *Surgery* 135: 326-351, 2004.

Cohen R, Pinheiro, J., Correa, J., Schiavon C. Laprascopic Roux-en-Y gastric bypass for BMI <35kg/m^2: a tailored approach. *Surg Obes Relat Dis* 2: 2006.

Covasa M, and Ritter RC. Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin. *Peptides* 19: 1407-1415, 1998.

Cummings D. Endocrine mechanisms mediating remission of diabetes after gastric bypass surgery. *International journal of obesity* 33: S33-S40, 2009.

Cummings DE, and Overduin J. Gastrointestinal regulation of food intake. *The Journal of clinical investigation* 117: 13-23, 2007.

Cummings DE, Overduin J, and Foster-Schubert KE. Gastric Bypass for Obesity: Mechanisms of Weight Loss and Diabetes Resolution. *Journal of Clinical Endocrinology & Metabolism* 89: 2608-2615, 2004.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, and Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 346: 1623-1630, 2002.

Dapri G, Cadiere GB, and Himpens J. Laparoscopic seromyotomy for long stenosis after sleeve gastrectomy with or without duodenal switch. *Obes Surg* 19: 495-499, 2009.

Dapri G, Vaz C, Cadiere GB, and Himpens J. A prospective randomized study comparing two different techniques for laparoscopic sleeve gastrectomy. *Obes Surg* 17: 1435-1441, 2007.

Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, and Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123: 1120-1128, 2002a.

Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, and Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123: 1120-1128, 2002b.

de Sa VC, Ferraz AA, Campos JM, Ramos AC, Araujo JG, Jr., and Ferraz EM. Gastric bypass in the treatment of type 2 diabetes in patients with a BMI of 30 to 35 kg/m2. *Obes Surg* 21: 283-287, 2011.

Demaria EJ, and Jamal MK. Surgical options for obesity. *Gastroenterol Clin North Am* 34: 127-142, 2005.

Dixon AF, Dixon JB, and O'Brien PE. Laparoscopic adjustable gastric banding induces prolonged satiety: a randomized blind crossover study. *J Clin Endocrinol Metab* 90: 813-819, 2005.

Drucker D. The role of gut hormones in glucose homeostasis. *J Clin Invest* 117: 24-32, 2007.

Eisendrath P, Cremer M, Himpens J, Cadiere GB, Le Moine O, and Deviere J. Endotherapy including temporary stenting of fistulas of the upper gastrointestinal tract after laparoscopic bariatric surgery. *Endoscopy* 39: 625-630, 2007.

Emond M, Schwartz GJ, and Moran TH. Meal-related stimuli differentially induce c-Fos activation in the nucleus of the solitary tract. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 280: R1315-R1321, 2001.

Farrell T, Haggerty S, Overby D, Kohn G, Richardson W, and Fanelli R. Clinical application of laparoscopic bariatric surgery: an evidence-based review. *Surgical Endoscopy* 23: 930-949, 2009.

Florentin M, Liberopoulos EN, and Elisaf MS. Sibutramine-associated adverse effects: a practical guide for its safe use. *Obesity Reviews* 9: 378-387, 2008.

Frezza EE. Laparoscopic Vertical Sleeve Gastrectomy for Morbid Obesity. The Future Procedure of Choice? *Surg Today* 37: 275-281, 2007.

Frezza EE, Herbert H, and Wachtel MS. Combined laparoscopic gastric banding and stomach reduction (GBSR): initial experience after 1 year. *Obes Surg* 18: 690-694, 2008.

Gagner M, and Rogula T. Laparoscopic Reoperative Sleeve Gastrectomy for Poor Weight Loss after Biliopancreatic Diversion with Duodenal Switch. *Obesity Surgery* 13: 649-654, 2003.

Galvani C, Gorodner M, Moser F, Baptista M, Chretien C, Berger R, and Horgan S. Laparoscopic adjustable gastric band versus laparoscopic Roux-en-Y gastric bypass. *Surgical Endoscopy* 20: 934-941, 2006.

