BEHAVIOURAL AND NEUROIMAGING STUDIES OF FOOD REWARD AFTER BARIATRIC SURGERY FOR OBESITY

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Thesis for the Degree of Doctor of Philosophy

2013

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"We all woke up this morning and we had with it the amazing return of our conscious mind. We recovered minds with a complete sense of self and a complete sense of our own existence — yet we hardly ever pause to consider this wonder."

Antonio Damasio

ABSTRACT

BACKGROUND

Roux-en-Y gastric bypass (RYGB) surgery is the most effective treatment for obesity and has greater efficacy for weight loss than gastric banding (BAND) surgery. The superior weight loss seen after RYGB may result from profoundly different effects on food hedonics and reward brought about by physiological changes secondary to the distinct manipulations of gut anatomy.

AIMS

To compare body mass index (BMI) matched patients after RYGB or BAND surgery and unoperated controls using comprehensive phenotyping of brain structure and function, eating behaviour and metabolism.

METHODS

In these cross-sectional studies, un-operated controls and patients after RYGB and BAND surgery had functional and anatomical neuroimaging of food reward systems. Reward responses to food were assessed with a functional magnetic resonance imaging (fMRI) food picture evaluation task. Anatomical differences in grey and white matter were assessed using voxel-based morphometry (VBM) and diffusion tensor imaging (DTI). Eating behaviour, food appeal and palatability, potential mediators, and post-ingestive effects were compared between groups using questionnaires, test meals, food diaries and assay of plasma hormones and metabolites. Surgical patients were compared in both the fasted and fed state, and after administration of the somatostatin analogue, Octreotide, to suppress anorexigenic gut hormone responses after RYGB.

RESULTS

Obese patients after RYGB had healthier gut-brain-hedonic responses to food than patients after BAND surgery. RYGB patients had lower activation than BAND patients in brain reward systems, particularly to high-calorie foods, including the orbitofrontal cortex, amygdala, caudate nucleus, nucleus accumbens and hippocampus. This was associated with lower palatability and appeal of high-calorie foods, and healthier eating behaviour, including less fat intake, in RYGB compared to BAND patients and/or BMI-matched unoperated controls. These differences were not explicable by differences in hunger or psychological traits between the surgical groups, or by differences in brain structure as measured by VBM and DTI. However anorexigenic plasma gut hormones (GLP-1 and PYY), plasma bile acids and symptoms of dumping syndrome were increased in RYGB patients. Octreotide increased nucleus accumbens activation to food pictures, increased food appeal and decreased post-meal satiety in patients after RYGB, but not BAND surgery. The preliminary nature of this small study precludes extensive interpretation especially of the difference between surgical groups. Patients in the operated groups (RYGB and BAND) had lower grey matter density in areas involved in reward processing, including the amygdala, nucleus accumbens and hippocampus compared to BMI-matched controls. There was no difference between the groups in white matter tract integrity.

CONCLUSIONS

Identification of these differences in the gut-brain axis and hence food hedonic responses as a result of altered gut anatomy/physiology provides a novel explanation for the more favorable long-term weight loss seen after RYGB than BAND surgery. This supports targeting of gut-brain reward systems for future treatments of obesity.

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ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Tony Goldstone, Prof. Carel W le Roux, and Prof. Jimmy Bell for their continuing support, mentorship and teaching during this PhD. I would also like to thank my mentor, Prof. Richard Wise for his guidance and support and friends Ella Knight, psychotherapist, and Charl Botha, engineer, for their interest and insights from their respective fields. The completion of these projects would not have been possible without the help and support from my colleagues and in particular Dr. Alexander Miras, Dr. Christina Prechtl, Dr. Navpreet Chhina, Dr. Martin Schmidt and all the students who assisted with the studies. I would like to thank the staff at the Imperial Weight Centre Dr. Lisa Cotter, Ms Debbie O'Rourke, Sister Karen O'Donnell, Torsten Olbers and the staff at the Robert Steiner MRI Unit Ms Giuliana Durighel and Dulcie Rodrigues, and the staff at the Sir John McMichael Research Unit, in particular, Ms Jeanette Davis. I would also like to thank the funding sources including the Wellcome Trust Charity (Clinical Research Training Fellowship WT087745MA), Medical Research Council and Imperial College Healthcare Charity. Finally, my special thanks to the patients and volunteers who took part in these experiments.

This PhD is dedicated to my partner, Albert Du Plessis (for his sacrifices of time, emotional and financial support as well as his insights from a non-medical perspective), my parents (for being tirelessly encouraging and for all the babysitting), to my son Lucas Du Plessis, and my unborn son, as yet unnamed.

STATEMENTS OF WORK BY CANDIDATE

All work described in the thesis was performed by the author. All collaborations and assistance are described below.

Dr. Alex Miras (assisted with recruitment, scanning, data collection and analysis and Table 1.1)

Dr. Navpreet Chhina (assisted with scanning and data collection)

Dr. Christina Prechtl (assisted with dietary record analysis)

Ms Giuliana Durighel and Emer Hughes (radiographers who performed the MRI scanning)

Dr. Michelle Sleeth and Dr. Norlida Mat Daud (assisted with scanning)

Dr. Vincent Royce and Prof. Jamie Alghband-Zadeh (who performed bile acid assays)

Dr. Bruce Gaylinn and Michael Thorner (who performed ghrelin assays)

Mr Paul Beck (who assisted with GLP-1 and PYY assays)

Dr. David Larkman, Prof. Jo Hajnal and Rita Nunes (technical advice and help)

Dr. Adam Waldmann (radiologist who reported the MRI structural scans)

Ms Natalie White (BSc student who assisted with Table 1.2 and whose unpublished work is cited in Chapter 3)

Ms Waaka Mona-Nwinia, Ms Sharzhad Deliran and Ms Koeun Choi (BSc students who assisted with data collection and entry)

Ms Priya Shah (BSc student under my supervision who assisted with data collection and entry)

Ms Alanna Brown (MSc student under my supervision who assisted with data collection and entry)

Dr. Martin Schmidt (assisted with data collection and entry)

PUBLICATIONS PERTAINING DIRECTLY TO WORK IN THIS THESIS

Scholtz S, Miras AD, Chhina N, Prechtl CG, Sleeth M, Daud NM, Ismail N, Durighel D, Ahmed AR, Olbers T, Vincent RP, Alaghband-Zadeh J, Ghatei MA, Waldman AD, Frost GS, Bell JD, le Roux CW, Goldstone AP. Obese patients after gastric bypass surgery have lower brain hedonic responses to food than after gastric banding. *Gut* (in press) doi:10.1136/gutjnl-2013-305008

PUBLICATIONS RELATED TO WORK IN THIS THESIS

Goldstone AP, Prechtl CG, **Scholtz S**, Miras AD, Chhina N, Durighel G, Deliran SS, Beckmann C, Ghatei MA, Ashby D, Waldman AD, Gaylinn BD, Thorner MO, Frost GS, Bloom SR, Bell JD. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex and hippocampal responses to food. *Gastroenterology* (in review)

Stevens T, Spavin S, **Scholtz S**, McClelland L. Your patient and weight-loss surgery. *Advances in Psychiatric Treatment*, 18:418-425, 2012

Fenske, W. K, Pournaras, D. K, Aasheim, E. T, Miras, A. D, Scopinaro, N, **Scholtz, S**, le Roux, C. W. Can a protocol for glycaemic control improve type 2 diabetes outcomes after gastric bypass? *Obesity Surgery*, 22: 90-6, 2012

Scholtz S, le Roux C, Balen A. The role of bariatric surgery in the management of female fertility. *Human Fertility* 13(2): 67-71, 2010.

Scholtz S, Morgan J. Obesity and Psychiatry, *Psychiatry* 8(6), 198-202, 2009

ABSTRACTS PERTAINING DIRECTLY TO WORK IN THIS THESIS

Goldstone AP, Prechtl de Hernandez CG, **Scholtz S**, Durighel G, Deliran SS, Wong T, Ashby D, Frost G, Bloom SR & Bell JD. Ghrelin mimics fasting in biasing food appeal towards high-calorie foods. *OR40-5, Abstracts 92nd Annual Meeting Endocrine Society, San Diego, USA June 2010*

Scholtz S, Le Roux CW, Prechtl de Hernandez CG, Deliran S, Ahmed A, Bell JD & Goldstone AP. Gastric bypass surgery has beneficial effects on eating behaviour and mood. *T3:PO-118, 11th International Congress on Obesity, Stockholm July 2010. Obesity Reviews* 11 Suppl 1: 259, 2010

Scholtz S, Chhina N, Miras A, Prechtl CG, Durighel G, Ahmed A, Olbers T, Mona-Nwinia W, le Roux C, Bell JD, Goldstone AP. Obese patients after gastric bypass surgery have different brain reward responses to high-calorie food and healthier eating behaviour compared to gastric banding. *29th Annual Meeting of the Obesity Society, Orlando USA, October 2011 (Nominated for Ethan Sims Young Investigator Award). Obesity 19 Suppl 1: S59, 2011*

Scholtz S, Miras AD, Chhina N, Prechtl CG, Sleeth M, Mat Daud N, Ismail N, Durighel G, Ahmed AR, Olbers T, Vincent RP, Alaghband-Zadeh J, Ghatei MA, Waldman AD, Frost GS, Bell JD, le Roux CW & Goldstone AP. Obese patients after gastric bypass have healthier gut-brain-hedonic responses to high-calorie food than after gastric banding surgery. *British Neuroscience Association, London 7-10th April 2013*

ABSTRACTS RELATED TO WORK IN THIS THESIS

Goldstone AP, Prechtl de Hernandez CG, **Scholtz S**, Durighel G, Ashby D, Beckmann C, Frost G, Bloom SR & Bell JD. Ghrelin mimics fasting in preferentially increasing reward responses to high-calorie foods. *5-OR, 28th Annual Meeting of the Obesity Society, San Diego USA, October 2010 (Plenary Session). Obesity* 18 suppl 2 S4, 5-OR, 2010

Prechtl de Hernandez CG, Beaver JD, Croese C, Muhammed K, Durighel G, Hughes E, Waldman AD, **Scholtz S**, Frost G, Bell JD & Goldstone AP. Dietary restraint predicts neural responses in executive self-control and affective reward systems to foods of different caloric value. *T4:PO-42, 11th International Congress on Obesity, Stockholm July 2010 (nominated for poster prize). Obesity Reviews* 11 Suppl 1: 312, 2010

Goldstone AP, Prechtl de Hernandez CG, **Scholtz S**, Durighel G, Deliran SS, Wong T, Ashby D, Frost G, Bloom SR & Bell JD. Ghrelin mimics fasting in biasing food appeal towards high-calorie foods. *T2:PO-40, 11th International Congress on Obesity, Stockholm July 2010. Obesity Reviews* 11 Suppl 1: 187, 2010

Prechtl CG, **Scholtz S**, Durighel G, Chhina N, Frost G, Bell JD, Goldstone AP. Insula and orbitofrontal cortex activation to food stimuli predicts human food intake. *T3:P.028, 18th European Congress on Obesity, Istanbul, Turkey, May 2011. Obesity Reviews 12 Suppl 1:135, 2011*

Prechtl CG, **Scholtz S**, Chhina N, Durighel G, Frost G, Bell JD, Goldstone AP. Insula and orbitofrontal cortex activation to food stimuli predicts human food intake depending on nutritional state and caloric density. *29th Annual Meeting of the Obesity Society, Orlando USA, October 2011. Obesity 19 Suppl 1: S172, 2011*

Starke JA, Prechtl CG, **Scholtz S**, Chhina N, Miras A, Durighel G, Leech R, Sharp DJ, Beckmann CF, Frost GS, Bloom SR, Bell JD & Goldstone AP. Lack of modulation of brain resting

state networks by fasting and ghrelin in humans. *42nd Annual Meeting Society for Neuroscience, New Orleans USA, October 2012*

Goldstone AP, Prechtl CG, Starke JA, **Scholtz S**, Chhina N, Miras A, Durighel G, Leech R, Sharp DJ, Beckmann CF, Frost GS & Bell JD. Salience resting state network integrity in the orbitofrontal cortex predicts task activation to viewing high-calorie foods when fasted. *42nd Annual Meeting Society for Neuroscience, New Orleans USA, October 2012*

Goldstone AP, Prechtl CG, **Scholtz S**, Miras AD, Starke JA, Chhina N, Durighel G, Frost GS, Bloom SR & Bell JD. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex and hippocampal responses to food. *British Neuroscience Association, London 7-10th April 2013*

Starke JA, Anjari MA, Prechtl CG, **Scholtz S**, Miras AD, Chhina N, Sleeth M, Frost GS, Bell JD & Goldstone AP. Salience resting state network integrity is increased in obesity and predicts orbitofrontal cortex activation to high-calorie food cues. *British Neuroscience Association, London 7-10th April 2013*

Prechtl CG, **Scholtz S**, Miras AD, Chhina N, Durighel G, Frost GS, Bell JD & Goldstone AP. Amygdala and orbitofrontal activation to visual food cues predicts food intake depending on nutritional state. *British Neuroscience Association, London 7-10th April 2013*

Starke JA, Anjari MA, Prechtl CG, **Scholtz S**, Miras AD, Chhina N, Sleeth M, Frost GS, Bell JD & Goldstone AP. Salience resting state network integrity is increased in obesity and predicts orbitofrontal cortex activation to high-calorie food cues. *21st Annual Meeting of Society for the Study of Ingestive Behavior, New Orleans, USA August 2013*

FREQUENTLY USED ABBREVIATIONS

ANOVA	Analysis Of VAriance
AUC	Area Under the Curve
BAND	Gastric banding
BMI	Body Mass Index
BMI-M	Body Mass Index matched
BOLD	Blood Oxygen Level Dependent
CCK	Cholecystokinin
DRD2/3	Dopamine Receptor D2 or D3
DTI	Diffusion tensor imaging
FDG	^{18}F-fluorodeoxyglucose
fMRI	functional Magnetic Resonance Imaging
FSL	Functional mri of the brain Software Library
GLP-1	Glucagon Like Peptide 1
MOR	Mu-opioid receptor
OFC	Orbitofrontal cortex
PYY	Peptide tyrosine tyrosine
PET	Positron Emission Tomography
rCBF	Regional cerebral blood flow
ROI	Region(s) Of Interest
RYGB	Roux-en-Y Gastric Bypass
VBM	Voxel based morphometry

CHAPTER 1: INTRODUCTION

1.1. The obesity epidemic

Over 300 million people worldwide are obese, and nearly 1 billion adults are overweight, with huge associated medical and socio-economic costs, including diabetes mellitus, cardiovascular disease, certain cancers and psychiatric morbidity (van Hout et al. 2004; Haslam et al. 2005; WHO 2005; Kopelman 2007). The Department of Health predicts prevalence rates of 60% for obesity in adult men, 50% in women, and 25% of children by 2050 in the UK, with consequent annual costs to the NHS of nearly £50 billion (McPherson et al. 2007).

Obesity is defined as a body mass index (BMI = weight (kg) / height $(m)^2$) of more than 30 kg/m^2, using BMI as a marker for adiposity (WHO 2005). Mortality risk is nearly double that of those with normal weight (BMI 20-25 kg/m^2) for people with a BMI above 35 kg/m^2 with risk increasing exponentially above a BMI of 40kg/m^2 (Berrington de Gonzalez et al. 2010), leading to the term morbid obesity for this category.

A full discussion of the reasons for such high rates of obesity in Western, and more recently in developing societies too, is complex and beyond the remit of this thesis. Increasingly, however, the interaction between an "obesogenic" environment where an abundance of highly palatable, high-calorie foods are readily available and factors which make certain individuals more susceptible to consume in excess of their needs in this environment has been blamed for these epidemic levels of obesity (Carnell et al. 2008; Berthoud 2012).

1.2 Treatment of obesity

1.2.1 Lifestyle and medication compared to bariatric surgery

Currently the only successful long-term strategy to treat obesity is bariatric surgery. More traditional forms of weight loss treatment, such as behavioural therapy, or dietary interventions have proven disappointing in comparison (Sjostrom 2008), and have often not been evaluated for long term outcomes (Shaw et al. 2005).

Studies of behavioural and dietary interventions show weight loss of about 8-10% is achieved in 6 -12 months, but that weight regain is common and about half of patients return to baseline weight at 5 years (Shaw et al. 2005; Wadden et al. 2007; Middleton et al. 2012; Wadden et al. 2012). Data from the National Health and Nutrition Examination Survey (NHANES), a home-interview based national survey, with demographics representative of the United States of America (USA) population, reflect these long-term results. In this study, whilst about one out of every six adults (17.3%) who had ever been overweight or obese has accomplished weight loss maintained for 1 year of at least 10%, only 4.4% managed to maintain the 20% weight loss seen in bariatric surgery (Kraschnewski et al. 2010). High levels of weight regain were seen. A study of the same cohort over a shorter 2-year period found that of patients who had lost substantial weight in the previous year, one third had regained more than 5% of their body weight 2 years later (Weiss et al. 2007). Similarly, a meta-analysis of dietary intervention studies in the USA in obese individuals found only modest maintained weight losses of around 3.0 kg, representing a reduced weight of about 3.2% below initial body weight after 5 years (Anderson et al. 2001). There are no meta-analyses of lifestyle intervention studies conducted in the UK.

Large studies conducted in Europe and USA aimed at diabetes prevention, usually in overweight or obese patients have found similar results to the above. The Finnish Diabetes Prevention Study, found 3.5kg weight loss in intensive lifestyle interventions and in the Diabetes Prevention Programme, conducted in USA, lifestyle interventions in conjunction with metformin were more effective than metformin alone in reducing the risk of diabetes (Lagerros et al. 2013).

Most dietary interventions, regardless of regime, appear to be similar in the degree of weight loss they achieve, with only the Mediterranean diet showing greater weight loss than control diets (Mean difference in weight loss -1.84 kg (95% CI: 2.54, 21.15 kg; P<0.00001) (Ajala et al. 2013). On the other hand high-protein, low glycaemic index diets appear to promote weight maintenance after weight loss. In a large European study (773 participants), participants randomized to high protein and low glycaemic index diets had lower study dropout rates, and improved weight maintenance over 6 months after (high-protein diets led to 2.7kg less weight regain than low-protein, and low-glycaemic index led to 0.48g less weight regain than high-glycaemic index diets)(Larsen et al. 2010)

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Very low calorie diets (450-800kcal/day) are effective at producing greater weight loss than low calorie diets alone (17% vs. 10%) after 12 weeks. However these are not recommended for most obese patients, except in specific circumstances (pre-bariatric surgery, or as a catalyst for change in severely obese patients with multiple comorbidity prior to lifestyle intervention) due to the lack of evidence for long term weight maintenance (Baker et al. 2009; 2012)

In comparison, bariatric surgery patients lose on average 20-30% (equivalent of 30-50kg) of their weight in the first year following surgery, and maintain around 16% weight loss at 10-year follow up (Elder et al. 2007; Sjostrom 2008). Roux-en-Y gastric bypass surgery (RYGB) produces more weight loss compared to gastric banding (BAND): 25% vs. 14% at 10 year follow up (Sjostrom 2008; Tice et al. 2008).