Garb J, Welch G, Zagarins S, Kuhn J, and Romanelli J. Bariatric Surgery for the Treatment of Morbid Obesity: A Meta-analysis of Weight Loss Outcomes for Laparoscopic Adjustable Gastric Banding and Laparoscopic Gastric Bypass. *Obesity Surgery* 19: 1447-1455, 2009.

Ghatei M, Uttenthal L, Christofides N, Bryant M, and Bloom S. Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. *Journal of Clinical Endocrinology & Metabolism* 57: 488-495, 1983.

Goodrick GK, Poston WS, 2nd, and Foreyt JP. Methods for voluntary weight loss and control: update 1996. *Nutrition* 12: 672-676, 1996.

Grundlingh J, Dargan P, El-Zanfaly M, and Wood D. 2,4-Dinitrophenol (DNP): A Weight Loss Agent with Significant Acute Toxicity and Risk of Death. *J Med Toxicol* 7: 205-212, 2011.

Guillot E, Coste A, and Angel I. Involvement of capsaicin-sensitive nerves in the regulation of glucose tolerance in diabetic rats. *Life Sciences* 59: 969-977, 1996.

Habegger KM, Kirchner H, Yi C-X, Heppner KM, Sweeney D, Ottaway N, Holland J, Amburgy S, Raver C, and Krishna R. GLP-1R agonism enhances adjustable gastric banding in diet-induced obese rats. *Diabetes* 62: 3261-3267, 2013.

Hahn TM, Breininger JF, Baskin DG, and Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nature neuroscience* 1: 271-272, 1998.

Hanusch-Enserer U, Cauza E, Brabant G, Dunky A, Rosen H, Pacini G, Tuchler H, Prager R, and Roden M. Plasma Ghrelin in Obesity before and after Weight Loss after Laparoscopical Adjustable Gastric Banding. *J Clin Endocrinol Metab* 89: 3352-3358, 2004.

Harris JL, Pomeranz JL, Lobstein T, and Brownell KD. A Crisis in the Marketplace: How Food Marketing Contributes to Childhood Obesity and What Can Be Done. *Annual Review of Public Health* 30: 211-225, 2009.

Hayes MR, and Covasa M. Gastric distension enhances CCK-induced Fos-like immunoreactivity in the dorsal hindbrain by activating 5-HT3 receptors. *Brain Research* 1088: 120-130, 2006.

Henderson DC, Fan X, Copeland PM, Borba CP, Daley TB, Nguyen DD, Zhang H, Hayden D, Freudenreich O, Cather C, Evins AE, and Goff DC. A double-blind, placebo-controlled trial of sibutramine for clozapine-associated weight gain. *Acta Psychiatrica Scandinavica* 115: 101-105, 2007.

Holzer HH, Turkelson CM, Solomon TE, and Raybould HE. *Intestinal lipid inhibits gastric emptying via CCK and a vagal capsaicin-sensitive afferent pathway in rats*. 1994, p. G625-G629.

Holzer P. II. The elusive action of capsaicin on the vagus nerve. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 275: G8-G13, 1998.

Huang CK, Shabbir A, Lo CH, Tai CM, Chen YS, and Houng JY. Laparoscopic Roux-en-Y gastric bypass for the treatment of type II diabetes mellitus in Chinese patients with body mass index of 25-35. *Obes Surg* 21: 1344-1349, 2011.

Huo L, Maeng L, Bjorbaek C, and Grill HJ. Leptin and the control of food intake: neurons in the nucleus of the solitary tract are activated by both gastric distension and leptin. *Endocrinology* 148: 2189-2197, 2007.

IASO IAftSoO. Overweight and Obesity – The Global Picture. 2010.

Jackness C, Karmally W, Febres G, Conwell IM, Ahmed L, Bessler M, McMahon DJ, and Korner J. Very Low–Calorie Diet Mimics the Early Beneficial Effect of Roux-en-Y Gastric Bypass on Insulin Sensitivity and β-Cell Function in Type 2 Diabetic Patients. *Diabetes* 62: 3027-3032, 2013.

Jackson PA, Cardin S, and Coffey CS. *Effect of hepatic denervation on the counterregulatory response to insulin-induced hypoglycemia in the dog*. 2000, p. E1249-E1257.