One of the largest available sources of information on dietary weight loss and maintenance is the National Weight-Control Registry (NWCR) in the USA. Entry onto the NWCR requires members to be ≥ 18 years of age and to have lost and maintained at least 13.6 kg for ≥ 1 year. Its more than 4,000 members are predominately white (95%), married (64%), college educated (82%) women (77%) in their late 40's. Entrants lost an average of 33 kg and maintained the minimum weight loss (13.6 kg) for an average of 5.7 years. Data from the NWCR are not representative of the general population, and the demographics differ considerably from bariatric surgery patient populations in general. In addition, entry to the register is dependent on successful weight loss in the first place. However it does provide a rare database of patients who have maintained weight loss in the long term that is comparable with bariatric surgery.

From the NWCR a subset of individuals who maintained 10% weight loss for more than five years were studied in more detail. They engaged in high levels of strenuous physical activity (approximately 1 hour/day) and maintained a low-fat, low-calorie diet. They ate breakfast regularly, and maintained a high level of vigilance over their weight and diet. 44% weighed themselves daily and 33% weekly, and they had high levels of dietary restraint (as measured by the Eating Inventory), indicating a need to maintain vigilance over the desire to eat high-calorie foods. They maintained a consistent rigid eating pattern even on weekends (McGuire

et al. 1999; Wing et al. 2005). Similarly, a recent review of lifestyle interventions for weight loss found that low-fat, low-calorie diets (1500-1800 kCal/day and <7% saturated fat/day), vigorous exercise (200-300 minutes/week), regular monitoring of food intake and weight, and regular contact with group or individual therapist to reinforce dietary restraint, were key components to maintaining weight loss of between 4 and 7% for 1 year (Anderson et al. 2001).

By contrast, bariatric surgery patients engage in negligible strenuous physical activity and remain largely sedentary after surgery, though even a small increase in physical activity is associated with improved weight loss (Egberts et al. 2012; Liu et al. 2012). Although significant dietary changes are reported after bariatric surgery, these are not associated with a high degree of dietary restraint in RYGB patients (Kalarchian et al. 1999; Capuron et al. 2011; Laurenius et al. 2012), although BAND surgery patients appear to differ in this respect (Lang et al. 2002; Schindler et al. 2004).

When 105 bariatric surgery patients (58% RYGB, 18% BAND, 24% unspecified) were compared with 210 dieting patients from the NWCR who had lost and maintained a similar amount of weight (>13.6kg over approximately 5 years), surgical patients engaged in significantly less exercise and employed less dietary restraint than their non-surgical counterparts (Bond et al. 2009).

Pharmaceutical interventions are also not as effective as bariatric surgery. Currently only Orlistat, a lipase inhibitor is licensed for treatment of overweight and obesity. This achieves around 3kg more weight loss than placebo by preventing the absorption of fat in the intestine. Steatorrhoea, faecal urgency and faecal incontinence occur if fat is consumed,

thereby acting as a dramatic behavioural deterrent to their ingestion (Padwal et al. 2004; Ara et al. 2012). Orlistat has also shown beneficial effects on cardiovascular risk factors including fasting cholesterol, low density lipoprotein (LDL) and glucose and blood pressure (Zhou et al. 2012). Agents which act on the central nervous system to reduce appetite, such as Sibutramine (a serotonin and nor-adrenalin reuptake inhibitor) and Rimonobant (selective cannabinoid receptor CB1 antagonist) have been withdrawn from the market due to unacceptable side effects (Christensen et al. 2007; James et al. 2010). Sibutramine and Rimonobant achieved similar weight loss (3.73kg and 3.66kg more than placebo respectively), (Burch et al. 2009; Zhou et al. 2012) whereas Sibutramine appeared more cost-effective than either Rimonobant or Orlistat in the longer term (Ara et al. 2012; Gray et al. 2012). Even drugs that are in development and may soon be licensed, including combination and gut hormone therapies do not appear to result in the degree of weight loss that is seen in bariatric surgery. For example, Qnexa (Phentermine-Topiramate) results in 7-11% weight loss over placebo (Gadde et al. 2011) Contrave (Naltrexone-Bupropion) 5-7% (Greenway et al. 2010) and Liraglutide (GLP-1 agonist) 3-5% (Astrup et al. 2009).

Taken together, these data suggest that: (i) successful weight loss of more than 10% of initial body weight sustained over more than 5 years by any means other than bariatric surgery is rare, (ii) in those individuals where successful weight loss and its maintenance is achieved without surgery, regular strenuous physical exercise and a high degree dietary and weight vigilance are required, (iii) bariatric surgery, and in particular RYGB, achieves superior sustained weight loss to both traditional weight loss methods and medication, and (iv) in RYGB surgery, this appears to be achieved without requiring the same levels of conscious dietary restraint and vigilance as other weight loss methods. Understanding how different bariatric procedures bring about weight loss may therefore uncover important and possibly novel mechanisms for successful weight loss and maintenance.

1.2.2 Bariatric surgery

The four most commonly performed bariatric surgery procedures are: Roux-en-Y gastric bypass (RYGB) surgery, laparoscopic gastric banding surgery (BAND), sleeve gastrectomy and biliopancreatic diversion/duodenal switch. In 2011, nearly half of all weight loss procedures performed worldwide were RYGB and 18% BAND (Buchwald et al. 2009; Buchwald et al. 2013). In Europe there has been a sharp decline in the use of BAND surgery, from 63 to 43 to 18% of all bariatric procedures performed in 2003, 2008 and 2011 respectively. RYGB, on the other hand increased from 11 to 39 to 43% over the same years. These trends are different in the USA/Canada, where BAND surgery increased from 9 to 44% between 2003 and 2008, but then fell to 27% in 2011, whereas RYGB decreased from 85 to 51 to 47% in the same period. Sleeve gastrectomy has gained popularity and has increased from 0% in 2003, to nearly a third of all procedures in 2011, in Europe. The biliopancreatic diversion/duodenal switch are rarely used (<2% of procedures) (Astrup et al. 2009; Buchwald et al. 2009).

1.2.2.1 Roux-en-Y gastric bypass (Fig. 1.1)

RYGB is the oldest form of bariatric surgery, invented by Cesar Roux in 1897. It was first performed for the purpose of weight loss in 1969 (Mason et al. 1969), after observations that patients who had undergone partial gastrectomy for other illnesses such as peptic ulcer disease, lost weight. It involves partial gastrectomy, anastomosis of the stomach pouch to the jejunum and entero-entero anastomosis of the excluded biliary and alimentary limbs of the small intestine (See Fig. 1.1). Importantly as a smaller gastric pouch now receives food, the fundus of the stomach (gastric remnant) no longer sees food although it retains its blood supply and secretion of stomach acid continues as normal. Food therefore reaches the distal ileum almost immediately after swallowing, a process that would have taken at least 20 minutes in unaltered anatomy. This early delivery of food into the small intestine is thought

to have important and dramatic effects on appetitive gut hormone secretion which are elaborated on below (le Roux et al. 2006; Dixon et al. 2012; Pournaras et al. 2012). In addition, since the gallbladder continues to secrete bile into the duodenum, bile is delivered via the entero-entero anastomosis (also known as the Roux-en-Y anastomosis) into the small intestine undiluted by food. This alteration of bile flow is recognized to have important effects on intestinal pH, gut intestinal flora and may also have effects on satiety hormone secretion (Li et al. 2011; Pournaras et al. 2012). The vagus nerve is also disrupted by the surgery itself, which is thought to have possible knock-on effects on satiety (Tadross et al. 2009).

Figure 1.1 Roux-en-Y gastric bypass surgery

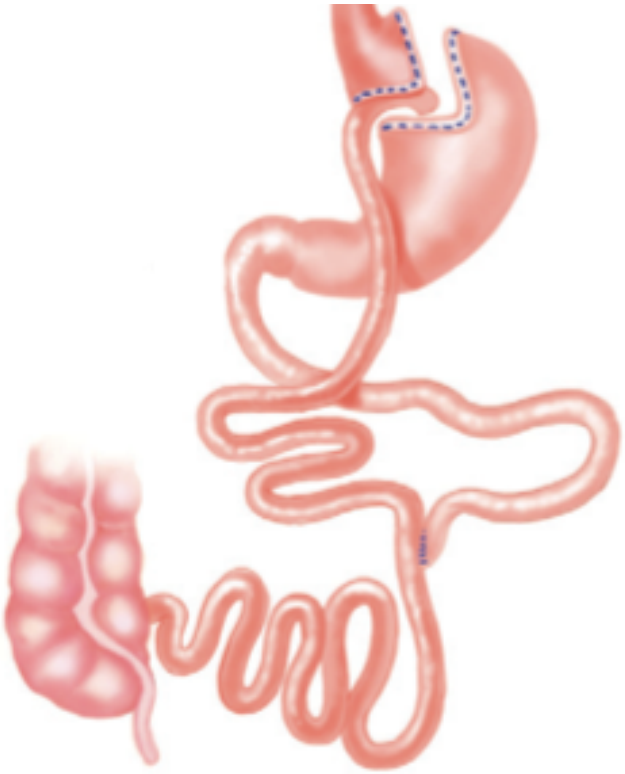


Figure from (Dixon et al. 2012), with copyright permission

1.2.2.2 Adjustable laparoscopic gastric banding (Fig.1.2)

In BAND surgery, a silicon saline-filled band is placed around the proximal pouch of the stomach, creating a much smaller receptacle for food. The stomach is essentially reduced from the size of a fist to the size of a thumb. This restricts the amount and type of food that can be eaten in one sitting and increases intraluminal pressure on vagal afferents (Dixon et al. 2005; Burton et al. 2010). The amount of restriction can be adjusted via a port under the skin through which saline is injected. After food has passed into the fundus of the stomach, its digestion is not altered. Horizontal gastroplasty (HGP), where the proximal stomach is stapled horizontally, and vertical banded gastroplasty (VBG), where it is stapled vertically and a permanent band placed around the entrance to the distal stomach, both create a smaller stomach pouch connected to the distal stomach by a narrow stoma (Doherty 2001). They have been superseded by BAND due to its lower complication rate (Chapman et al. 2004). Due to similarities in the mechanism of weight loss between VBG, HGP and BAND, they are collectively referred to as restrictive operations.

Figure 1.2 Adjustable gastric banding surgery

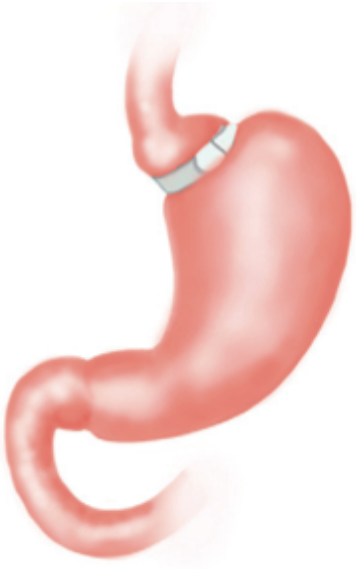


Figure from (Dixon et al. 2012), with copyright permission

1.2.2.3 Sleeve gastrectomy (Fig. 1.3)

The sleeve gastrectomy was initially only performed as part of a two-stage operation in patients with severe morbid obesity (BMI >60 kg/m^2), where a complete RYGB was deemed too dangerous (Brethauer et al. 2009). Partial gastrectomy was performed to reduce stomach volume, whilst maintaining the normal anatomy of the rest of the gastrointestinal tract, including the pylorus. The operation was performed to aid initial weight loss so that a full RYGB or duodenal switch could follow. However it has gained popularity as a stand-alone operation in view of its low surgical risk, and similar favourable metabolic effects and weight loss to RYGB surgery (Brethauer et al. 2009; Gill et al. 2010; Fischer et al. 2012; Rosenthal et al. 2012; Stefater et al. 2012).

Figure 1.3 Sleeve gastrectomy

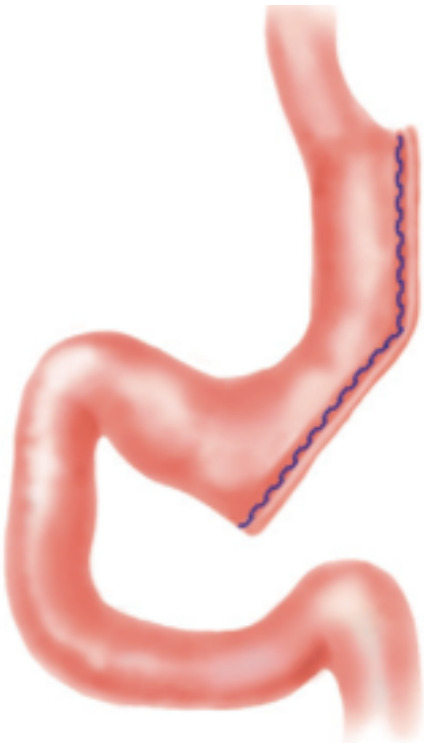
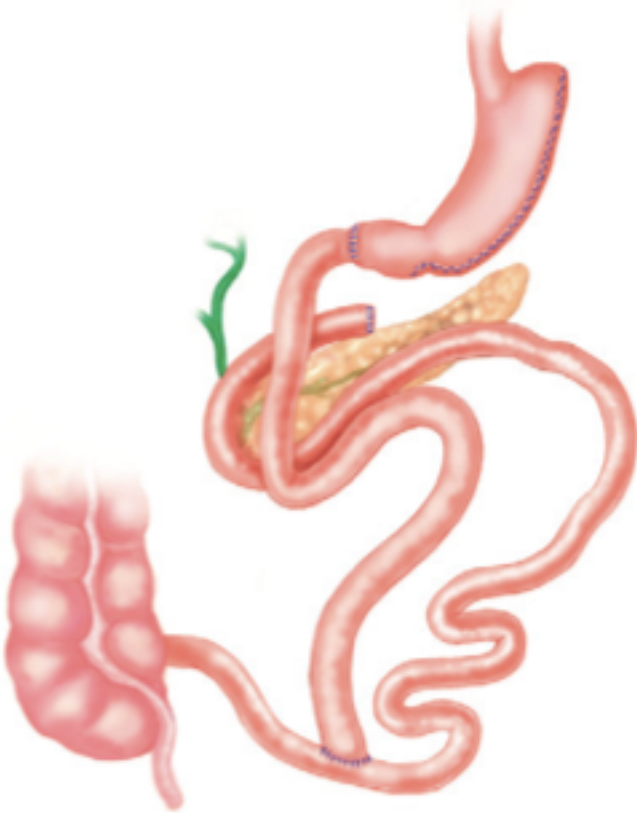


Figure from (Dixon et al. 2012), with copyright permission

1.2.2.4 Biliopancreatic diversion / Duodenal switch (Fig. 1.4)

The original form of this complex operation (biliopancreatic diversion) has been largely replaced by a modification known as duodenal switch (BPS/DS). In this operation, a partial sleeve gastrectomy is performed, and the remaining stomach pouch and duodenum are connected to the distal part of the small intestine (ileum) so that the jejunum is bypassed. Pancreatic and biliary secretions are diverted to the distal part of the ileum. Malabsorption of micro- and macronutrients and consequent unpleasant side effects such as diarrhoea and complications such as metabolic bone disease make this operation much less popular than the others (Dixon et al. 2012), although it is still considered the gold standard by some surgeons (Hess et al. 2005).

Figure 1.4 Biliopancreatic diversion / Duodenal switch



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1.2.2.5 Improvements in mortality and morbidity after bariatric surgery

All forms of bariatric surgery have been shown to lead to weight loss that is sustained in the long-term (Buchwald et al. 2004; Sjostrom et al. 2007). 15 years after surgery RYGB patients have recorded weight loss of 27% of their original body mass and LAGB patients 14% (Sjostrom 2013). In sleeve gastrectomy surgery and BPS/DS respectively, the longest studies show between 20-30% and 30-40% weight loss respectively after 5-6 years (Dixon et al. 2011).

All have been shown to improve mortality and obesity related co-morbidity such as diabetes, cardiovascular disease, sleep apnoea and cancer (Adams et al. 2007; Sjostrom et al. 2007; Adams et al. 2009). They have also been shown to be cost-effective treatments (Picot et al. 2009; Finkelstein et al. 2011; Padwal et al. 2011).

In a meta-analysis of approximately 44,000 patients, entered into 5 trials of RYGB surgery and 3 of BAND or VBG surgery compared to non-surgical controls, surgery was associated with a reduced risk of global mortality (odds ratio (OR) =0.55, CI=0.49-0.63) (Pontiroli et al. 2011). There were no significant differences in global mortality between RYGB and BAND surgery (OR = 0.55 (0.47–0.64) vs.0.57 (0.44–0.73)), but cardiovascular mortality was better in the RYGB group (OR = 0.48 (0.35 – 0.66) vs. 0.71 (0.51-1.00)).

A Cochrane review (Colquitt et al. 2009) included 26 studies comparing different types of surgery with each other and with non-surgical interventions. Surgery was found to bring about superior weight loss compared to conventional treatments, and this was sustained up

to 15 years in the largest study, although many studies only reported 2 year follow up. Improvements in metabolic disease, including diabetes, hypertriglyceridaemia and hypertension were statistically significant for patients undergoing surgery compared with conventional treatment. Most studies showed improvements in physical and emotional aspects of quality of life in the first 2 years for surgery patients above those treated non-surgically. These were variably sustained at 10-year follow up. Peri-operative risk of death was around 0.25% and complication rates around 13%.

In the same review, most studies found RYGB to produce more weight loss than restrictive surgeries (such as BAND or VBG). Peri-operative risk was lower in the less complex restrictive procedures, but in the longer term 2 studies found high rates of conversion to other procedures in patients who underwent VBG (Colquitt et al. 2009).

Sleeve gastrectomy has similar outcomes to RYGB surgery in 2 year follow up studies (Fischer et al. 2012; Rosenthal et al. 2012), and greater weight loss outcomes and fewer complications compared to BAND in the only RCT comparing the two procedures (Himpens et al. 2006). Longer-term outcomes have not been investigated (Dixon et al. 2012; Rao et al. 2012).

1.3. Mechanisms of weight loss in RYGB and BAND surgery

All bariatric surgeries do not produce the same amount of weight loss and do not produce weight loss in the same way (Stefater et al. 2012). Some of the variance in achieved weight loss and improvement in metabolic illness may be explained by the way that the differing

anatomical manipulations result in different effects on appetite, eating behaviour and metabolism through neuroendocrine pathways. Different types of bariatric surgery therefore provide useful models for investigating these pathways in more detail.

Animal models of surgery, especially RYGB, are proving to be instrumental in elucidating the mechanisms underlying their effectiveness. Rats have strikingly similar gastrointestinal anatomy to humans, and some strains (particularly Wistar and Sprague Dawley) become obese and develop metabolic syndromes when exposed to high-fat chow similar to humans. Changes in food intake, weight, glucose metabolism, gut and other hormones and peptides regulating appetite (e.g. ghrelin, leptin, peptide YY (PYY), gastric insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), adiponectin, cholecystokinin) mirror those seen in humans following RYGB surgery (Rao et al. 2010). Rodents also respond to behavioural testing in a predictable manner, so that food preference and progressive ratio task paradigms can be used to assess eating behaviour and food reward (Mathes et al. 2012). Rat models of gastric sleeve and BAND have also been developed, although in the case of BAND these are more difficult to perform and less successful (Monteiro et al. 2006). Pig and dog models of the various procedures have also been successfully developed (Rao et al. 2010; Escareno et al. 2012).

Malabsorptive procedures such as the BPD/DS have been largely replaced by RYGB surgery, gastric sleeve and BAND due to fewer side effects. Sleeve gastrectomy as a standalone procedure is relatively new, and has not been evaluated in the longer term to the same extent as RYGB and BAND surgery, although it provides an intriguing model for gastric fundus exclusion and retention of the pylorus. For the purposes of this thesis therefore,

discussion will focus on mechanistic differences between RYGB and BAND surgery (or other restrictive procedures).

RYGB brings about more weight loss than BAND (Tice et al. 2008; Pontiroli et al. 2011) and has favourable effects on glycaemic control, that have been shown to be independent of weight loss, at least initially (Laferrere et al. 2008). The mechanism underlying the greater weight loss seen in RYGB are not fully understood, but are thought to involve important neuroendocrine pathways regulating eating behaviour and glucose homeostasis. Interrogation of the differences in these underlying mechanistic pathways between RYGB and BAND are therefore an important step toward the improved use of current treatments and the development of new treatments.

1.3.1 Gastric restriction

Originally it was thought that both RYGB and BAND surgery functioned primarily as a restrictive procedures. However it has been increasingly recognized that if restriction does play a role in weight loss following RYGB, it appears to be a minor one. In animal models, rats who have undergone RYGB surgery consume 50% less than sham-operated rats and eat smaller meals, but without the expected increase in meal frequency that would occur if the reduction in food intake were due to purely restriction of food volume (Zheng et al. 2009). Similarly, in human studies, RYGB patients reduce their calorie intake, eat smaller meals and feel full quickly, but they do not necessarily compensate by increasing meal frequency (Halmi et al. 1981; Trostler et al. 1995; Laurenius et al. 2012).

In BAND surgery, the mechanism of weight loss is thought to be primarily through restriction of food intake by a reduced gastric pouch, although again there is little evidence to support this assumption. Decreased food intake, increased feeding frequency, and compensatory calorie intake after food deprivation, have been demonstrated in a rat model of BAND. However since decreased food intake was not sufficient to decrease body weight after BAND in the first place, and was also not sustained in the long run, the model was not considered a success. It is therefore not a useful comparison to RYGB rat models (Monteiro et al. 2006).

In humans, BAND reduces food intake, but not to the same extent as RYGB (Naslund 1987; Kenler et al. 1990). There are no studies which demonstrate increased meal frequency after BAND surgery. Increased satiety is reported both at fasting and post-prandially when the band is optimally inflated (Dixon et al. 2005) and food is cleared from the small gastric pouch 1-2 minutes after eating (Burton et al. 2011). This suggests that restriction alone is unlikely to account for weight loss after BAND surgery, and peripheral effects on satiety may also be an important mechanism for weight loss in BAND surgery, although the exact pathway remains obscure (Burton et al. 2010; Burton et al. 2011). However, soft foods are more easily consumed than coarse after BAND surgery, and larger boluses of food tend to induce regurgitation even in weight stable BAND patients, suggesting that mechanical obstruction of certain foods may play a role in modifying eating behaviour after BAND surgery (Burton et al. 2010). In VBG patients, but not RYGB, the size of intestinal stoma (although similar between groups) and gastric pouch, was only related to weight loss in the VBG group (Naslund 1987) suggesting that there are additional factors at play in RYGB surgery to account for the difference in intake and weight loss.

1.3.2 Gastric emptying

Alterations in gastric emptying could contribute to weight loss in both RYGB and BAND surgery. For instance in BAND, delay in emptying of the small gastric pouch by the band might result in increased satiety, via mechanisms such as stretch receptors that activate vagal efferent nerves. On the other hand, the lack of a pylorus to delay stomach contents entering the small intestine in RYGB surgery might result in increased gastric emptying and expedite delivery of nutrients to the small intestine, with knock-on effects on the secretion of anorexigenic gut hormones.

However, studies have shown no change in gastric emptying after VBG (although the proximal pouch is emptied quickly) (Mistiaen et al. 2000) or BAND (de Jong et al. 2009), and no relation between weight loss and satiety with gastric emptying rate following BAND (de Jong et al. 2009). In RYGB surgery, the presence of dumping syndrome supports the possibility of increased gastric transit, but in studies directly measuring gastric emptying, one has shown *decreased* gastric emptying (Suzuki et al. 2005), whilst two others showed *increased* gastric emptying of liquids, but not solids (Horowitz et al. 1986; Wang et al. 2012). These reported inconsistencies may be a result of different surgical techniques and pouch sizes, but also may be due to possible disruption of the vagus nerve by the various surgeries. In any event the most likely contribution of gastric emptying in weight loss in RYGB is induction of exaggerated gut hormone responses by earlier nutrient delivery to the ileum.

1.3.3 Vagal tone

Vagal afferent fibres may be disrupted by RYGB surgery. In this case, there may be increased satiety or increased vasovagal release of gastrin, which may influence gastric emptying. In

addition, the vagus nerve appears to be important in the effect of anorexigenic ghrelin on the central nervous system (Huda et al. 2010). However, in surgical techniques where the vagus nerve is deliberately spared in RYGB surgery, no difference in satiety is observed, suggesting that it plays a minor role if at all (Bueter et al. 2010). In BAND surgery, the vagus nerve is not usually affected. However, although the experimental addition of truncal vagotomy to the surgical technique did not lead to more weight loss, it did reduce the number of patients requiring band adjustments to produce weight loss in the first year (Angrisani et al. 2009). Vagal tone may however be affected by increased intraluminal pressure. In successful BAND patients intraluminal pressure in the range of 25–30mmHg is consistently observed at the level of the band (Burton et al. 2009; Burton et al. 2011).

1.3.4 Malabsorption of nutrients

It is rare to see macronutrient deficiency after RYGB surgery, and most patients remain in the obese or overweight category, which would not be possible if significant malabsorption of macronutrients were induced by RYGB surgery. Rats which have undergone RYGB surgery and achieve similar weight loss compared to humans (30% over 5 months) have no change in the energy density of faeces after surgery, suggesting that malabsorption is not responsible for the weight loss (Furnes et al. 2008; Furnes et al. 2008; Furnes et al. 2009; Bueter et al. 2010). Two studies have demonstrated gut hypertrophy in RYGB rats, suggesting a possible compensatory measure of the gut to reduce malabsorption (le Roux et al. 2010; Taqi et al. 2010).

There are surprisingly few studies in humans examining malabsorption of macronutrients after RYGB surgery, although several have shown that some micronutrients, such as iron,

calcium, and vitamins A, B_{12} and D are not absorbed effectively (Chaves et al. 2007; Decker et al. 2007; Marinella 2008; Dewey et al. 2011). A recent study showed no carbohydrate malabsorption after RYGB surgery (Wang et al. 2012), and another examining faecal fats found little evidence of fat malabsorption (MacLean et al. 2001). However, a study linking fat malabsorption to the side effect of nephrolithiasis after RYGB surgery suggests otherwise (Kumar et al. 2011). Surgeons have developed modified versions of RYGB surgery specifically to introduce significant malabsorption in order achieve greater weight loss in super-obese patients. In these modifications, bile and pancreatic secretions are introduced more distally into the ileum via a longer jejunal limb (Brolin et al. 1992; Brolin et al. 2002). In patients with a BMI <50 kg/m^2, the length of the common channel being less than 100cm is the most important determinant of additional weight loss, suggesting that for most ordinary RYGB surgeries, where the common channel is well over 100cm, malabsorption plays a minor role in weight loss (Brolin et al. 1992; Stefanidis et al. 2011). In these modified procedures, malabsorption accounts for 6-11% of weight loss (Odstrcil et al. 2010).

1.3.5 Adipokines

1.3.5.1 Leptin

Leptin, a hormone secreted mainly by white adipose tissue, received much attention following its discovery due to the observed resolution of obesity in treatment of leptin-deficient children suggesting a potential therapeutic target for obesity. In normal adults reduced adipose tissue following weight loss causes a reduction in circulating leptin levels which signals increased appetite centrally and has profound neuroendocrine, metabolic and immunological effects. This is one of the main drivers of rebound weight gain after dieting. Obese people have been found to have paradoxically high leptin levels, and are thought to be leptin resistant (Frederich et al. 1995; Seeley et al. 1996). Treatments for obesity

incorporating leptin therefore require additional agents that promote leptin sensitivity; most recently the leptin-amylin combination has shown promising results in pre-clinical and clinical phase II trials (Tam et al. 2011; Rodgers et al. 2012).

Leptin levels decrease following bariatric surgery in line with loss of adipose tissue (Jacobsen et al. 2012). After RYGB surgery, leptin levels fall by approximately 50%, below those seen in weight-matched controls, becoming comparable to lean controls. The reduction in leptin is also greater than that seen in BAND patients, which may simply reflect greater loss of adipose tissue. However, the reduction in leptin is also observed over a longer period (up to a year in RYGB compare to the first 2 weeks only in BAND surgery) (Korner et al. 2006; Korner et al. 2009; Woelnerhanssen et al. 2011). This differential pattern may be because in BAND, the gastric fundus, where some leptin is produced, is still seeing nutrients, whereas in RYGB surgery the fundus is bypassed by nutrients. Increased post-prandial insulin secretion and more efficient glucose metabolism seen in RYGB surgery may also affect adipocyte function and therefore inhibit leptin secretion in the longer term (Chen et al. 2012; Stefater et al. 2012).

Such large decreases in leptin would ordinarily increase hunger, but RYGB patients lose weight and describe decreased hunger. It has been postulated that RYGB may therefore induce a mechanism to increase leptin sensitivity, similarly to increased insulin sensitivity, or even that further weight loss could be achieved in these patients by the addition of leptin replacement therapy (Korner et al. 2009). Further studies are needed to confirm this.

1.3.5.2 Other adipokines

Adipose tissue in obesity is characterized by enlarged adipocytes, impaired proliferation and differentiation of adipocytes, dysfunctional secretion of adipose tissue derived cytokines (adipokines), and altered inflammatory signaling from adipocytes. Adipokines affect energy homeostasis and glucose metabolism and may interact with leptin and/or insulin signaling in peripheral tissues including the liver and brain. These have been linked to insulin resistance, chronic vascular inflammation, oxidative stress, and activation of the renin-angiotensin system (RAS), eventually leading to type 2 diabetes mellitus (T2DM).

A reduction in metabolically active visceral adipose tissue and adipocyte size have been observed following bariatric surgery as in other forms of weight loss (Lofgren et al. 2005; Pontiroli et al. 2009). This reduction in visceral adiposity, as well as a reduction in lipotoxic free fatty acids, and reduction in inflammatory markers generated by pathogenic adipocytes, are thought to contribute to improvements in metabolic parameters (Bays et al. 2009). Although leptin has been studied in some depth, less is known about the function of the many other adipokines. These include adiponectin, resistin, chemerin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), retinol binding protein 4 (RBP4), tumor necrosis factor-alpha (TNFα) and visfatin. Not much is known about how different bariatric procedures affect secretion of adipokines, other than leptin. However, reduced adiposity and improved adipocyte function on a cellular level contributes to normalized adipokine secretion, which may be important in the resolution of metabolic disease after RYGB surgery. Most other adipokines decrease after surgery, as with leptin, probably as a function of decreased fat mass which follows RYGB surgery, and to a lesser degree BAND surgery (Vilarrasa et al. 2007; Garcia de la Torre et al. 2008; Swarbrick et al. 2008; Trakhtenbroit et al. 2009). Adiponectin (involved in glucose metabolism) which unlike the other adipokines is

lower in obese people normalizes after RYGB surgery (Whitson et al. 2007; Guijarro et al. 2008), although one study showed it to decrease (Woelnerhanssen et al. 2011). Improvements in inflammatory signaling after bariatric surgery has not fully been explored as yet, but inflammatory cytokines are likely to mirror fat mass loss after bariatric surgery (Kohli et al. 2011). Upregulation of the RAS-related gene expressions in adipose tissue seen in obesity, resolves in post-RYGB subjects (Chen et al. 2012). Most of the changes in adipokines would seem to be a result loss of fat mass after RYGB surgery, without evidence for an independent role in appetite regulation.

1.3.6 Gut hormones

Increasingly the distinct and specific effects of RYGB on post-prandial gut hormone secretion have been implicated in its superior weight loss. There are early and exaggerated increases in post-prandial blood levels of anorexigenic peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and oxyntomodulin which promote satiety and which, when reversed, increase food intake (le Roux et al. 2005; Borg et al. 2006; Korner et al. 2006; le Roux et al. 2006; le Roux et al. 2007).

1.3.6.1 PYY

PYY is secreted mainly by L cells in the mucosa of the ileum and colon (Adrian et al. 1985). It acts on the hypothalamic arcuate nucleus to inhibit neuropeptide Y (NPY) neurons to reduce appetite and food intake (Schwartz et al. 2000; Schwartz et al. 2002; Riediger et al. 2004; Sloth et al. 2007). Furthermore it also increases ghrelin levels which may enhance anorexia (Batterham et al. 2003), and reduces gastrointestinal motility and increases water absorption in the gut, which may also induce satiety (Wang et al. 2010). In obese people,

post-prandial PYY secretion is attenuated compared with lean individuals (le Roux et al. 2006) and PYY given intravenously to lean and obese humans reduces food intake and increases satiety (Batterham et al. 2002; Batterham et al. 2003). Following RYGB surgery, but not BAND surgery, post-prandial secretion of PYY is increased (Bose et al. 2010; Jacobsen et al. 2012), PYY is secreted earlier and to a greater level than that even seen in lean people (Korner et al. 2006; le Roux et al. 2006), and remains higher than pre-operative levels even 2 years after surgery (Pournaras et al. 2010). Attenuated PYY secretion is associated with poor weight loss in RYGB patients (le Roux et al. 2007). Administration of exogenous PYY to RYGB rats causes increased weight loss (Fenske et al. 2012). RYGB in PYY knock-out (KO) mice induces less weight loss than gastric bypass in wild type mice. Furthermore, there was no difference in weight loss between sham-operated and gastric bypass PYY KO mice, suggesting that PYY is an important mediator of weight loss in RYGB (Chandarana et al. 2011). Reduction of PYY secretion by administration of somatostatin or an analogue increases food intake in rats (le Roux et al. 2006) and humans that have undergone RYGB surgery (le Roux et al. 2007; Fenske et al. 2012), although somatostatin will suppress secretion of many gut hormones. Injection of a PYY neutralizing antibody into mice who had undergone a jejeno-ileal bypass (a similar procedure to RYGB), led to increased food intake the following day (le Roux et al. 2006). Direct blockade of PYY via receptor antagonists has not been tested in RYGB yet, but has been shown to reduce anorexic responses to gastric infusions of protein and long-chain fatty acids (Reidelberger et al. 2013).

1.3.6.2 GLP-1, oxyntomodulin and GLP-2

Oxyntomodulin (OXM), GLP-1 and GLP-2 are known as enteroglucagons, a family of peptides produced by differential splicing of the products of the preproglucagon gene by proconvertase enzymes. Oxyntomodulin and GLP-1 are anorexigenic, whereas GLP-2

influences the gut hypertrophic response (Turton et al. 1996; Wynne et al. 2006; Janssen et al. 2013). GLP-1 and oxyntomodulin are secreted by ileal L-cell after a meal, and GLP-1 inhibits gastric secretion and motility. This means that macronutrient absorption is protracted and delayed, leading to satiety and a reduction in food intake. Peripheral infusion of GLP-1 in normal weight and obese people reduces appetite and food intake (Naslund et al. 1999; Flint et al. 2001)., and Liraglutide, a GLP-1 agonist has been shown to reduce weight in obese people without T2DM (Astrup et al. 2009). Post-prandial GLP-1 and oxyntomodulin release is exaggerated after RYGB in pre-clinical (Bueter et al. 2010) and clinical studies (le Roux et al. 2006; Morinigo et al. 2006; le Roux et al. 2007; Laferrere et al. 2008; Jacobsen et al. 2012), and suppression of GLP-1 release hormones by somatostatin increases food intake in patients who have undergone RYGB but not banding, although specificity of effect of GLP-1 cannot be assumed in this case since many other gut hormones are also suppressed by somatostatin (le Roux et al. 2007) . Raised GLP-2 levels and associated gut hypertrophy are found in pre-clinical models of RYGB surgery (le Roux et al. 2010).

GLP-1 is also a potent anti-hyperglycemic hormone, which acts by stimulating insulin secretion and suppressing glucagon secretion, in a glucose dependent manner (Kreymann et al. 1987). When plasma glucose concentration is in the normal fasting range, GLP-1 no longer stimulates insulin so that reactive hypoglycemia does not result. GLP-1 also appears to restore the glucose sensitivity of pancreatic β-cells (D'Alessio et al. 1995). Alterations in GLP-1 positively affect glucose metabolism after RYGB surgery (Laferrere et al. 2008; Pournaras et al. 2010; Van der Schueren et al. 2012).

There are two theories as to why the gut hormone response and glucose metabolism are altered after RYGB surgery. The foregut theory proposes that because nutrients bypass the

foregut (gastric fundus, duodenum and jejunum) nutrient-dependent actions that would negatively affect glucose metabolism and limit gut hormone responses are prevented (Pories et al. 1982; Rubino et al. 2004). In support of this theory, an intraluminal sheath that is attached to the pylorus of the stomach and extends to the jejunum, thereby delivering nutrients more distally without altering the anatomy of the gastrointestinal tract, leads to similar effects on glucose metabolism as RYGB surgery in animals and humans (Aguirre et al. 2008). As counter-evidence, in a case series of 5 patients, greater plasma insulin, GLP-1, and PYY responses were induced by oral ingestion of glucose following RYGB surgery, compared with glucose loading by way of the gastrostomy tube, where the proximal small bowel was not bypassed (Pournaras et al. 2012).

The hindgut theory on the other hand proposes that it is the rapid delivery of concentrated nutrients to the distal ileum that results in the release of GLP-1 and PYY, which improves glucose metabolism, and that shunting of the duodenum is not important. This is supported by studies using ileal interposition surgery, where the distal ileum is repositioned to the proximal jejunum, whilst maintaining continuity of the gastrointestinal tract and preserving neurovascular connections, thereby isolating this part of the mechanism. Ileal interposition results in exaggerated postprandial PYY and GLP-1 release, improved glucose metabolism and weight loss in rodents (Kohli et al. 2010). Studies which show gastric emptying is increased in RYGB surgery support this theory (Wang et al. 2012). The success of the gastric sleeve (VSG), which produces similar effects on gut hormones without bypassing the duodenum and in which rapid gastric emptying has been shown, also seems to support this theory (Gill et al. 2010; Chambers et al. 2011). Raised PYY following RYGB surgery may also be an appropriate response to a reduced small bowel thereby ensuring increased contact of nutrients with an absorptive lining, as is seen in intestinal disease (Adrian et al. 1986). Other

authors suggest that foregut vs. hindgut theory is an impedance to understanding the mechanisms of RYGB surgery by oversimplifying the matter. They argue for the use of rodent models and comparisons of the similarities and differences between RYGB, BAND and VSG to unpick the effect of each aspect of the mechanism (Stefater et al. 2012).