Jagger A, Grahn J, and C. Ritter R. Reduced vagal sensory innervation of the small intestinal myenteric plexus following capsaicin treatment of adult rats. *Neuroscience Letters* 236: 103-106, 1997.

James J, Thomas P, and Kerr D. *Preventing childhood obesity: two year follow-up results from the Christchurch obesity prevention programme in schools (CHOPPS)*. 2007, p. 762.

Jancsó G, and Such G. Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat. *The Journal of Physiology* 341: 359-370, 1983.

Jong JR, Ramshorst B, Gooszen HG, Smout AJPM, and Tiel-Van Buul MMC. Weight Loss After Laparoscopic Adjustable Gastric Banding is not Caused by Altered Gastric Emptying. *Obesity surgery* 19: 287-292, 2009.

Kampe J, Brown W, Stefanidis A, Dixon J, and Oldfield B. A Rodent Model of Adjustable Gastric Band Surgery—Implications for the Understanding of Underlying Mechanisms. *Obesity Surgery* 19: 625-631, 2009.

Kampe J, Stefanidis A, Lockie SH, Brown WA, Dixon JB, Odoi A, Spencer SJ, Raven J, and Oldfield BJ. Neural and humoral changes associated with the adjustable gastric band: insights from a rodent model. *Int J Obes* 36: 1403-1411, 2012.

Karlsson S. *Involvement of capsaicin-sensitive nerves in regulation of insulin secretion and glucose tolerance in conscious mice*. 1994, p. R1071-R1077.

Kelley DE, Wing R, Buonocore C, Sturis J, Polonsky K, and Fitzsimmons M. Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 77: 1287-1293, 1993.

Khoursheed M, Al-Bader I, Al-asfar F, Mohammad A, Shukkur M, and Dashti H. Revision of Failed Bariatric Procedures to Roux-en-Y Gastric Bypass (RYGB). *Obesity Surgery* 21: 1157-1160, 2011.

Klaiber C, Metzger A, and Forsell P. [Laparoscopic gastric banding]. *Chirurg* 71: 146-151, 2000.

Korner J, Bessler M, Inabnet W, Taveras C, and Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. *Surgery for Obesity and Related Diseases* 3: 597-601, 2007.

Korner J, Bessler, M., Inabnet, W., Taveras, C., Holst, J. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. *Surg Obes Relat Dis* 3: 597-601, 2007.

Korner J, Inabnet W, Febres G, Conwell IM, McMahon DJ, Salas R, Taveras C, Schrope B, and Bessler M. Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass. *Int J Obes (Lond)* 33: 786-795, 2009.

Kral JG, Görtz L, Hermansson G, and Wallin GS. Gastroplasty for obesity: Long-term weight loss improved by vagotomy. *World Journal of Surgery* 17: 75-78, 1993.

Ladabaum U, Minoshima S, Hasler WL, Cross D, Chey WD, and Owyang C. Gastric distention correlates with activation of multiple cortical and subcortical regions. *Gastroenterology* 120: 369-376, 2001.

Laferrere B, Heshka, S., Wang, K., Khan, Y., McGinty, J, Teixeira, J. Incretin levels and effect are markedly enhanced 1 month after Rouz-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care* 30: 1709-1716, 2007.

Laferrère B, Teixeira J, McGinty J, Tran H, Egger JR, Colarusso A, Kovack B, Bawa B, Koshy N, Lee H, Yapp K, and Olivan B. Effect of Weight Loss by Gastric Bypass Surgery Versus Hypocaloric Diet on Glucose and Incretin Levels in Patients with Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism* 93: 2479-2485, 2008.

Laffin M, Chau J, Gill RS, Birch DW, and Karmali S. Sleeve Gastrectomy and Gastroesophageal Reflux Disease. *Journal of Obesity* 2013: 6, 2013.

Langer FB, Bohdjalian A, Felberbauer FX, Fleischmann E, Reza Hoda MA, Ludvik B, Zacherl J, Jakesz R, and Prager G. Does gastric dilatation limit the success of sleeve gastrectomy as a sole operation for morbid obesity? *Obes Surg* 16: 166-171, 2006.