1.3.6.3 Ghrelin

Ghrelin is secreted mainly in mucosa of the gastric fundus and to a lesser degree by the rest of the stomach and duodenum and is released in response to fasting and chronic negative energy balance. The active form is created by acylation by the ghrelin O-acyltransferase (GOAT) enzyme (Cummings et al. 2002; Korbonits et al. 2004; Goldstone et al. 2005; Sumithran et al. 2011). Ghrelin stimulates hunger and food intake in normal weight and obese subjects and patients with cancer- and renal dialysis-associated anorexia (Neary et al. 2004; Druce et al. 2005; Druce et al. 2006; Ashby et al. 2009) and is thought to play a role in meal initiation (Nakazato et al. 2001; Cummings et al. 2001). These actions appear to be mediated through actions on hypothalamic feeding neuropeptides either directly or via the vagus nerve (le Roux et al. 2005; Kuo et al. 2007).

Obese people have lower circulating levels of ghrelin (Tschop et al. 2001), and the post-prandial release of ghrelin in obese people is not suppressed to the same degree as in normal weight people (le Roux et al. 2005).

It would be expected, given that the gastric mucosa remains intact in both surgeries, that the reduction in both weight and calorie intake would result in increased ghrelin levels after

RYGB and BAND surgery. Therefore the finding of paradoxically low 24 hour, fasting and post-prandial ghrelin levels after RYGB surgery in three pre- vs. post-RYGB longitudinal and three cross-sectional studies (RYGB vs. matched obese patients) sparked interest in a possible novel and important mechanism for weight loss maintenance in these patients. However four other studies showed no change, and one an increase in plasma ghrelin after RYGB (Cummings et al. 2004; Pournaras et al. 2009; Tymitz et al. 2011; Jacobsen et al. 2012).

Various hypotheses have been put forward to explain these results and their inconsistencies, but they appear to be largely explained by inaccurate measurement techniques. For instance, only acyl ghrelin is reduced by prolonged fasting, whereas the inactive form increases. Therefore these forms need to be measured separately, which the above studies did not always do (Cummings et al. 2002; Faraj et al. 2003; Geloneze et al. 2003; Leonetti et al. 2003; Lin et al. 2004; Morinigo et al. 2004; Chan et al. 2006; Sundbom et al. 2007). In those that did measure active ghrelin, two showed reduced fasting active ghrelin 2 weeks and 6 months post-surgery (Fruhbeck et al. 2004; Jacobsen et al. 2012) and one showed increased fasting active ghrelin at 6 and 12 months post-surgery (Holdstock et al. 2003)

The handling of particularly acyl ghrelin requires temperature control, chelation and protease inhibitors to ensure accuracy, which was variably performed. Furthermore, since ghrelin is inversely proportionate to fat mass, determining whether an effect was dependent or independent of weight loss requires correction for BMI or body fat percentage. One study investigated the relationship between weight loss and ghrelin levels in RYGB patients and found no association, (Fruhbeck et al. 2004) whereas another found that weight-stable patients had no change in ghrelin levels between two points after surgery, whereas patients actively losing weight did have elevated ghrelin levels (Holdstock et al. 2003).

An intact vagus nerve is also required for ghrelin to have an effect on appetite, and differing operative techniques (vagus-sparing vs. non vagus-sparing) may therefore also play a role in discrepancies between studies (Sundbom et al. 2007).

In BAND surgery ghrelin levels may be unchanged or increased in post-operative compared to pre-operative patients (Hanusch-Enserer et al. 2003; Cummings et al. 2004; Fruhbeck et al. 2004; Stoeckli et al. 2004; Langer et al. 2005; Busetto et al. 2006; Stefater et al. 2012).

In gastric sleeve surgery, the mucosa of the stomach is resected, and ghrelin levels have been shown to be lower after surgery compared to RYGB (Lee et al. 2011). However hypoghrelinaemia may have no role in appetite, weight loss and food choice, since ghrelin knockout mice behaved in the same way as standard mice on these measures, implying that even if ghrelin was reduced in RYGB surgery, it is not likely to be a contributing mechanism for weight loss (Stefater et al. 2012).

1.3.6.4 Cholecystokinin (CCK)

CCK is secreted by the duodenum and jejunum in response to particularly high fat and high protein meals. It inhibits gastric emptying, which is thought to be the main mechanism of its satiating effects, delays intestinal transit, and is a catalyst for the digestion of fat, protein and carbohydrates in the duodenum. It may also act centrally to promote satiation, and can cause nausea and anxiety via central or vagal stimulation. Its satiating effects appear to be reduced in obesity (Fink et al. 1998). In total gastrectomy, CCK is increased and has been

shown in mice knockout studies to reduce food intake. The role of CCK in RYGB or BAND surgery has not been clearly elucidated, but so far three studies have reported no change in CCK after RYGB (Rubino et al. 2004; Suzuki et al. 2005; Jacobsen et al. 2012). Additionally, CCK knockout mice have a favourable outcome after RYGB suggesting no role (Hajnal et al. 2010). One study showed that although there was no change in baseline levels, after VBG the post-prandial CCK peak was increased compared with weight matched controls suggesting a possible satiety role for CCK in restrictive operations, although one study found no change after BAND surgery (Kellum et al. 1990).

1.3.6.5 Amylin

Amylin is co-secreted by the pancreas with insulin and plays a role in satiety, and the development of T2DM. Following RYGB surgery, no change in post-operative fasting secretion of amylin has been seen at 2 weeks (Jacobsen et al. 2012), whilst another study showed decreased post-prandial amylin levels in RYGB but not banding (Bose et al. 2010). Post-prandial amylin secretion is increased both in pre-clinical (Shin et al. 2010) and clinical studies in the short- and long-term, in keeping with improved β-cell function (Bose et al. 2010).

1.3.7 Glucose and insulin

Insulin, produced by β-cells of the pancreas, is a hormone that regulates carbohydrate and fat metabolism in the body. It facilitates glucose absorption from the blood in the liver and skeletal tissues, where it is stored as glycogen, and in the fat cells where it is stored as triglycerides.

Insulin inhibits the release of glucagon, thereby reducing the use of fat as an energy source. Under normal conditions, insulin release is controlled by glucose levels. When blood glucose levels fall below a certain level, the body begins to use stored sugar as an energy source by facilitating the breakdown of glycogen stored in the liver and muscles, resulting in increased glucose, which can then be utilized as an energy source. In T2DM, the feedback mechanism by which glucose levels are controlled is disrupted due to insulin resistance, whereby insulin cell receptors are changed in such a way as to no longer respond to insulin. This means that increasing amounts of insulin are secreted, resulting in hyperglycaemia and eventual end-organ damage, including cardiovascular disease, stroke, renal failure, vascular dementia and limb amputation (Steiner 1981; Santiago 1986).

The worldwide increase in T2DM is associated with increasing rates of obesity and overweight (Unwin et al. 2010). More than 60% of patients with T2DM are obese (Kramer et al. 2010).

RYGB is known to improve glycaemic control in T2DM, independent of the effect of weight loss and potentially mediated by increased incretin secretion (such as GLP-1)(Laferrere et al. 2008; Laferrere 2011). These improvements are sustained in the long-term (Sjostrom et al. 2004). Reduction in peripheral insulin resistance occurs in accordance with weight loss, but hepatic insulin resistance can change earlier (Lim et al. 2011). Other factors that have been proposed to play a role in improvement of glycaemic control in both RYGB and BAND surgery are decreased caloric intake and improved β-cell function due to decreased lipotoxic, glucotoxic and inflammation effects of obesity resulting from weight loss (Wajchenberg

2007; Weir et al. 2009; Isbell et al. 2010; Nannipieri et al. 2011). In RYGB, dietary changes resulting from a shift in food preference away from high-glycaemic-index, high-fat foods may also play an important role in improving glycaemic control (Olbers et al. 2006; Mathes et al. 2012).

1.3.8 Bile acid secretion

RYGB modifies the anatomical location at which bile enters the upper gastrointestinal tract via the bilio-pancreatic limb of the Roux-en-Y construction. Several studies have found increased serum bile acid concentrations after RYGB surgery (Nakatani et al. 2009; Patti et al. 2009; Jansen et al. 2011; Pournaras et al. 2012). Decreased faecal bile acid secretion has been found in rats after RYGB compared with sham procedures (Li et al. 2011). Bile acids are known to have significant effects on glucose metabolism through a variety of pathways and may also reduce appetite through modulation of gut hormone secretion. Bile acids stimulate production of fibroblast growth factor 19 (FGF19), a regulator of hepatic lipid and glucose metabolism. Both bile acids and FGF19 were increased 3 months after RYGB but not BAND surgery (Pournaras et al. 2012).

In a rat model delivery of bile into the ileum rather than the duodenum resulted in greater release of GLP-1 and PYY, reduced food intake and body weight (Pournaras et al. 2012). This study suggested that the delivery of undiluted bile (not bound up in micelles created by progressing through the stomach and proximal intestine and combining with food) to the terminal ileum stimulates bile acids to produce PYY and GLP-1 via TGR5 receptors on L-cells.

Additionally FGF19 (which regulates bile acid secretion and stimulates of metabolic rate and inhibition of gluconeogenesis in the liver) may also be important in the mechanism of weight loss and improved glucose metabolism seen after RYGB surgery. FGF19 receptors have been found in the rat hypothalamus and their expression is reduced in high fat fed animals compared to lean. Acute administration of intracranial FGF19 reduces food intake and body weight in rats, whereas an FGF19 receptor inhibitor has the opposite effect (Ryan et al. 2013).

1.3.9 Increased resting energy expenditure

Some studies suggest that RYGB may increase resting energy expenditure (Flancbaum et al. 1997; Carrasco et al. 2007; Bueter et al. 2010), whilst others show decreased or little effect on energy expenditure (Das et al. 2003; Carrasco et al. 2007). In BAND there is an expected decrease in energy expenditure in line with weight loss (Coupaye et al. 2005). However even a decrease in energy expenditure needs to be interpreted with caution in the context of the large amount of weight loss that has taken place and therefore comparison with a control group which has lost similar amounts of weight is required, as animal studies allow. Accurate interpretation of these data requires correction for either body weight, lean body mass, or body surface area. In studies where this has been done, one study suggested resting energy expenditure is increased (Stylopoulos et al. 2009) and another did not find the expected decrease, but found no change (Zheng et al. 2009).

Overall, rodent models seem to support either an increase in energy expenditure, or a decrease that is not of the magnitude expected with the degree of weight loss seen following RYGB surgery.

The suggested mechanism for increased energy expenditure is diet induced thermogenesis and gut hypertrophy which has been observed in RYGB compared to sham-operated rats (Bueter et al. 2010).

In human studies, a recent prospective study of 13 RYGB patients found a reduction in resting metabolic rate and fat mass after surgery (Liu et al. 2012). Variance in negative energy balance did not explain variance in fat loss, and the authors suggest that the capacity of diverting glucose to oxidation, leaving less of it available to make fat was important. They found that there was little increase in physical activity in this cohort, but that even small increases had large effects fat loss, since even low-intensity exercise is associated with more utilization of glucose as fuel. This may be particularly relevant in this population, since systematic reviews have shown that exercise is increased after bariatric surgery and associated with better weight loss (Egberts et al. 2012; Livhits et al. 2012)

1.3.10 Altered gut microbiota

Gut microbiota are increasingly seen as another important information pathway between the gut and the brain. They modulate the generation of cytokines in the intestinal immune system and release signaling molecules such as lipopolysaccharide (LPS) and peptidoglycan components that can directly act on the central nervous system. Therefore they are thought to play a role in the regulation of digestion, nutrition, mucosal function and intestinal immunity, as well as metabolic homeostasis, systemic immunity and brain function (emotion, mood, cognition)(Holzer et al. 2012).

Obesity is associated with reduced levels of certain gut microbiota species (Lactobacillus, Bacteroides, Bifidobacterius) and increased levels of Firmicutes (Ley et al. 2006) and changes in levels of these found in human faeces studies of RYGB patients are associated with weight loss (Furet et al. 2010; Li et al. 2011; Sweeney et al. 2013) and in rats with alterations in gut peptide synthesis (Osto et al. 2013). Furthermore caecal transplant from RYGB-mice to unoperated mice decreased their weight and adiposity, whereas caecal transplant from sham-operated mice had no effect on weight and adiposity (Liou et al. 2013).

1.4 Food hedonics and reward

1.4.1 Homeostatic and non-homeostatic food intake control

Appetite regulatory systems are often divided into homeostatic and non-homeostatic (or hedonic) control systems, although the divide can be artificial since these are interlinked. Homeostatic control refers to the control of food intake and meal termination in response to physiological hunger and satiety signaling. These are largely controlled by anorexigenic (e.g. ghrelin) and orexigenic (e.g. PYY, GLP-1, CCK, oxyntomdulin) hormones as well as vagal afferent responses to gastric distension, the effects of insulin and glucose, and in the longer term adipokines such as leptin (Saper et al. 2002; Flier 2004). The main gateway for these mechanisms within the central nervous system is the hypothalamus (Schwartz et al. 2000; Sam et al. 2012). Non-homeostatic mechanisms include various individual and environmental factors that govern the intake of food in addition to physiological hunger. These are primarily the individual hedonic and emotional reactions to food governed by brain food reward systems, including dopaminergic and opioid cortico-limbic pathways as well as prefrontal decision making areas and memory systems (De Silva et al. 2012).

There is increasing recognition that there are societal and environmental factors within Westernized countries, which have contributed to the epidemic levels of obesity currently seen (de Castro 2010). Highly palatable highly calorific food is cheaply and easily accessible and a high social value is placed on immediate personal gratification and reward. Furthermore an evolutionary legacy of defense of a higher rather than a lower body weight, to favour survival in periods of cyclical starvation and plenty, makes humans ill-suited suited to an "obesogenic" environment of continuous plenty. Homeostatic and non-homeostatic systems most likely function in synergy with cross-modulation between systems taking place particularly during periods of food deprivation. However, in an "obesogenic" environment, the influence of palatable food cues on brain food reward systems, may override homeostatic satiety signals, and/or exaggerate hunger signals, contributing to weight gain, and also hindering weight loss during attempted reduced caloric intake (Berthoud 2012).

1.4.2 Food reward and executive control systems in the brain

The hedonic appeal of food is used to describe how rewarding the anticipated or experienced pleasure of a particular food is perceived to be. For instance, palatable, or high-calorie foods are usually perceived to be more hedonically appealing and are consumed more than bland or unappetizing foods, and may be perceived as even more so in obese and dieting people (Herman et al. 2008). Food reward encompasses the concept of hedonic appeal but also integrates the influence of learning and memory on behaviour that is cue-elicited. In situations where hedonic appeal is high, approach and consummatory behaviour is elicited, at the expense of other ongoing behaviour. Food intake induces subjective feelings of pleasure, which in turn has a positively reinforcing effect on the behaviour.

This pattern of behaviour is thought to be mediated by reward and cognitive control systems in the brain, and dopamine, opioid and other neuroreceptor pathways (5HT, noradrenaline, endocannabanoid) (Franken 2003; Cools et al. 2008; Bermudez-Silva et al. 2012). In obesity, similarly to addictive behaviour, eating occurs despite negative consequences, and is driven by subjective feelings of compulsion or craving and negative affect arises if the craving is not satisfied. Dopamine pathways in particular are thought to play an important role in the processing of reward and primarily food reward (Martel et al. 1996; Schultz 2001; Kishi et al. 2005; de Araujo et al. 2012). Reward from natural (eg. food and sex) and non-natural (e.g. drugs of addiction, which supplant natural rewards in valence, and have no beneficial evolutionary purpose) sources, both lead to increased dopamine release in the nucleus accumbens the ventral striatum. This is an important site for processing pleasure from reward and crucially involved in the pathology of addiction, particularly to certain drugs, considered stimulants, such as nicotine, cocaine and methamphetamine.

Dopamine projections run from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens, and to the dorsal striatum where consolidation of the efficient actions to obtain reward occur (eg. learned behaviour, formation of habits, stimulus response) (Hernandez et al. 1988; Schultz 2001; Vanderschuren et al. 2005). The VTA also projects to the amygdala (governing emotional responses), the hippocampus (memory formation), the OFC (which encodes the predicted reward value of a cue) and prefrontal cortex (where reward representations are consolidated and suppression of maladaptive responses or initiation of behaviour to obtain a desired goal takes place). Dopamine pathways appear to be particularly important in processing the hedonic appeal rather than appetitive drive for

food, for example preference for sugary food as opposed to hunger for any type of food (Szczypka et al. 2001; Volkow et al. 2008).

When a stimulus is as rewarding as expected, tonic dopamine release occurs in the nucleus accumbens. Dopamine is fired in phasic bursts when reward exceeds expectation. If the reward does not reach expected levels of pleasure, there are pauses in dopamine release. Unpredictable or unexpected rewards have a more reinforcing effect than predictable rewards. In addiction states, dopamine is released *regardless* of actual reward, but in keeping with the expectation of reward. Memory and learning play a role in this since gains are remembered and losses forgotten (Schultz 2001; Bellebaum et al. 2008; Park et al. 2010). Bello et al in their review of the role of dopamine in binge eating, suggest that sustained stimulation of the dopamine systems by bingeing, promoted by pre-existing conditions (e.g. genetic traits (D2 receptor polymorphisms), dietary restraint, stress, etc.) results in progressive impairments of dopamine signaling (Bello et al. 2010) which perpetuate the behaviour.

In addition to dopamine, opioid pathways have also been shown to be important in the processing of reward valence of food. Mu opioid receptors (MORs) are largely distributed within brain regions mediating food intake and reward including nucleus accumbens and amygdala (Mansour et al. 1995). Animal studies have shown MOR activation in VTA enhances hedonic reaction to sweet and fatty foods (Taber et al. 1998; MacDonald et al. 2003; MacDonald et al. 2004; Olszewski et al. 2007), and opioid agonists and antagonists injected into VTA respectively increase or decrease food intake (Yeomans et al. 1997; Echo et al. 2002; Will et al. 2003; Smith et al. 2007). In humans, opioid antagonists have shown mixed results: some show reduced food intake (Yeomans et al. 2002) and reduced

palatability of sugary foods (Fantino et al. 1986; Yeomans et al. 1991; Yeomans et al. 1996; Arbisi et al. 1999) and reduced bingeing (Drewnowski et al. 1992; Drewnowski et al. 1995), whereas others have shown no reduction in bingeing (Mitchell et al. 1987; Mitchell et al. 1989; Alger et al. 1991; Marrazzi et al. 1995; Marrazzi et al. 1995).