Lantz H, Peltonen M, Ågren L, and Torgerson JS. A dietary and behavioural programme for the treatment of obesity. A 4-year clinical trial and a long-term posttreatment follow-up. *Journal of Internal Medicine* 254: 272-279, 2003.

Lauer MS. Lemons for obesity. *Ann Intern Med* 157: 139-140, 2012.

Le Roux CW. Attenuated Peptide YY Release in Obese Subjects Is Associated with Reduced Satiety. *Endocrinology* 147: 3-8, 2006.

le Roux CW, Neary NM, Halsey TJ, Small CJ, Martinez-Isla AM, Ghatei MA, Theodorou NA, and Bloom SR. Ghrelin Does Not Stimulate Food Intake in Patients with Surgical Procedures Involving Vagotomy. *Journal of Clinical Endocrinology & Metabolism* 90: 4521-4524, 2005.

Lee C, Cirangle P, and Jossart G. Vertical gastrectomy for morbid obesity in 216 patients: report of two-year results. *Surgical Endoscopy* 21: 1810-1816, 2007.

Lee WJ, Chong K, Chen CY, Chen SC, Lee YC, Ser KH, and Chuang LM. Diabetes remission and insulin secretion after gastric bypass in patients with body mass index <35 kg/m2. *Obes Surg* 21: 889-895, 2011.

Leonetti F, Silecchia G, Iacobellis G, Ribaudo MC, Zappaterreno A, Tiberti C, Iannucci CV, Perrotta N, Bacci V, Basso MS, Basso N, and Di Mario U. Different Plasma Ghrelin Levels after Laparoscopic Gastric Bypass and Adjustable Gastric Banding in Morbid Obese Subjects. *J Clin Endocrinol Metab* 88: 4227-4231, 2003.

Maggard MA, Shugarman LR, Suttorp M, Maglione M, Sugerman HJ, Livingston EH, Nguyen NT, Li Z, Mojica WA, Hilton L, Rhodes S, Morton SC, and Shekelle PG. Meta-Analysis: Surgical Treatment of Obesity. *Annals of Internal Medicine* 142: 547-559, 2005.

Mann T, Tomiyama AJ, Westling E, Lew AM, Samuels B, and Chatman J. Medicare's search for effective obesity treatments: diets are not the answer. *Am Psychol* 62: 220-233, 2007.

Marciani L, Pfeiffer JC, Hort J, Head K, Bush D, Taylor AJ, Spiller RC, Francis S, and Gowland PA. Improved methods for fMRI studies of combined taste and aroma stimuli. *Journal of Neuroscience Methods* 158: 186-194, 2006.

Martin MB, and Earle KR. Laparoscopic adjustable gastric banding with truncal vagotomy: any increased weight loss? *Surg Endosc* 27: 27, 2011.

Martinez V. Distension of the proximal colon induces FOS in the central nervous system in conscious rats: Role of Capsaicin sensitive afferents: V. Martinez, L. Wang, J.-C. Marvizón, Y. Taché CURE/GBC, VA Med. Ctr., Brain Res. Inst. and Dept. med., UCLA. Los Angeles, CA 90073. *Gastroenterology* 108: A645, 1995.

Martínez V, Wang L, and Taché Y. Proximal colon distension induces Fos expression in the brain and inhibits gastric emptying through capsaicin-sensitive pathways in conscious rats. *Brain Research* 1086: 168-180, 2006.

Martling C-R, Saria A, Andersson P, and Lundberg JM. Capsaicin pretreatment inhibits vagal cholinergic and non-cholinergic control of pulmonary mechanics in the guinea pig. *Naunyn-Schmiedeberg's Archives of Pharmacology* 325: 343-348, 1984.

McCann MJ, Verbalis JG, and Stricker EM. Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats. *Journal of the Autonomic Nervous System* 23: 265-272, 1988.

McLaughlin CL, and Baile CA. Decreased sensitivity of Zucker obese rats to the putative satiety agent cholecystokinin. *Physiology & Behavior* 25: 543-548, 1980.