Gene expression studies in animals support the role of MOR in food hedonics (Welch et al. 1996; Chang et al. 2004; Chang et al. 2007; Barnes et al. 2008), but there is considerable variation in specific hypothalamic and striatal region peptide expression following high fat food intake, which appear to be modulated by duration of food intake. MOR gene expression has not been linked to obesity as yet, but has been linked to alcohol and other drug dependency, especially for substances like alcohol and heroin (Davis et al. 2009).

It is generally accepted that dissociation exists between the hedonic preference for food "liking" and the reinforcing value of food "wanting". These appear to be independently affected by homeostatic systems, so that in some studies hunger appears to increase "wanting" but not necessarily "liking" (Epstein et al. 2003). In addition, the interaction between homeostatic and hedonic systems in their control of energy intake does not appear to be symmetrical. For example, increased palatability of food reduces hunger at a slower rate and brings earlier satiety and a quicker return of hunger, whereas decreased hunger does not necessarily reduce the perceived palatability of food, although increased hunger does improve it (Blundell et al. 2004).

Dopamine pathways have been implicated in "wanting" or desire to seek out / incentive salience for food which in turn is linked to food intake (Berridge et al. 2009) whereas opioid

pathways have been linked to “liking”, i.e. a pleasant experience, e.g. from food intake (Davis et al. 2009). This is probably an oversimplification of the matter, and an alternative explanation may be that increasing the hedonic properties of food via the MOR pathway may then translate via associative learning into “wanting”. For instance, opioid drugs inhibit GABAergic activity in midbrain leading to increased dopamine outflow to nucleus accumbens and inhibit synaptic input from dopaminergic transmission (Will et al. 2003). Opioid action in nucleus accumbens is also associated with increased food intake or “wanting” of food. Conversely MOR antagonists’ effects may be mediated by inhibiting MORs in the VTA, leading to disinhibition of GABAergic interneurons and subsequently leading to decreased dopamine release in the shell of the nucleus accumbens (Taber et al. 1998; MacDonald et al. 2003; MacDonald et al. 2004).

1.4.3 Food reward and food addiction

Studies have shown increased cue-reactivity to drug cues in drug dependency in areas associated with memory, learned association and behavioural reinforcement through reward (amygdala, hippocampus, VTA and nucleus accumbens) as well as areas involved in cognitive control and craving (ACC, OFC, dorsolateral pre-frontal cortex (DLPFC)) (Jentsch et al. 1999; Goldstein et al. 2002; See 2002; Franken 2003; Wilson et al. 2004). Long-term drug dependency has been associated with changes in neural circuitry in the VTA and nucleus accumbens. Prolonged dopamine elevation such as seen in drug taking may also affect reward-related behaviour in other arenas, such as eating or sex.

Several theories have been put forward regarding parallels between obesity and addiction, prompted in part by tantalizing evidence of similarities in reward network activation. Akin to

other addictive behaviours, obese people have been found to have reduced striatal dopamine receptor availability (Volkow et al. 2008), suggesting down-regulation of these receptors in response to feeding-induced increase in dopamine release (see Section 1.7.5 for elaboration). This finding, along with findings from some, but not all, neuroimaging studies that obese people have increased reward system activation to high-calorie foods (see Table 1.1), have resulted in a resurgence of interest in the parallels between obesity and drug addiction.

It has been posited that certain foods may be "addictive", for example highly processed sugary foods or fat (Shriner 2011). In fact, the evidence for this in humans is scant. For example, whilst rodents will demonstrate behaviour consistent with addiction to sucrose (increased lever pressing, withdrawal symptoms on removal of the sucrose, dopamine released in the nucleus accumbens and the induction of withdrawal symptoms by opioid antagonists) in paradigms designed to induce sucrose preference followed by periods of food deprivation, attempts to replicate this in humans have failed (Avena et al. 2008; Garber et al. 2011). Even within animal models, the patterns of dopamine release from sugar differed significantly from those seen addictive drugs (e.g. reduced release upon satiation vs. continued release)(Corwin et al. 2011). Furthermore activation of reward circuitry by potentially "addictive" foods may well indicate increase salience, but this does not necessarily translate into addiction, any more than increased salience of attractive faces might.

Similarly, although greater activation of reward pathways to food cues in obese people (for which the evidence is contradictory to say the least (Ziauddeen et al. 2012)) *may* indicate a

greater salience of the food cue in obese individuals, this is not necessarily associated with addictive behaviour as such.

The behavioural phenotype of obesity has been aligned with drug addiction and intriguing behavioural parallels do exist in this respect. The American Psychiatric Association criteria for the clinical diagnosis of abuse and dependence is "maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the seven-point criteria, occurring at any time in the same 12-month period (1) Tolerance, as demonstrated by a need for markedly increased amounts of the substance to achieve the desired effect or a markedly diminished effect with continued use of the same amount of the substance (2) Withdrawal, as manifested by either a characteristic withdrawal syndrome for the substance or consumption of the same (or a closely related) substance to relieve or avoid withdrawal symptoms (3) The substance is often taken in larger amounts or over a longer period than was intended (4) There is a persistent desire or unsuccessful efforts to cut down or control substance use (5) A great amount of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects (6) Important social, occupational, or recreational activities are given up or reduced because of substance use (7) The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption)" (*Diagnostic and Statistical Manual-IV (DSM-IV).*

It is not difficult to see how in certain obese individuals, the diagnosis of food addiction may fit very well (Allen et al. 2012) and that many suffer occupational, social, interpersonal and physical consequences from their obesity (Abiles et al. 2010), despite which overeating persists. The potential neurobiological overlap in the mechanism of drug addiction and obesity, particularly the reward deficiency hypothesis (see Section 1.7.3.7 (Volkow et al. 2011), provide a mechanism for the development of tolerance and resulting increased intake to achieve the same level of reward. In addition, psychological symptoms of withdrawal to refined foods, such as sugar, have been reported (Ifland et al. 2009), although physical symptoms not. Refined carbohydrates in particular may sensitise certain individuals to increased use over time (Ifland et al. 2009). Weight relapse is commonly seen in obesity after dieting (Anderson et al. 2001), and chronic dieting or restraint appears to induce paradoxical counter-regulatory (disinhibitory) eating behaviour, particularly in the context of stress and negative affect (Herman et al. 1984; Ruderman 1985; Haynes et al. 2003; Chaput et al. 2009). Furthermore the finding that substance or alcohol dependency seldom co-exists with obesity (Kleiner et al. 2004; Sarwer et al. 2004), and that after weight loss through gastric bypass surgery, the prevalence of alcohol misuse increases (King et al. 2012) suggests that food may compete with drugs for addictive potential and salience, potentially along the same neural pathway. However it is not likely that all obese individuals would fulfil the above criteria if applied to food, or even certain types of food (Rogers et al. 2000). In addition, heterogeneity of this population and the relative lack of supporting evidence for common mechanism in neuroimaging studies means that rigid comparison of addiction and obesity may not be appropriate (Ziauddeen et al. 2012) (see section 1.7.3).

Psychopathology in patients with binge eating disorder (BED) is perhaps more easily aligned with the addiction model. For instance binge eaters appear sensitised to food after eating

and increase the amount of work they are prepared to do to obtain more food by 40%, compared to non-binge eaters who reduced their work effort for food by 30% (Nasser et al. 2008). Furthermore binge-eaters have greater reward sensitivity and activation in the OFC to visual food cues compared to non-binge-eaters (Schienle et al. 2009).

1.4.4 Measurement of food hedonics and reward

Individual reactivity to palatable food and food cues is measured in a number of ways. Food appeal and palatability can be measured using visual analogue (VAS) or Likert scales, and food preference using food choice paradigms (Blundell et al. 2010). Visual analogue scales have been shown to have good inter-relater reliability and give best results in experimental controlled conditions, although the results may not transfer to real-life scenarios (Stubbs et al. 2000), whereas food preference paradigms allow comparison between animal models and humans (le Roux et al. 2011). Dietary records and questionnaires give information about food choice and actual dietary behaviour but suffer from the vagaries of being subjective in nature, and therefore subject to distortion by observation and underreporting (Barrett-Connor 1991). This appears to be particularly true of obese patients (Lissner 2002). Food diaries appear to be more accurate when recording intake over at least 3 day periods (Burrows et al. 2010).

More objective measures of individual reward responsiveness toward palatable food are progressive ratio tasks (which measure how hard a participant is willing to work to obtain a food reward) (Miras et al. 2012) and implicit measures of attentional bias to food or food cues such as eye movement (Yokum et al. 2011), Stroop tests (Nijs et al. 2010) and reaction times when rating food cues (Meule et al. 2012).

Neuroimaging of food reward pathways offers the additional advantage over these methods of providing objective information about the biological underpinnings on a neural level of behaviour and cognition. Functional magnetic resonance imaging (fMRI) is increasingly being used to investigate particularly non-homeostatic control of appetite in the brain (Carnell et al. 2012).

Homeostatic control is not as reliably assessed by fMRI due to the small size of the hypothalamus, and its anatomical proximity to air sinuses in the head which distort magnetic signal and therefore reduce signal obtained, motion artefacts due to proximity to the 3rd ventricle, and physiological noise from cardiac cycles, although there are techniques which aim to improve visualization of this area with modest success . In animals, techniques such as measurement of c-Fos (a transcription factor and indirect marker of neuronal activity) and manganese enhanced MRI (MEMRI) (which utilizes the calcium binding and paramagnetic properties of manganese to indirectly measure calcium influx and therefore neuronal activity in vivo in animal experiments) are therefore useful in this respect (Chaudhri et al. 2006; Parkinson et al. 2009; Hankir et al. 2011).

In addition to the hedonic or motivating aspects of food and its effect on food intake, other neurocognitive factors that govern behaviour also play a role, and may be important in the development of obesity. These include aspects of executive functioning, which govern decision-making, risk taking, response inhibition/self-control, compulsivity, impulsivity and attentional bias. There exist many validated ways of measuring these, including the Stroop test, Go/No Go test, Wisconsin card sorting, Iowa Gambling task and Delay discounting tests (Fig. 1.5).

Figure 1.5 Measurement of food hedonics

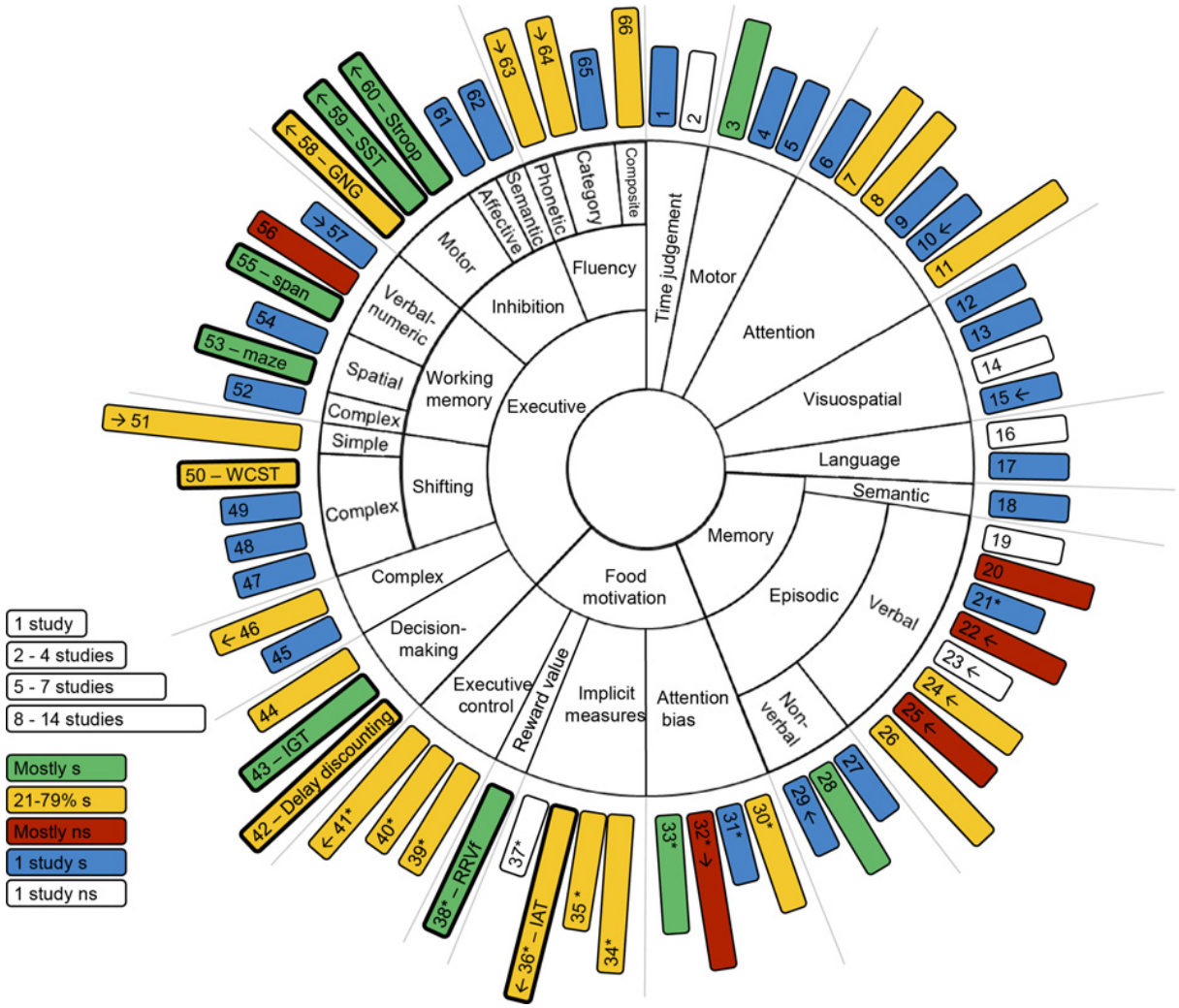


Figure from (Vainik et al. 2013), with copyright permission

Visual overview of neurocognitive measures and their possible links with obesity and weight-related appetitive behaviours. The results of the systematic search are depicted in this Figure. The major domains are positioned in the centre of the circle. Some of the domains are further broken into subdomains, when necessary. Each rectangle corresponds to a single neurobehavioural task. The length of the rectangle reflects the number of studies conducted with this task, and the colour reflects the overall outcome. Studies with replications have a separate colour scheme from studies with no replications. Asterisks indicate tasks that use food stimuli, as opposed to generic stimuli, and rectangles in bold indicate tasks that are discussed in more detail in this paper. Arrows indicate if task has been tested in a longitudional design. * = task uses food stimuli; ↑↓ = Outward arrow–task performance has been tested as a predictor of BMI change or discrepancy between planned and conducted behaviour. Inward arrow–BMI change has been tested as a predictor of task performance; GNG= go/no go; IAT = Implicit Association Test; IGT = Iowa Gambling Task; maze = Austin Maze; ns = not significant; RRVf = Relative reinforcing value of food; s = significant; span = Computational span; SST = stop-signal test; WCST = Wisconsin Card Sorting Test.

1.5 Neuroimaging techniques

1.5.1 Functional MRI

fMRI measures blood oxygen level-dependent (BOLD) changes in contrast to map neural activity. The difference in magnetic properties of oxygen-rich (oxygenated) and oxygen depleted (de-oxygenated) blood is exploited by fMRI. In MRI, a strong permanent static magnetic field (B_0) aligns hydrogen nuclei in the brain, and another (the gradient field or radio field) is applied at 90 degrees at regular intervals to move the nuclei in its path to a higher magnetization level. When the gradient field is removed, the nuclei move back to their original state and the energy emitted is measured with a coil and converted into images. In anatomical MRI, different tissues can be identified and localized according to the energy they emit, a function of how long their nuclei take to return to baseline. The strength of the signal obtained depends primarily on the proton density of the particular tissue.

Figure 1.6 Magnetic field manipulation in fMRI

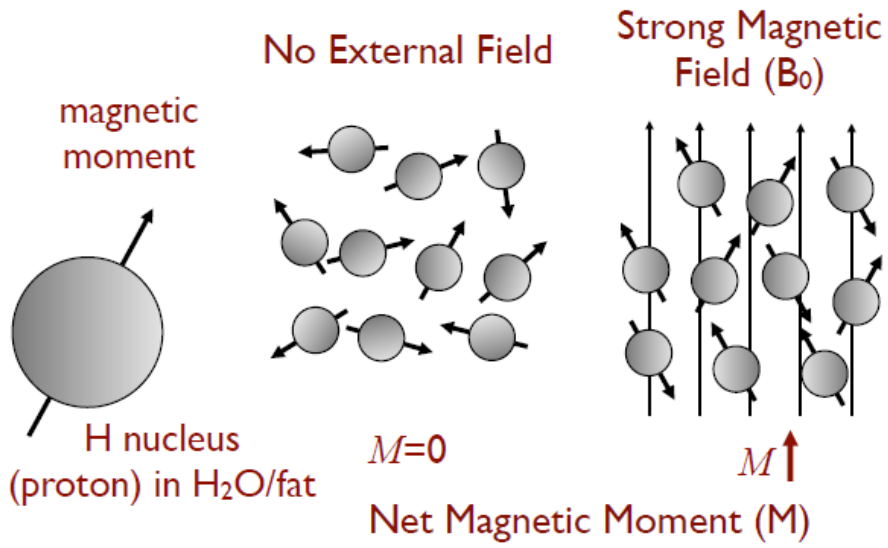
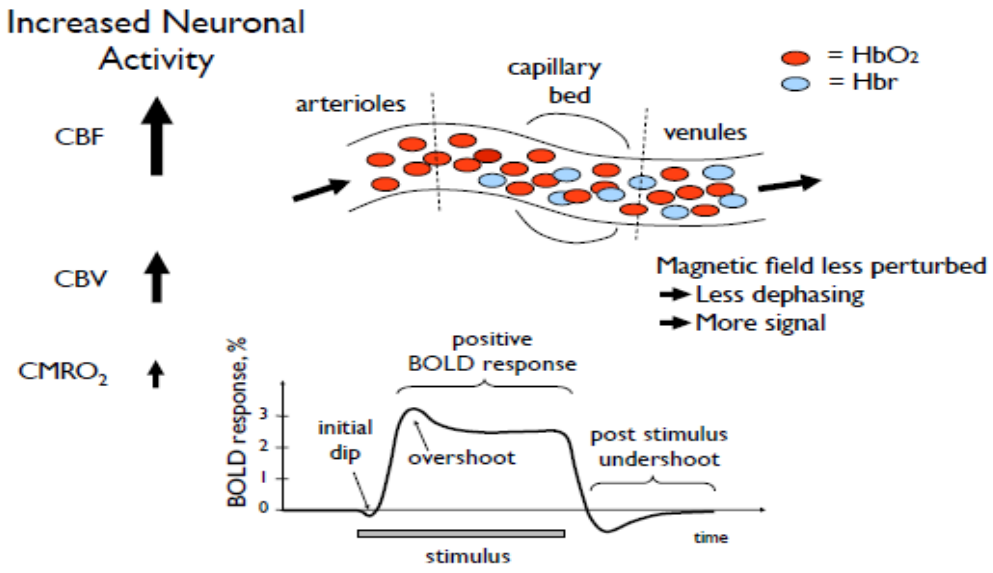


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

In fMRI, the principles of MRI are used to assess changes in blood flow to brain regions that are active, as a marker of neural activity. Increased metabolic activity within an active neuron results in localized increased oxygenated blood to that area, as a result of local vasodilatation increasing cerebral blood flow (neurovascular coupling). The oxygenated non-paramagnetic haemoglobin displaces magnetically active deoxygenated haemoglobin. In areas where more oxygenated blood flows, less interference of the gradient field signal will be registered by the coil, leading to an increase in signal and therefore increase in visible contrast. This is assumed to be an indirect measure of increased neuronal activity in that area, and can be linked a specific trigger event or stimulus being tested. This is termed the BOLD hemodynamic response function (HRF). It lags the triggering event by 1 to 2 seconds, and takes about 5 seconds to peak, after which a plateau is achieved whilst the neurons remain active. Following termination of activity, BOLD signal falls below the original level (post stimulus undershoot) and then returns to baseline (Fig. 1.7). In total the HRF duration is approximately 16 seconds.

Figure 1.7 BOLD signal

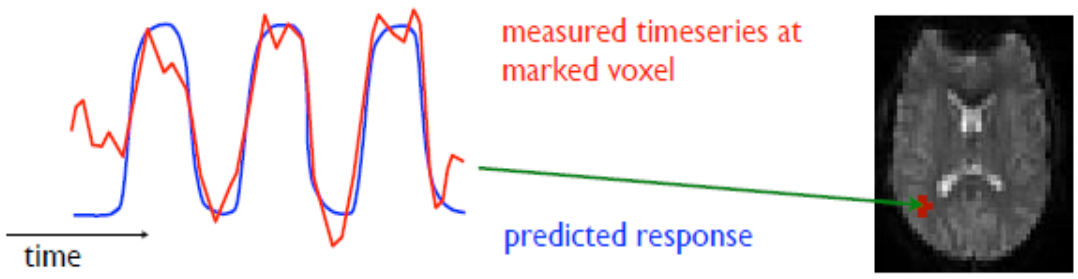


HbO_2= oxyhaemoglobin; Hbr = Deoxyhaemoglobin; CBF = cerebral blood flow; CBV = cerebral blood volume; $CMRO_2$ = cerebral metabolic rate for oxygen. Figure taken from FSL course, with kind permission of Prof. Stephen Smith

As MRI hardware has advanced, more rapid acquisition of a large number of images is possible. An example of this is echo-planar imaging (EPI), which allows the entire brain to be functionally imaged within the same timeframe as the BOLD hemodynamic response.

In order to increase the strength of signal obtained, stimuli are usually presented in multiples. For example, pictures are shown in blocks lasting 18 or more seconds. This results in continued activation of the same area of the brain, which is assumed to increase the signal strength in that area. By mapping the hemodynamic response in time against a task undertaken whilst in the scanner, changes in BOLD contrast during that time period give an indication of how brain activation changes during the task. This is then mapped against a predicted response allowing statistical analysis of the change in BOLD signal in response to a task (Fig.1.8). Since fMRI is only able to measure changes in BOLD and not a quantifiable BOLD measurement, a baseline condition is important as a reference point.

Figure 1.8 Haemodynamic response function



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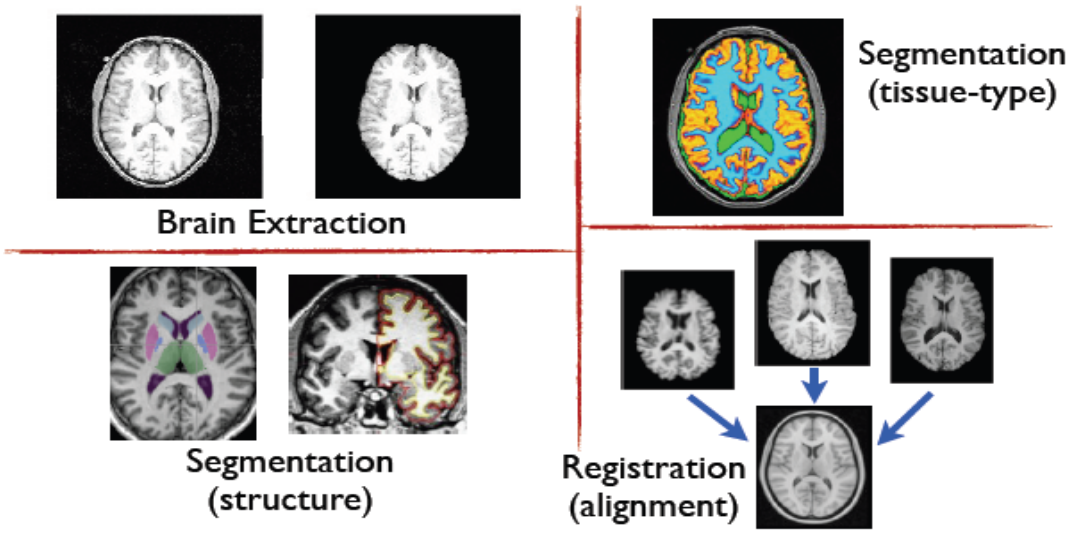
Before changes in BOLD can be interpreted, a number of pre-processing steps need to be undertaken including isolating and segmenting brain tissue, correction for motion, and aligning all images to a standardized space (see Fig.1.9)

Figure 1.9 Pre-processing in fMRI



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BOLD changes can be quantified in statistical maps, and presented visually by colour-coding the strength of activation across the whole brain or within certain pre-determined regions of interest (ROI).

The brain is divided into hundreds of thousands of voxels, each assigned signal intensity. Statistical analysis of each voxel or cluster of voxels can ascertain whether the signal intensity in that particular voxel or cluster of voxels is greater than the signal intensity in another part the brain, in response to a particular stimulus. A statistical threshold can then be applied to either the whole brain or to ROIs. In analyzing 100,000 units of brain (or voxels) at the same time in whole brain analysis, the problem of multiple comparisons is evident. Even small structures such as the amygdala contain around 50 voxels. This can be corrected for in a number of ways. For instance the overall statistical threshold can be raised (e.g. Using a P value of <0.001 as threshold instead of $P<0.05$) or Bonferroni correction can be made. Further methods include using FDR (false discovery rate) or FWE (family-wise error) corrections, which are less stringent but equally valid ways of making

corrections for multiple comparisons, using Bayesian cluster statistics. ROI analysis has a greater chance of finding statistically significant results but runs the risk of missing activated areas of the brain that were not included in the original hypothesis. Scans can be performed either when the subject is not doing anything in particular (at rest) or during a specific task.

Rest scans are used to analyze functional connectivity between different brain regions, assuming that distinct neural networks function in a coordinated brain response and that these are altered in various conditions (e.g. disease or aging or even state-dependent). Rest scans are also used for pharmacological fMRI studies in which continuous resting fMRI is measured before and after administration of a drug or hormone (Batterham et al. 2007; Vidarsdottir et al. 2007; Jones et al. 2012).

In task related studies, subjects are asked to complete a task whilst in the scanner. For example, a common approach in appetite studies is for subjects to be presented with images, smells or tastes of food (with further subdivision into high-calorie, or low-calorie food, and anticipation or actual receipt of a tastant) or non-food items. A subtraction analysis is performed to see whether the difference in regional brain activation between viewing images of food vs. non-food or high-calorie vs. low-calorie food is altered in different pathological states (eg. obese vs. lean, high vs. low psychological or eating behaviour trait), or physiological state (e.g. fasted vs. fed, before vs. after bariatric surgery, drug/hormone vs. placebo).

1.5.2 Positron emission tomography (PET)

Another popular imaging technique is positron emission tomography (PET). PET involves detecting changes in neuronal activity from a baseline state, by measuring degeneration of an unstable nucleus radioactive tracer injected intravenously. The decaying nucleus emits a positron which collides with surrounding tissue electron to emit a ray which is recorded using detectors around the head. Since the half life of decay is known, within subject differences can be quantified between states. The temporal resolution can be accurate, but spatially signal can be up to 6mm from actual neuronal activity. These differences in the indirect measure of neuronal activity are then mapped onto standard space structural MRI maps, and statistical parametric maps of the average activation across subjects can be created.

PET can measure state-dependent differences but not task related. By varying the tracer, different neuronal populations can be targeted, offering the advantage of obtaining information about neuronal metabolism (e.g. ^{15}O-water for the measurement of regional cerebral blood flow (rCBF) related to neuronal activity; ^{18}F-fluorodeoxyglucose (FDG) for the measurement of cerebral glucose uptake) and neurotransmitters (e.g. Dopamine (DRD2/3), MOR or uptake of precursors e.g. L-DOPA). Neurotransmitter function can be deduced by receptor binding which depends on the specificity of the ligand, receptor availability and neurotransmitter release (e.g. dynamic changes in dopamine release induced by amphetamine or drug challenges) (Booij et al. 2012).

1.5.3 Advantages and limitations of fMRI

The advantages of fMRI compared with PET is its ability to acquire task-related information, non-invasively (i.e. without radionucleotide injections as in PET) and its superior spatial and

temporal resolution over PET, although temporal resolution is dependent on blood flow changes and therefore lags the task by some seconds. Spatial resolution of about 8-27 mm^3 (2-3mm x 2-3mm x 2-3mm)(approximately the size of a peppercorn) (termed voxel size) is possible, but still accounts for millions of neurons and billions of synapses.

fMRI's limitations are due to factors which limit the interpretation of data obtained. These are influenced by the choice of experimental design and how fastidiously the paradigm is carried out. Unwanted signal (noise) from various sources including from the scanner itself, inhomogeneities in the magnetic field strength, head movement, physiological changes in blood flow independent of the task, neuronal activity not related to the task, and various other sources corrupts the data obtained. Experimental designs therefore need to minimize noise as far as possible, for instance by reducing head movement, making corrections for inhomogeneities in the magnetic field and physiological changes in blood brain flow not related to the task, as well as applying filters to remove frequencies not of interest and to obtain averaging of voxels neighbouring each other.

It is also important to make sure that the baseline condition is sufficiently different to the stimulus presented to obtain a meaningful change in signal. Even rest involves activity that may detract from interpretation of signal obtained during the task. A fundamental assumption of fMRI interpretation is that increased signal means increased activity, whereas increased signal may in fact represent metabolic activity in line with deactivation of the neurons. Neighbouring neurons may be also performing different tasks during the same stimulus presentation that cancel each other out, or decrease the observed signal. This is particularly true of the hypothalamus, where adjacent nuclei perform opposite functions. In addition, if specific areas are shown to be active during a given task, and previous studies

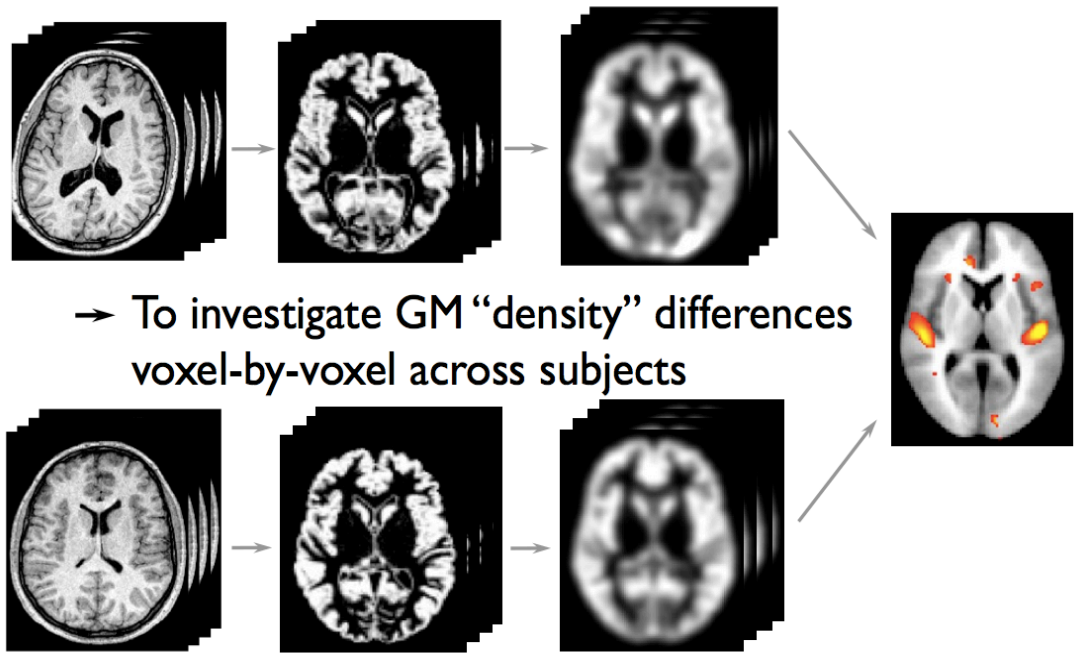
have ascribed a particular function to that same area based on another task, the inference is sometimes made that the new task is therefore engaging the same cognitive process. This is termed reverse inference and is logically flawed (Poldrack 2006). Furthermore increased metabolic activity is now known to result more from post-synaptic rather than pre-synaptic activation

Most of these limitations can however be overcome with good experimental design and judicious and cautious interpretation of results. fMRI remains the current mainstay of brain imaging particularly in the area of cognitive research. Indeed functional neuroimaging techniques such as PET and fMRI are now established tools in the study of food reward (Neary et al. 2010; Carnell et al. 2012; Smeets et al. 2012).

1.5.4 Voxel based morphology (VBM)

Voxel-based morphology (VBM) is a neuroimaging analysis technique which allows measurement of differences in particularly grey (but also white) matter density in the brain within subjects longitudinally, or between groups of subjects. It makes use of structural MRI scans (T1 scans) and computes the grey matter density in each voxel across the brain once placed in standard space. Voxelwise comparisons across individuals are then made correcting for multiple comparisons.

Figure 1.10 Voxel based morphometry



Abbreviations: GM: grey matter Figure taken from FSL course, with kind permission of Prof. Stephen Smith

Several steps in the analysis of MRI structural scans prepare the data for comparison between subjects. For instance, brain tissue is extracted from scans to exclude superfluous tissue, such as skull tissue, and the images undergo tissue-type segmentation to separate out grey matter from white matter and cerebrospinal fluid. Each individual's structural scan is then registered to a template constructed from a standardized brain (either from a reference brain e.g. MNI152, or from a study specific template brain) to allow for comparison within the same standard space. A technique called smoothing averages out the concentration of each voxel and surrounding voxels, to correct for registration errors and reduced signal to noise ratio. The grey matter density is then calculated for each voxel in each subject and comparisons made between subjects.

Figure 1.11 Processing steps in VBM

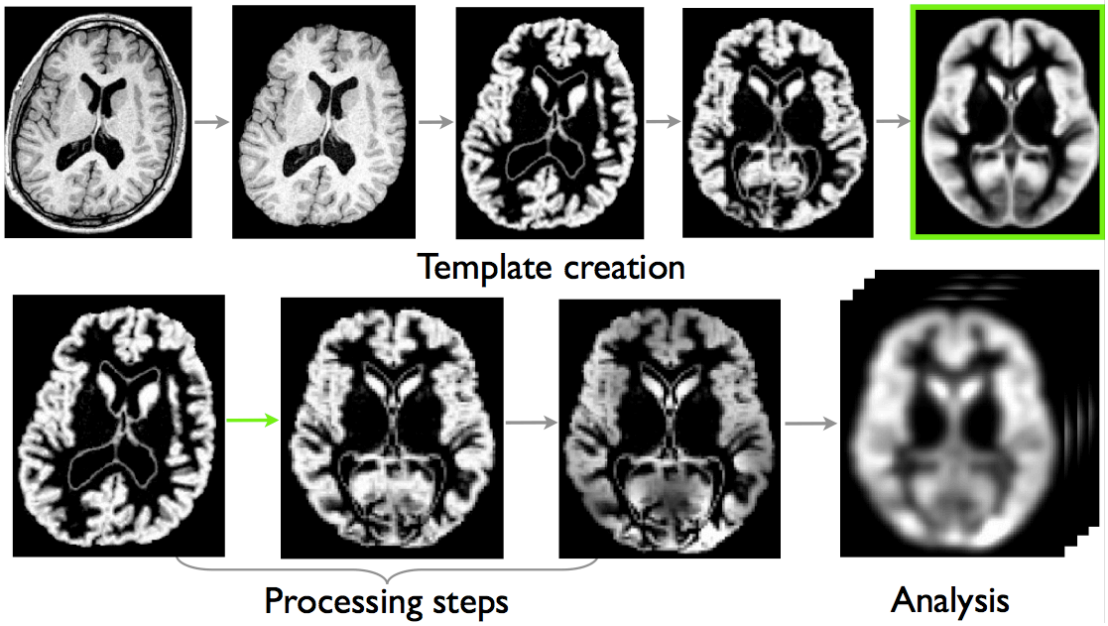


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

Subcortical areas of interest can also be pre-selected and volumetric differences between groups in these areas of interest calculated using T1 images in subject as opposed to standard brain space, using FFMRIB's Integrated Registration & Segmentation Tool (FIRST). This model-based FSL tool uses a priori knowledge of the structure and shape of previously learned models of subcortical regions to calculate the most probable shape instance of those regions in study populations, given the observed intensities in a T1-weighted image. In this way, regions of interest for specific conditions, traits or functions can also be assessed.

There are several methodology differences in VBM according to the analysis software technique employed, and the number of regions investigated and number and nature of correction included in the analysis, thresholding and use of optimized or standard protocol.

This makes comparison between studies difficult, and has hampered interpretation of previous studies.

Factors which independently affect grey and white matter volume and density such as age, gender and ethnicity should also be controlled or corrected for. Interpretation of differences between populations in grey matter density using VBM can also be complicated by the fact that such differences may be as a result of either grey matter volume differences or of differences in gyrification, contrast or registration of grey matter. The clinical relevance of these scenarios remains unclear.