McLaughlin CL, and Baile CA. Obese mice and the satiety effects of cholecystokinin, bombesin and pancreatic polypeptide. *Physiology & Behavior* 26: 433-437, 1981.

McLaughlin CL, Baile CA, Della-Fera MA, and Kasser TG. Meal-stimulated increased concentrations of CCK in the hypothalamus of Zucker obese and lean rats. *Physiology & Behavior* 35: 215-220, 1985.

Mistry SB, Omana JJ, and Kini S. Rat models for bariatric surgery and surgery for type 2 diabetes mellitus. *Obes Surg* 19: 655-660, 2009.

Mönnikes H, Rüter J, König M, Grote C, Kobelt P, Klapp BF, Arnold R, Wiedenmann B, and Tebbe JJ. Differential induction of c-fos expression in brain nuclei by noxious and non-noxious colonic distension:: role of afferent C-fibers and 5-HT3 receptors. *Brain Research* 966: 253-264, 2003.

Monteiro M, Monteiro J, Águas A, and Cardoso M. A Rat Model of Restrictive Bariatric Surgery with Gastric Banding. *Obesity Surgery* 16: 48-51, 2006.

Moo T-A, and Rubino F. Gastrointestinal surgery as treatment for type 2 diabetes. *Current Opinion in Endocrinology, Diabetes and Obesity* 15: 153-158, 2008.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, and Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 409: 194-198, 2001.

Narayan KMV, Boyle JP, Thompson TJ, Gregg EW, and Williamson DF. Effect of BMI on Lifetime Risk for Diabetes in the U.S. *Diabetes Care* 30: 1562-1566, 2007.

Niijima A. Glucose-sensitive afferent nerve fibers in the liver and their role in food intake and blood glucose regulation. *Journal of the Autonomic Nervous System* 9: 207-220, 1983.

Nisoli E, and Carruba MO. An assessment of the safety and efficacy of sibutramine, an anti-obesity drug with a novel mechanism of action. *Obesity Reviews* 1: 127-139, 2000.

Noel P, Nedelcu M, Nocca D, Schneck AS, Gugenheim J, Iannelli A, and Gagner M. Revised sleeve gastrectomy: another option for weight loss failure after sleeve gastrectomy. *Surg Endosc* 28: 1096-1102, 2014.

Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia* 43: 533-549, 2000.

O'Brien P, McPhail, T., Chaston, T., Dixon, J. Systematic Review of Medium-Term Weight Loss after Bariatric Operations. *Obesity Surgery* 16: 1032-1040, 2006.

O'Brien PE, MacDonald L, Anderson M, Brennan L, and Brown WA. Long-term outcomes after bariatric surgery: fifteen-year follow-up of adjustable gastric banding and a systematic review of the bariatric surgical literature. *Ann Surg* 257: 87-94, 2013.

O'Brien P, Brennan L, Laurie C, and Brown W. Intensive Medical Weight Loss or Laparoscopic Adjustable Gastric Banding in the Treatment of Mild to Moderate Obesity: Long-Term Follow-up of a Prospective Randomised Trial. *Obesity Surgery* 1-9, 2013.

O'Hara B, Phongsavan P, Eakin E, Develin E, Smith J, Greenaway M, and Bauman A. Effectiveness of Australia's Get Healthy Information and Coaching Service®: maintenance of self-reported anthropometric and behavioural changes after program completion. *BMC Public Health* 13: 1-14, 2013.

Ogden Cl FKMCMDJCL. PRevalence and trends in overweight among us children and adolescents, 1999-2000. *JAMA* 288: 1728-1732, 2002.

Okazaki H, Tanaka K, Nagase H, and Inoue S. Modulation of insulin secretion by hepatic vagotomy in cirrhotic rats. *Physiology & Behavior* 53: 521-525, 1993.

Oster G, Thompson D, Edelsberg J, Bird AP, and Colditz GA. Lifetime health and economic benefits of weight loss among obese persons. *American Journal of Public Health* 89: 1536-1542, 1999.

Page AJ, Symonds E, Peiris M, Blackshaw LA, and Young RL. Peripheral neural targets in obesity. *British Journal of Pharmacology* 166: 1537-1558, 2012.