Figure 1.12 Interpretation of VBM results

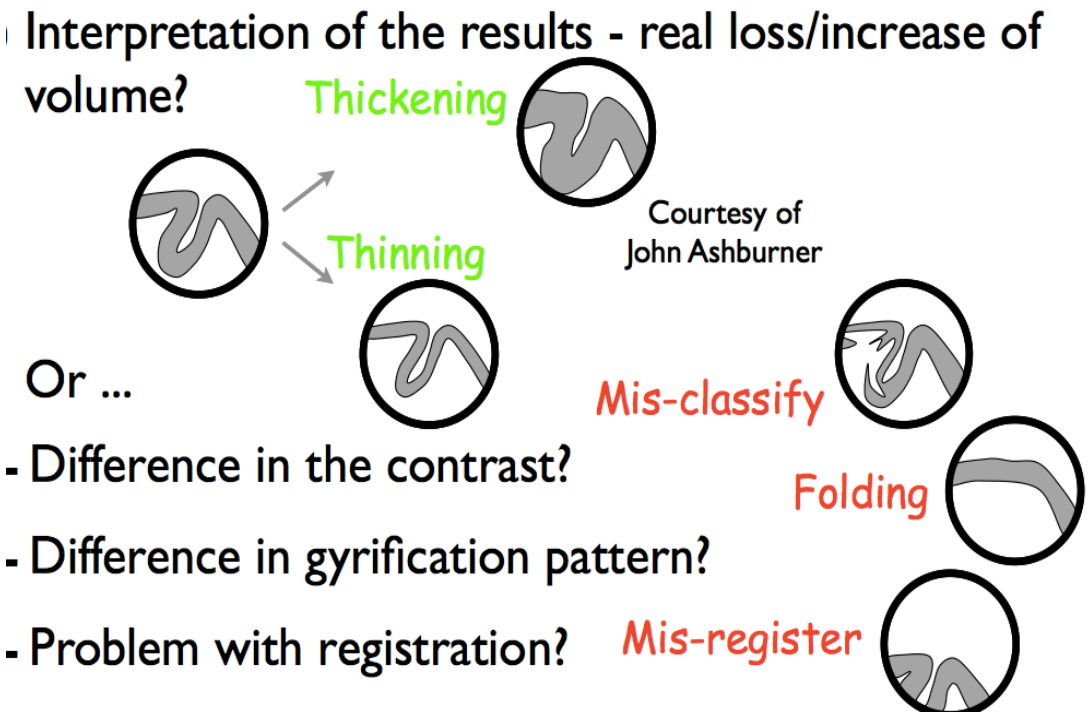


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

However, correlations of grey matter density with clinical or psychological scores, e.g. dietary restraint or depression scores, may be particularly useful in making associations of psychological factors with neuronal structure in particular areas of the brain. In addition interpretation of co-existing functional MRI data is strengthened by finding that there are no structural differences between the populations being examined which may account for the fMRI results. Grey matter density/volume in areas involved in the processing of reward have been associated with increased BMI (Pannacciulli 2006, Walther 2010, Ho 2010, Taki 2008. Raji 2010, Gunstad 2008, Orsi 2011, Widya 2011, Jagust), leptin levels (Pannacciulli 2007, Horstmann 2011), and obesity associated genes e.g. FTO (Ho 2010) (See Table 1.2).

1.5.5 Diffusion tensor imaging (DTI)

DTI is a technique used to quantitatively measure the integrity of white matter in the brain (Alexander et al. 2007). MRI is used to infer the structure of white matter tracts based on the pattern of diffusion of water molecules within the brain. In white matter, unlike grey matter and cerebrospinal fluid, diffusion occurs predominantly along one axis (Fig. 1.13).

Figure 1.13 Diffusion of water within neurons

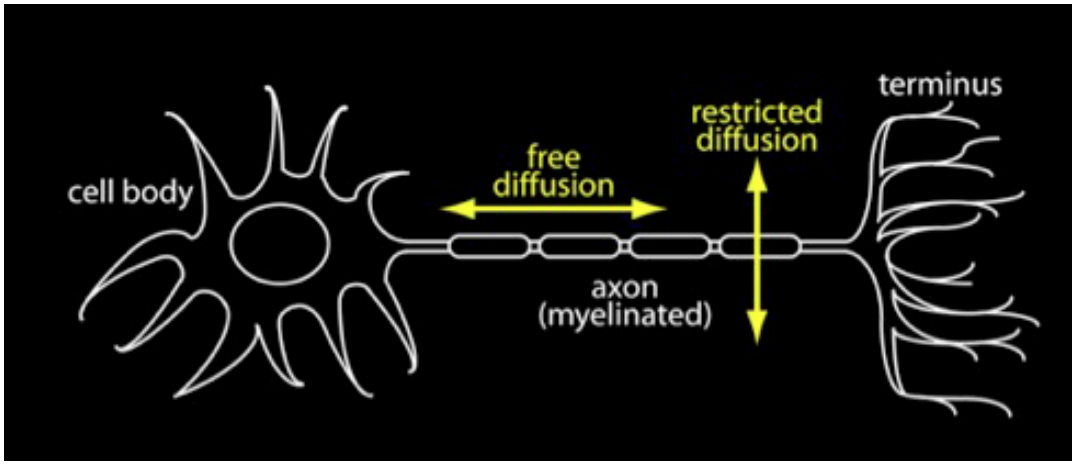


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

This is termed anisotropy, and occurs preferentially along intact white matter parallel to the direction of the tract. Diffusion perpendicular to the tract is limited by the presence of cell membranes and myelin. Both the rate and direction of diffusion of water molecules carries information and are depicted in cartography images which make use of colour-coding and weighting to depict tracts according to their fractional anisotropy and direction (Fig. 1.1.4).

Figure 1.14 Colour coded images in of white matter tracts

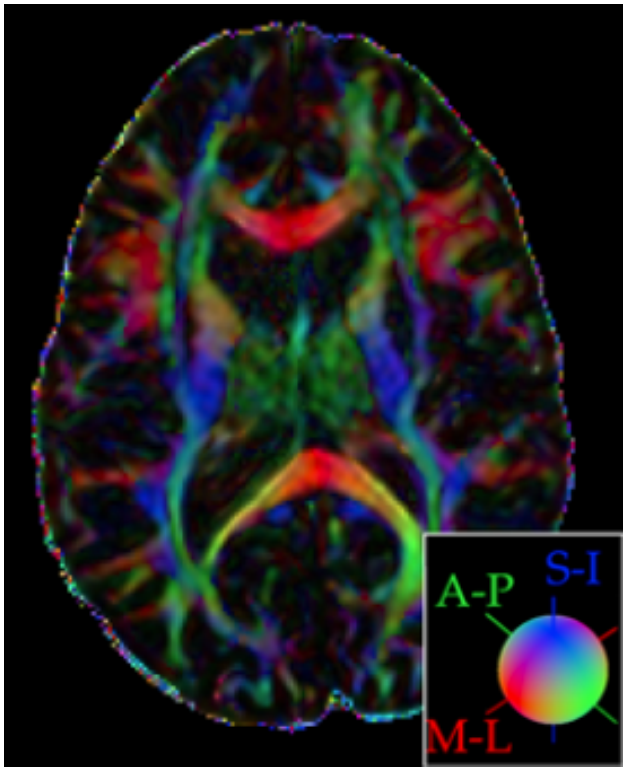


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

Each voxel's properties are calculated by vector math using six or more different diffusion weighted acquisitions, each obtained with a different orientation of the diffusion sensitizing magnetic gradients. A fibres' direction is calculated using the main or so-called eigenvector.

Figure 1.15 Depiction of eigenvector within neural fibres

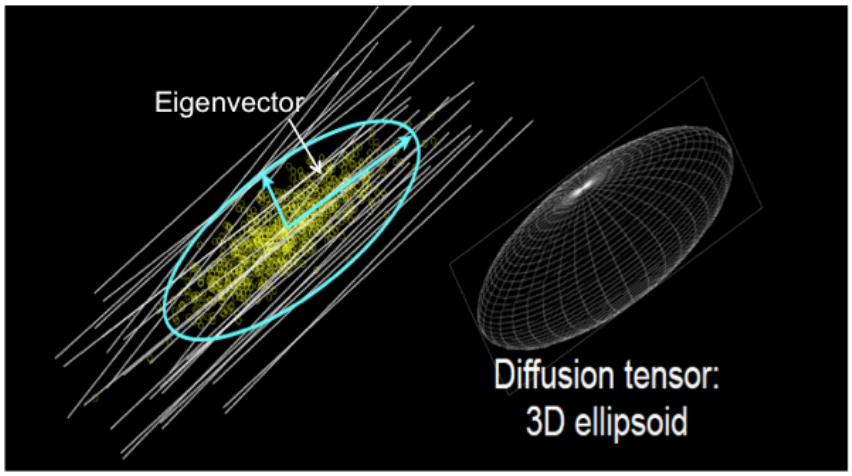


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

Several DTI measures can be used to assess white matter integrity, including the degree of anisotropy within a voxel known as the fractional anisotropy (FA) (on a scale of 0 to 1, where 1 is parallel to the tract), and the degree of total diffusion within a voxel known as the mean diffusivity (MD) (measured in μm/μsec) (Fig.1.15 and 1.16).

Figure 1.16

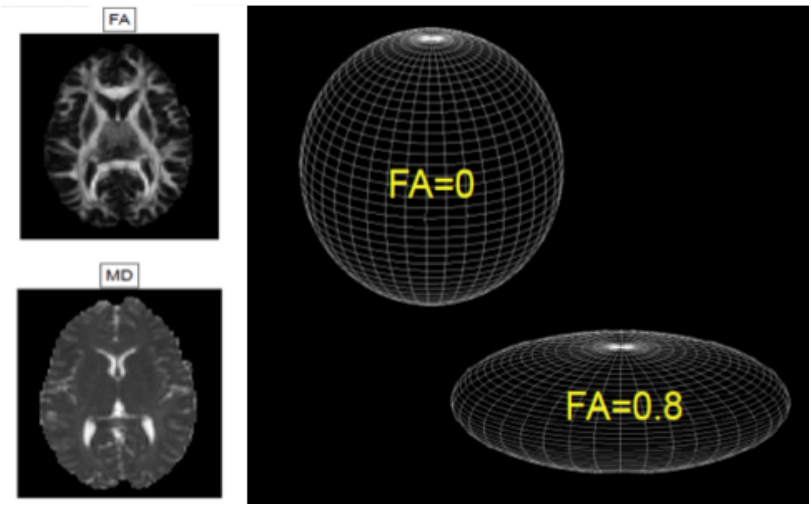


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

Inferences may be made about structural connectivity of different regions depending on the structural integrity of white matter tracts connecting these regions, using tractography, which track the structural integrity of white matter fibres along their lengths (Fig. 1.17)

Figure 1.17 Tractography of white matter tracts

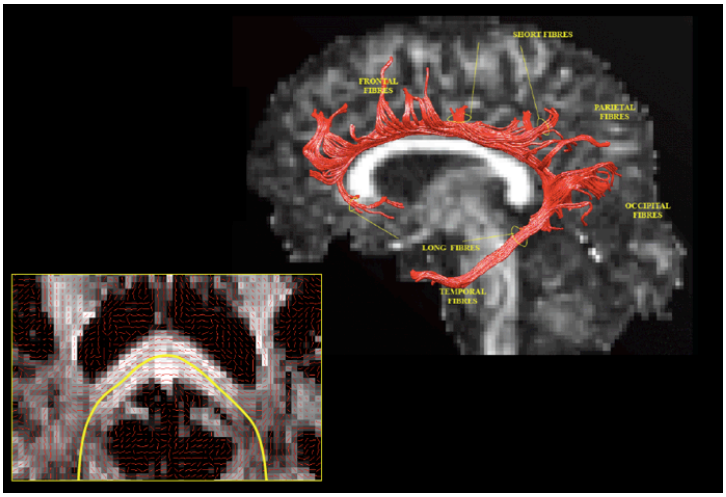


Figure taken from FSL course, with kind permission of Prof. Stephen Smith

For example, reduced integrity in fibres connecting corticolimbic reward areas may imply structural, connectivity and therefore functional deficits in these areas (Delgado et al. 2012).

Reduced FA appears to be a marker of reduced integrity of white matter tracts and is seen in Parkinson's disease (Cochrane et al. 2013), Alzheimer's disease (Stebbins et al. 2009), traumatic brain injury (Maller et al. 2010), autistic spectrum disorder (Travers et al. 2012), bipolar disorder (Vederine et al. 2011) and obsessive compulsive disorder (Peng et al. 2012) amongst others. Conversely increased FA or connectivity may be a marker of improved cognitive functioning (Sato et al. 2013) or compensatory alternative neural pathways (Palacios et al. 2013).

Studies using DTI have also demonstrated white matter changes associated with depression (White et al. 2008), obesity (see section 1.8.2), diabetes (Hsu et al. 2012), metabolic syndrome (Segura et al. 2009), addictive behaviour (Bora et al. 2012) impulsivity (Olson et al. 2009) and reward sensitivity (Xu et al. 2012).

1.6 Behavioural studies of food hedonics in obesity and weight loss

1.6.1 Food hedonics in obesity

In animal models of obesity, genetically obese or obesity-prone rats and diet induced obese rats demonstrate an increased preference for high concentrations of sugar and fat, compared to lean rats (De Jonghe et al. 2005; Shin et al. 2011).

Behavioural studies in humans have shown that obese or overweight people appear to have a strong preference for high-calorie foods, which may either predispose them to or be a learned behavior as a result of overeating, although not all studies agree.

Obese subjects demonstrate an automatic attentional bias toward food measured by eye movement and the Stroop test (which measures selective attention and cognitive processing speed) (Braet et al. 2003; Castellanos et al. 2009; Nijs et al. 2010; Werthmann et al. 2011; Yokum et al. 2011), although not in one study of adolescents (Soetens et al. 2007). Obese people also show greater anticipatory orofacial reactions, galvanic skin responses and increases in cardiac rate toward palatable food cues such as pictures of food or smell compared to normal weight individuals (Schachter 1968; Soussignan et al. 2012). This increased appeal of food predicts weight gain in infants (van Jaarsveld et al. 2011) and is associated with higher BMI or adiposity in children (Carnell et al. 2008; Soussignan et al.

2012). Furthermore consumption of palatable foods is more strongly triggered by food cues such as smell or taste in obese individuals compared with normal weight individuals (Schachter 1968; Rodin et al. 1989; Jansen et al. 2003; Tetley et al. 2009; Ferriday et al. 2011). Several studies of food biases have indicated that preference for highly palatable, high-calorie foods is stronger among obese than among lean individuals and can predict weight gain (Drewnowski et al. 1992; Mela 2001; Rissanen et al. 2002; Salbe et al. 2004) In addition, degree of craving for high-calorie foods is positively correlated with BMI (Burton et al. 2007). Obese people may find palatable food more appealing than lean people (Stoeckel et al. 2008), but this is not always reflected in their rating of food pictures (Scharmuller et al. 2012), indicating a potential dissociation between implicit and explicit attitudes toward food or perhaps a dissociation between the anticipation vs. receipt of food (Roefs et al. 2002; Czyzewska et al. 2008).

1.6.2 Food hedonics after non-surgical weight loss

There are few studies that examine the effect of dietary methods of weight loss on food preference, and studies are contradictory in their results. Fasting has been shown to increase the desire to eat as measured by visual analogue scale and also increased food intake in adults (Doucet et al. 2000). Other studies using progressive ratio tasks have also shown an increase in the amount of work participants will do for preferred foods after food deprivation (Epstein et al. 2003; Raynor et al. 2003). On the other hand, low calorie diets have been shown to *reduce* cravings for foods (Lappalainen et al. 1990; Harvey et al. 1993; Martin et al. 2006). In children at any rate, an observed shift in preference toward high-calorie foods is reversed following a dietary intervention (Epstein et al. 1989). Given the high rates of recidivism following dietary induced weight loss in adults, it is unlikely that in adults these changes are sustained in the longer term. It may be that subsequent increase in

appetite and food craving is mediated by gut hormones. For example, in a recent study, overweight and obese patients who underwent a 10-week weight loss program had significantly lower levels of leptin, PYY, CCK, insulin, and amylin and significant increases in ghrelin levels from baseline (Sumithran et al. 2011). These differences persisted at one year and were accompanied by significant increases in appetite and pre-occupation with food, that may contribute to the long-term failure of dietary restriction for weight loss. In addition, taste acuity for sweet foods decreases after weight loss, possibly mediated by reductions in plasma leptin, so that more sweet food may be needed to satisfy a craving for it (Yoshida et al. 2012). Increased attentional bias to high calorie foods is associated with poor weight loss attempts (Meule et al. 2012).

1.6.3 Food hedonics after RYGB or BAND surgery for obesity

In gastric bypass, but not gastric banding, a shift in preference away from high-calorie, particularly sweet and fatty, food has been reported. It has been postulated to contribute to the superior weight loss of RYGB (Shin et al. 2011). The mechanisms underlying this phenomenon remain unclear.

1.6.3.1 Animal studies

Decreased licking of high concentrations of sugar (Shin et al. 2011; Tichansky et al. 2011) and high fat liquids (Shin et al. 2011), has been observed in rats after gastric bypass surgery with a shift in preference toward low concentrations of fat and sugar. A reduction in preference for sucrose (at a low and high concentration) and decreased lick responses for sucrose (for a spectrum of concentrations) in rats after gastric bypass, compared with unoperated obese rats and lean rats, has been reported (Hajnal et al. 2010). A similar effect was noted for other sweet compounds but not for salty, sour, or bitter tastants. In this experiment lean

rats who underwent RYGB did not show altered responses to any stimulus tested.

Rats after gastric bypass also have reduced preference for high fat chow (le Roux et al. 2011; Shin et al. 2011), and increased preference for low fat chow (Zheng et al. 2009; le Roux et al. 2011). In addition, rats after gastric bypass show reduced acceptance of high fat liquid if previously conditioned to it, or no intake of high fat chow at all if never exposed to it pre-operatively, compared to sham-operated rats which consistently prefer high fat solid and liquid chow (Zheng et al. 2009).

Interestingly, obese rats took longer to complete a food reward task compared to lean rats, and rats after gastric bypass performed similarly to lean rats, again suggesting a change in hedonics following gastric bypass surgery, although these results might also indicate an improvement in concentration or cognitive ability after gastric bypass surgery (Shin et al. 2011).

In one study, a reduced preference for high fat liquids over 48 hours, was not replicated when the test was applied over a shorter period and animals were water-deprived to eliminate post-ingestive effects. An oral gavage of 1 ml corn oil after saccharin ingestion in rats after gastric bypass, but not sham-operated rats, induced a conditioned taste aversion, suggesting that post-ingestive effects may play a role in the taste preference shifts seen after gastric bypass surgery (le Roux et al. 2011). Unexpectedly and contrary to previous studies, the same group found that rats after gastric bypass licked *more* sucrose relative to water compared with sham-operated rats (Mathes et al. 2012).

Although it has been speculated that shifts in food preference may be mediated by increased gut hormone levels operating through a gut-brain axis, the only study to date to examine this found no effect of GLP-1 blockade on sucrose preference in less obese rats given gastric bypass surgery (Mathes et al. 2012).

Therefore it is currently unclear whether changes in taste acuity, alteration in reward value of food along the gut-brain axis or post-ingestive effects leading to aversion for sweet and fatty foods, or indeed a combination of these results in the observed shift in preference away from high fat, high sugar foods in rat models of gastric bypass.

1.6.3.2 Human studies

In humans, (i) altered macronutrient dietary intake i.e. protein, carbohydrate and fat intake, (ii) specific food preference changes i.e. shift away from sweet, fatty or unhealthy food, (iii) reduced reward responses to sweet and fatty food, and (iv) altered taste acuity have all been reported after gastric bypass surgery.