Papailiou J, Albanopoulos K, Toutouzas K, Tsigris C, Nikiteas N, and Zografos G. Morbid Obesity and Sleeve Gastrectomy: How Does It Work? *Obesity Surgery* 20: 1448-1455, 2010.

Parton LE, Ye CP, Coppari R, Enriori PJ, Choi B, Zhang C-Y, Xu C, Vianna CR, Balthasar N, Lee CE, Elmquist JK, Cowley MA, and Lowell BB. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature* 449: 228-232, 2007.

Patriti A, Facchiano E, Annetti C, Aisa MC, Galli F, Fanelli C, and Donini A. Early improvement of glucose tolerance after ileal transposition in a non-obese type 2 diabetes rat model. *Obes Surg* 15: 1258-1264, 2005.

Paxinos G, and Watson C. *The rat brain in stereotaxic coordinates: hard cover edition*. Academic press, 2006.

Perugini Ra MRCDR, and et al. Predictors of complication and suboptimal weight loss after laparoscopic roux-en-y gastric bypass: A series of 188 patients. *Archives of Surgery* 138: 541-546, 2003.

Philipson TJ, and Posner RA. The Long-Run Growth in Obesity as a Function of Technological Change. *National Bureau of Economic Research Working Paper Series* No. 7423: 1999.

Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J, and Group ftR-NAS. Effect of Rimonabant, a Cannabinoid-1 Receptor Blocker, on Weight and Cardiometabolic Risk Factors in Overweight or Obese Patients. *JAMA: The Journal of the American Medical Association* 295: 761-775, 2006.

Pink B. Australian Health Survey: First Results, 2011-12. 2012.
Podnos YD, Jimenez JC, Wilson SE, Stevens CM, and Nguyen NT. Complications After Laparoscopic Gastric Bypass: A Review of 3464 Cases. *Arch Surg* 138: 957-961, 2003.
Pories W, Swanason, MS., MacDonald, K., Long, S., Morris P., Brown, B., Barakat, H., deRamon, R., Israel, G., Dolezal, J., Dohm, L. Who would have thought it? An operation proves to be the most effective therapy for adult onset diabetes mellitus. *Ann Surg* 222: 339-350, 1995.
Powers P, Rosemurgy A, Boyd F, and Perez A. Outcome of Gastric Restriction Procedures: Weight, Psychiatric Diagnoses, and Satisfaction. *Obesity Surgery* 7: 471-477, 1997.
Puzziferri N, Roshek TB, 3rd, Mayo HG, Gallagher R, Belle SH, and Livingston EH. Long-term follow-up after bariatric surgery: a systematic review. *JAMA* 312: 934-942, 2014.
Rao WS, Shan CX, Zhang W, Jiang DZ, and Qiu M. A Meta-Analysis of Short-Term Outcomes of Patients with Type 2 Diabetes Mellitus and BMI </=35 kg/m Undergoing Roux-en-Y Gastric Bypass. *World J Surg* 27: 27, 2014.
Raybould HE. Capsaicin-sensitive vagal afferents and CCK in inhibition of gastric motor function induced by intestinal nutrients. *Peptides* 12: 1279-1283, 1991.
Raybould HE. Visceral perception: sensory transduction in visceral afferents and nutrients. *Gut* 51: i11-i14, 2002.
Raybould HE, and Taché Y. Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anesthetized rats. *European Journal of Pharmacology* 167: 237-243, 1989.
Rinaman L, Baker EA, Hoffman GE, Stricker EM, and Verbalis JG. Medullary c-Fos activation in rats after ingestion of a satiating meal. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 275: R262-R268, 1998.
Rinaman L, and Schwartz G. Anterograde Transneuronal Viral Tracing of Central Viscerosensory Pathways in Rats. *The Journal of Neuroscience* 24: 2782-2786, 2004.
Ritter S, and Dinh TT. Capsaicin-induced neuronal degeneration: Silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. *The Journal of Comparative Neurology* 271: 79-90, 1988.
Sabbatini M, Molinari C, Grossini E, Mary DASG, Vacca G, and Cannas M. The pattern of c-Fos immunoreactivity in the hindbrain of the rat following stomach distension. *Experimental Brain Research* 157: 315-323, 2004.
Sahu A. Minireview: A hypothalamic role in energy balance with special emphasis on leptin. *Endocrinology* 145: 2613-2620, 2004.
Sayegh AI, and Ritter RC. Vagus nerve participates in CCK-induced Fos expression in hindbrain but not myenteric plexus. *Brain Research* 878: 155-162, 2000.
Schauer P, Burguera, B.., Ikramuddin, S., Cottam, D., Gourash, W., Hamad, G., Eid, G., Mattar, S., Ramanathan, R., Barinas, E., Rao, H., Kuller, L. Effect of Laprascopic Roux En Y Gastric Bypass on type 2 diabetes mellitus. *Ann Surg* 238: 467-484, 2003.
Schirra J, and Göke B. The physiological role of GLP-1 in human: incretin, ileal brake or more? *Regulatory Peptides* 128: 109-115, 2005.
Schwartz GJ. The role of gastrointestinal vagal afferents in the control of food intake: current prospects. *Nutrition (Burbank, Los Angeles County, Calif)* 16: 866-873, 2000.
Schwartz GJ, and Moran TH. Duodenal nutrient exposure elicits nutrient-specific gut motility and vagal afferent signals in rat. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 274: R1236-R1242, 1998.
Scozzari G, Zanini M, Cravero F, Passera R, Rebecchi F, and Morino M. High incidence of trocar site hernia after laparoscopic or robotic Roux-en-Y gastric bypass. *Surg Endosc* 2: 2, 2014.
Serdula MK, Ivery D, Coates RJ, Freedman DS, Williamson DF, and Byers T. Do Obese Children Become Obese Adults? A Review of the Literature. *Preventive Medicine* 22: 167-177, 1993.

Serra C, Baltasar A, Andreo L, Perez N, Bou R, Bengochea M, and Chisbert JJ. Treatment of gastric leaks with coated self-expanding stents after sleeve gastrectomy. *Obes Surg* 17: 866-872, 2007.

Shapiro RE, and Miselis RR. The central organization of the vagus nerve innervating the stomach of the rat. *The Journal of Comparative Neurology* 238: 473-488, 1985.

Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, and Nakao K. Ghrelin, an Endogenous Growth Hormone Secretagogue, Is a Novel Orexigenic Peptide That Antagonizes Leptin Action Through the Activation of Hypothalamic Neuropeptide Y/Y1 Receptor Pathway. *Diabetes* 50: 227-232, 2001.

Smith GP, Jerome C, and Norgren R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *Am J Physiol* 249: R638-R641, 1985.

Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, Bays H, and Shanahan WR. Multicenter, Placebo-Controlled Trial of Lorcaserin for Weight Management. *New England Journal of Medicine* 363: 245-256, 2010.

Sobhani I, Buyse M, Goiot H, Weber N, Laigneau JP, Henin D, Soul JC, and Bado A. Vagal stimulation rapidly increases leptin secretion in human stomach. *Gastroenterology* 122: 259-263, 2002.

Sobocki J, Krolczyk G, Herman RM, Matyja A, and Thor PJ. Influence of vagal nerve stimulation on food intake and body weight--results of experimental studies. *J Physiol Pharmacol* 6: 27-33, 2005.

Stanley G, Kyrkouli SE, Lampert S, and Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. *Peptides* 7: 1189-1192.

Strader AD, and Woods SC. Gastrointestinal hormones and food intake. *Gastroenterology* 128: 175-191, 2005.

Stroh C, Weiner R, Wolff S, Knoll C, and Manger T. Influences of Gender on Complication Rate and Outcome after Roux-en-Y Gastric Bypass: Data Analysis of More Than 10,000 Operations from the German Bariatric Surgery Registry. *Obes Surg* 24: 1625-1633, 2014.

Suzuki S, Ramos EJB, Goncalves CG, Chen C, and Meguid MM. Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model. *Surgery* 138: 283-290, 2005.

Swartz TD, Duca FA, and Covasa M. Differential feeding behavior and neuronal responses to CCK in obesity-prone and -resistant rats. *Brain Research* 1308: 79-86, 2010.

Sweeney ME, Hill JO, Heller PA, Baney R, and DiGirolamo M. Severe vs moderate energy restriction with and without exercise in the treatment of obesity: efficiency of weight loss. *Am J Clin Nutr* 57: 127-134, 1993.

Tassone F, Broglio F, Destefanis S, Rovere S, Benso A, Gottero C, Prodam F, Rossetto R, Gauna C, van der Lely AJ, Ghigo E, and Maccario M. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. *J Clin Endocrinol Metab* 88: 5478-5483, 2003.

Taylor IL. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. *Gastroenterology* 88: 731-737, 1985.

Thorens B, and Larsen PJ. Gut-derived signaling molecules and vagal afferents in the control of glucose and energy homeostasis. *Current Opinion in Clinical Nutrition & Metabolic Care* 7: 471-478, 2004.

Thornton C, Rozen W, So D, Kaplan E, and Wilkinson S. Reducing Band Slippage in Laparoscopic Adjustable Gastric Banding: The Mesh Plication Pars Flaccida Technique. *Obesity Surgery* 19: 1702-1706, 2009.

van de Wall EHEM, Gram DX, Strubbe JH, Scheurink AJW, and Koolhaas JM. Ablation of capsaicin-sensitive afferent nerves affects insulin response during an intravenous glucose tolerance test. *Life Sciences* 77: 1283-1292, 2005.

Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, and Rössner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *The Lancet* 365: 1389-1397, 2005.

Varela JE. Laparoscopic sleeve gastrectomy versus laparoscopic adjustable gastric banding for the treatment severe obesity in high risk patients. *Jsls* 15: 486-491, 2011.
Wang G-J, Tomasi D, Backus W, Wang R, Telang F, Geliebter A, Korner J, Bauman A, Fowler JS, Thanos PK, and Volkow ND. Gastric distention activates satiety circuitry in the human brain. *NeuroImage* 39: 1824-1831, 2008.
Wang Y-H, Raybould HE, and Wei JY. Mechanosensitivity and chemosensitivity of vagal afferents innervating the duodenum in vitro. *Gastroenterology* 118: A173, 2000.
WHO. Obesity and overweight. World Health Organisation, 2011.
Wilding J. *Pharmacological Approaches for Treating Obesity*. John Wiley & Sons, Ltd, 2009, p. 421-446.
Williams DL, Grill HJ, Cummings DE, and Kaplan JM. Vagotomy Dissociates Short- and Long-Term Controls of Circulating Ghrelin. *Endocrinology* 144: 5184-5187, 2003.
Williams KW, and Elmquist JK. From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. *Nat Neurosci* 15: 1350-1355, 2012.
Willing AE, and Berthoud HR. Gastric distension-induced c-fos expression in catecholaminergic neurons of rat dorsal vagal complex. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 272: R59-R67, 1997.
Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, and Bergman RN. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* 17: 30-36, 1994.
Woods SC, Seeley RJ, Porte D, and Schwartz MW. Signals That Regulate Food Intake and Energy Homeostasis. *Science* 280: 1378-1383, 1998.
Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA, and Bloom SR. The Novel Hypothalamic Peptide Ghrelin Stimulates Food Intake and Growth Hormone Secretion. *Endocrinology* 141: 4325-4328, 2000.
Wynne K, Park A, Small C, Meeran K, Ghatei M, Frost G, and Bloom S. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *International journal of obesity* 30: 1729-1736, 2006.
Zagorodnyuk VP, Chen BN, and Brookes SJH. Intraganglionic laminar endings are mechano-transduction sites of vagal tension receptors in the guinea-pig stomach. *The Journal of Physiology* 534: 255-268, 2001.
Zhang J, and Ritter RC. Circulating GLP-1 and CCK-8 reduce food intake by capsaicin-insensitive, nonvagal mechanisms. *American journal of physiology Regulatory, integrative and comparative physiology* 302: R264-273, 2012.