Various observational studies have confirmed a reduction in overall calorie intake in both gastric bypass and restrictive procedures, such as BAND, VBG and HPG (Brown et al. 1982; Coughlin et al. 1983; Kenler et al. 1990; Brolin et al. 1994; Trostler et al. 1995; Trostler et al. 1995; Sjostrom et al. 2004; Olbers et al. 2006; Kruseman et al. 2010; Liu et al. 2012).

Additionally, two studies have shown that gastric bypass patients consume less calories than patients who have undergone a restrictive procedure (HPG or VBG) (Kenler et al. 1990; Brolin et al. 1994; Olbers et al. 2006).

1.6.3.2.1. Macronutrient diet composition

Changes in the macronutrient composition of diet (carbohydrate, fat or protein) after gastric bypass surgery, particularly in the longer term are not supported by robust evidence. In the short term, there is a risk of a confounding effect of post-operative dietary advice. Most studies show no change in the proportion of food intake accounted for by protein, carbohydrate or fats after gastric bypass surgery (Coughlin et al. 1983; Kruseman et al. 2010; Liu et al. 2012). There are no studies which show changes in macronutrient composition after BAND surgery.

One study showed modest shifts toward increased protein and reduced carbohydrate intake, maintained over a 24 month period after gastric bypass, although these patients were given dietary advice to eat more protein (Kenler et al. 1990). However, in the same study, when compared with patients who had undergone a restrictive procedure, given the same advice, increased protein intake was seen in both groups up until 18 months follow up, but persisted until 24 months only in gastric bypass patients. Similar results were seen in a subsequent similar study by the same group (Brolin et al. 1994).

In the only randomized controlled trial comparing dietary changes in gastric bypass with VBG, gastric bypass patients reported a significant lower proportion of calories ingested as fat (mean ± SD, 30.5% ± 5.5% vs. 35.2% ± 6.3%, P=0.0014) and higher proportion of calories from carbohydrates (52.0% ± 6.9% vs. 47.7% ± 7.6%, P=0.0149) compared with VBG patients. There was no difference between groups in protein intake (Olbers et al. 2006).

One study showed that a shift in preference from a high fat to low fat version of a savoury

snack after surgery only occurred in those RYGB patients who had a high index of genetic susceptibility to obesity, suggesting that a shift in food preference may involve an interaction of RYGB surgery on genetic factors (Thirlby et al. 2006).

There was no difference in macronutrient composition in patients who had successful weight loss after RYGB surgery compared to those who did not in another study (Warde-Kamar et al. 2004).

1.6.3.2.2 Shift away from sweet and fatty food

There is more robust evidence for a reduction in consumption specifically of sweet desserts and drinks in RYGB patients when compared with restrictive procedures such as BAND or VBG (Brown et al. 1982; Sugerman et al. 1987; Kenler et al. 1990; Brolin et al. 1994; Olbers et al. 2006; Ernst et al. 2009; Kruseman et al. 2010; Thomas et al. 2010).

Kenler found that obese patients after RYGB halved their intake of sweet foods, and milk and ice cream products over a 2 year period, compared to horizontal gastroplasty (HPG) patients in whom sweet food intake was unchanged and milk and ice cream intake *increased* by a third (Kenler et al. 1990). They found similar results in a further study, this time comparing RYGB and VBG patients (Brolin et al. 1994). Olbers et al similarly found that patients randomized to RYGB surgery had a lower proportional intake of sweet foods in their diet compared to VBG patients (Olbers et al. 2006). These results support Sugerman's earlier similar findings upon which he recommended that only RYGB surgery be offered to sweet eaters (Sugerman et al. 1987).

Other studies however showed no change in these parameters (Trostler et al. 1995) or rates of consumption similar to general population (Warde-Kamar et al. 2004), despite a reported intolerance to sweets in the latter study.

There was no conclusive evidence (most studies had no control group or comparison to previous intake) of increases in fruit and vegetable intake, with either no change or reduced intake reported (Halmi et al. 1981; Brown et al. 1982; Trostler et al. 1995). Two studies did find that fruit and vegetable intake was greater after RYGB compared to BAND or VBG surgery (Olbers et al. 2006; Ernst et al. 2009).

The above studies on food preference acquired data using dietary recall or the keeping of dietary records, and these methods have been criticized for their inaccuracy, which may account for some of the variability in results (Mathes et al. 2012).

Studies that measure actual behaviour in an experimental setting on the other hand, offer the advantage of avoiding recall bias and reducing the interpretive problems associated with scaling procedures.

1.6.3.2.3 Taste acuity

Changes in taste acuity have been reported but these are inconsistent and difficult to separate from shifts in preference (Tichansky et al. 2006; Miras et al. 2010; Thomas et al. 2010).

Scruggs et al. found that obese patients had no difference in taste acuity compared to lean

controls, but that following RYGB surgery, taste recognition thresholds showed a trend to be decreased for sweet, salty and sour tastes, and was significantly decreased for bitter taste (Scruggs et al. 1994) Burge et al. found that sweet but not bitter recognition thresholds decreased after RYGB surgery (Burge et al. 1995).

Using self-report measures, a comparison of 82 RYGB and 28 BAND patients, found 82% of RYGB compared to 46% of BAND patients answered yes to the question "Has the taste of food and beverages changed after surgery?" (Tichansky et al. 2006). There was no difference in reported loss of taste. 92% of RYGB vs. 59% of BAND patients reported a decrease in intensity of taste. 62% of BAND patients developed increased taste for sweet foods, compared to only 27% of RYGB. On the other hand 55% of RYGB patients developed an increased taste for salty food, compared to 23% BAND patients. In another study, RYGB patients reported a shift in preference toward salty foods, and HGB patients a shift toward sweet foods, and increased intake of milk and ice cream products at 6, 18 and 24 months post-surgery (Kenler et al. 1990).

Rodent studies have been inconclusive with regards to taste sensitivity measures, since these can be difficult to separate from reward-related behaviour (Mathes et al. 2012). In their review, the authors point to the confounding effects of dietary advice, differences in surgical technique and predominance of female patients but lack of control of hormonal factors such as stage of menstrual cycle which influences appetite. These factors make interpretation of these results difficult. In addition, they suggest that there may be different mechanisms at work at different stages of weight loss after surgery, which may lead to variability in results, eg. acute weight loss phase may be more influenced by restriction and dumping symptoms. They suggest future studies should make use of paradigms measuring

actual eating behaviour and reward as well as examining the effect of these surgeries on the gut-brain axis using neuroimaging and other techniques.

1.6.3.2.4 Food reward

In a unique study by our Group, the reward value of a sweet and fatty snack (M&M) was reduced after RYGB surgery, suggesting that the change in preference for this type of food may be mediated by a reduction in how rewarding the food is perceived to be (Miras et al. 2012). This study used a progressive ratio task, i.e. lever pressing to obtain the candy-coated chocolate or a vegetable snack. Progressively more lever presses are required to obtain the snack, until a "breakpoint" is reached. In RYGB patients, this "breakpoint" was reduced by 50% after surgery for the candy, but was unchanged for a vegetable snack. Test-retest reliability of the paradigm was confirmed by finding no change in breakpoint for either candy or vegetable in normal weight controls. As the snacks were bite-size, and total calorie intake during the experiment low, the reduced appeal of and work to obtain them is attributed to reduction in reward value rather than post-ingestive effects of dumping(Miras et al. 2012).

Ochner's group has also shown selective reduction in the desire to eat (wanting) and liking of high calorie foods, relative to low-calorie foods following RYGB surgery. Reduction in mesolimbic neural reactivity to high-calorie food cues was also associated with ratings wanting but not liking high-calorie food cues (Ochner et al. 2011; Ochner et al. 2012).

1.6.3.2.5 Dumping symptoms following gastric bypass surgery

It has been postulated unpleasant symptoms after typically sweet and fatty foods referred to as “dumping”, may play a role in decreased food intake after RYGB, but not BAND surgery. By experiencing unpleasant symptoms after eating, conditioned taste aversion to these foods may develop, so that patients avoid these high-calorie foods and thus reduce their overall calorie intake.

The term “dumping” was first used by Andrews and Mix in 1920, to describe the observation of rapid gastric emptying of radiographic contrast in patients who experienced typical unpleasant post-prandial symptoms after undergoing a gastrectomy (Wyllys E).

Symptoms of “dumping” include nausea, vomiting, abdominal pain, diarrhea, weakness, faintness and sweating shortly after a meal and a reactive reduction in glucose levels about 1-2 hours later. Rapid gastric emptying is defined as >50% emptying of stomach after 1 hour (based on analysis of the normal range data established for standardized GET). This results in hyperosmolar (especially sugars) contents of the stomach entering the small intestine resulting in fluid shift from the intravascular compartment into the intestinal lumen, leading to small bowel distention. This fluid shift causes a drop in blood volume and initiates the release of various humoral and neural mediators including vasoactive serotonin, leading to increased blood flow to the bowel and skin. The increased intestinal contractibility following the fluid shift is believed to be responsible for nausea, bloating, abdominal cramps, and can lead to urgency and diarrhoea in a subset of patients.

“Late dumping” occurs 1–2 h after a meal, and is a consequence of a reactive reduction in

glucose and hypokalemia, as glucose, accompanied by potassium, enters the cells after the initial hyperglycemia from rapid absorption of glucose, and release of insulin takes place. Late dumping is characterized by systemic vascular symptoms, orthostatic changes in blood pressure and increased heart rate, and symptoms include flushing, dizziness, weakness, faintness, and palpitations. (Ukleja 2005; Hejazi et al. 2010)

Intolerance to sweet foods (Warde-Kamar et al. 2004; Kruseman et al. 2010) and milk products (Coughlin et al. 1983; Kenler et al. 1990) and avoidance of fatty foods due to unpleasant effects (Olbers et al. 2006) have been all been reported after RYGB. "Dumping" symptoms have been reported in up to 50% of patients 2-3 years after RYGB surgery (Sugerman et al. 1987). However, the above observations of dumping symptoms were incidental or qualitatively acquired, and had not been formally measured. In the only study that did formally compare dumping in RYGB and restrictive procedures, 75% of RYGB patients reported dumping symptoms at a mean of 18 months after surgery, compared to 0% VBG, but dumping was not correlated with weight loss, and RYGB patients who did not have dumping symptoms still lost more weight than the VBG patients (Mallory et al. 1996).

After RYGB, symptoms of dumping are less clearly attributable to rapid gastric emptying alone. Various additional factors brought about by the anatomical changes induced by surgery, including disruption of vagal afferents, vascular and hormonal changes may play a role. The early delivery of nutrients to the distal intestine may also play an important role in causing these symptoms, since gut hormones that stimulate insulin secretion (such as GLP-1) have been implicated in the pathogenesis of dumping following gastrectomy. For example, higher post-prandial levels of the gut hormones such as pancreatic polypeptide, GLP-1, peptide YY, neurotensin and enteroglucagon as well as noradrenaline and serotonin have

been documented in the patients with dumping syndrome (Gebhard et al. 2001). This is particularly relevant in RYGB surgery where some of these hormones are elevated postprandially from 1 week after surgery (le Roux et al. 2005), and remain elevated in long term studies (Laferrere 2011). In addition, patients with T2DM have higher incidence of dumping after bariatric surgery (Padoin et al. 2009).

"Post bypass hypoglycaemia" has been described which needs to be differentiated from dumping. (Ritz et al. 2011). This is sometimes referred to as a type of "nesidioblastosis" or non-insulinoma pancreatogenous hypoglycaemic syndrome (NIPHS) (Service et al. 2005; Deitel 2008). Importantly McLaughlin showed that hyperinsulinaemic hypoglycaemia after RYGB is due to the accelerated mode of nutrient delivery to the lower intestine and not β-cell dysfunction, as was previously thought (McLaughlin et al. 2010). In a single patient, administering a liquid meal orally generated rapid release of GLP-1 and insulin resulting in hypoglycaemia. Administering the same meal via a gastrostomy into the bypassed gastric remnant connected to the duodenal limb did not trigger this exaggerated insulin response and hypoglycaemia A similar case series has also been recently published (Pournaras et al. 2012).

Refractory hyperinsulinaemia that persists sometimes requires pancreatic β-cell resection, a drastic solution. High insulin secretion can be treated in less severe cases with Verapamil and acarbose (Moreira et al. 2008), or diet modification (Bantle et al. 2007; Kellogg et al. 2008). Importantly hypoglycaemia in RYGB patients needs to be differentiated from other causes not related to the surgery, such as insulinoma (Zagury et al. 2004).

Eating small, dry protein-rich low-carbohydrate meals more frequently, results in

normalization of glucose levels and improved symptoms in at least half of patients with dumping symptoms (Deitel 2008). In severe cases treatment with a serotonin antagonist (cyproheptadine) or somatostatin analogue (Octreotide) can be helpful. Octreotide reduces secretion of many gut hormones including PYY and GLP-1 and reduces gastrointestinal motility. Interestingly, administration of octreotide has been shown to increase food intake in RYGB but not BAND surgery patients (le Roux et al. 2007). Octreotide in long term appears to be well tolerated in patients with dumping and continues to improve symptoms but causes weight gain (Vecht et al. 1999).

1.7 Studying food reward and hedonics using neuroimaging

1.7.1 fMRI and PET of food reward systems in normal weight subjects

$H_2{}^{15}O$ PET studies in normal weight adults, examining brain activation during food consumption, show changes in regional cerebral blood flow (rCBF) in prefrontal regions and insula cortical regions (Tataranni et al. 1999; Gautier et al. 2000; Gautier et al. 2001; Small et al. 2001; Del Parigi et al. 2002). In these studies, by manipulating nutrition state, responses to fasting and to receipt of a liquid meal (Tataranni et al. 1999; Gautier et al. 2000; Gautier et al. 2001; Del Parigi et al. 2002) or chocolate milkshake (Small et al. 2001) were measured. rCBF increased during hungry states in the hypothalamus, insula, and orbitofrontal cortex, while receipt of food increased rCBF in prefrontal regions.

Studies which make use of fMRI to examine food reward, usually use a subtraction paradigm in which food cues (mainly pictures but smells, tastes, tactile sensation, words, menus and auditory cues have all been used) are presented usually in a block design (though sometimes in an individual event-related design) alongside neutral cues such as non-food pictures, and differences in BOLD signal are measured either across the whole brain or in pre-determined

ROIs.

fMRI studies that use visual food cues when subjects are hungry elicit activation of brain food reward regions known to be involved in the expectancy, appraisal and receipt of reward, including the striatal nucleus accumbens (nucleus accumbens) and caudate nucleus (key to dopaminergic reward conditioning and learning, motivation and expectancy), amygdala (emotional responses to rewarding stimuli), anterior insula (integrating gustatory and other sensory information) and orbitofrontal cortex (OFC) (reward value appraisal, cognitive control and attention) (Carnell et al. 2012; De Silva et al. 2012; Ziauddeen et al. 2012). In normal weight subjects, a fasted and therefore hungry state elicits increased activation to food cues in these areas compared to being fed, and high calorie/palatable food pictures elicit more activation than low-calorie/bland/unappetizing food picures, with an interaction between the two conditions such that fasting biases food reward responses to high-calorie foods (LaBar et al. 2001; Killgore et al. 2003; Simmons et al. 2005; St-Onge et al. 2005; Goldstone 2006; Porubska et al. 2006; Uher et al. 2006; Cornier et al. 2009; Goldstone et al. 2009; Schur et al. 2009; Goldstone et al. 2010).

1.7.2 Individual factors influencing food reward

1.7.2.1 Dietary restraint

Dietary restraint is defined as cognitive control over eating, in an attempt to reduce and self-regulate calorie intake. Restraint can be subdivided into rigid or flexible restraint. Rigid restraint refers to dichotomous, all-or-nothing approach and a tendency to oscillate between periods of strict dieting and periods of overeating, particularly of high-calorie foods. Flexible restraint is a more graduated approach in which “fattening” foods are permitted in limited quantities rather than avoided entirely. Flexible restraint is associated

with a more consistent and sustainable dieting regime (Westenhoefer et al. 2013). Dietary restraint is usually measured via questionnaires, for example the Three Factor Eating (TFEQ) (Stunkard et al. 1985), Dutch Eating Behaviour (DEBQ) (van Strien 1986) and Eating Disorders Examination (EDE-Q) (Fairburn et al. 1994) questionnaires.

Although dietary restraint is commonly practiced by overweight and obese individuals, in an attempt to lose weight or maintain weight loss, it can be paradoxically associated with weight gain. Several studies suggest that dietary restraint predicts weight gain, binge eating and bulimia nervosa, particularly in women (Hill 2004). Particularly the all-or-nothing approach taken in restrained eaters is thought to lead to overeating of 'forbidden' foods under emotional distress, commonly termed disinhibition. Repeated disinhibition-induced overeating appears to cause a shift of the boundaries at which satiety is felt, termed the boundary model for regulation of eating (Herman et al. 1984; Polivy et al. 1999). In other words, once dietary restraint is overridden, food intake continues beyond the point at which further food intake would normally be inhibited. In these circumstances, primed by recent food intake, restrained eaters may in fact find food more rewarding and hence overeat ('counter-regulatory eating'). In support of this, dietary restraint did not predict food intake in acute feeding studies or observational studies over weeks and months, suggesting that dietary restraint is therefore not equivalent to dietary restriction (Herman et al. 1984; Stice et al. 2004; Herman and Polivy, 1984; Heatherton et al., 1992).

However caution should be exercised when interpreting the apparent relationship between dietary restraint and obesity. Dietary restraint may also simply be a consequence of obesity, in the same way that all attempts to reduce weight are more likely to be engaged in by obese people (Hill 2004).

Providing support for the boundary model of eating regulation, individuals who score higher on measures of dietary restraint have been shown to have greater neural activation to food cues in reward areas compared to unrestrained eaters, but only after eating (Coletta et al. 2009; Demos et al. 2011).

Colletta et al. (2009) compared 9 restrained normal weight eaters and 10 unrestrained eaters viewing high-calorie and low-calorie food pictures. When fasted, viewing high-calorie food pictures elicited less BOLD activation in superior temporal gyrus, parahippocampal gyrus, dorsolateral prefrontal cortex (DLPFC), lentiform nucleus (putamen), superior temporal gyrus, and parahippocampal gyrus in restrained eaters compared to unrestrained eaters, who had more activation than unrestrained eaters only in the cerebellum. By contrast when fed, high-calorie food pictures elicited more activation in the OFC, DLPFC and insula in restrained eaters compared to unrestrained eaters, whereas unrestrained eaters had more activation in areas for satiation and memory (left cingulate gyrus) (Coletta et al. 2009).

Similarly, 50 dieters (assumed to be exercising dietary restraint) showed greater nucleus accumbens BOLD activation to high-calorie food pictures after a milkshake preload than after water, compared to 50 non-dieters who had lower nucleus accumbens activation after a milkshake compared to water (Demos et al. 2011). Activation in the amygdala followed an opposite pattern however, such that activity in the amygdala was greatest for dieters who received water and non-dieters who received the milkshake.

On the other hand, in their study of 10 mono-zygotic twins discordant for restrained vs. unrestrained eating, Schur et al, found that in the fasted state, twins scoring high on dietary

restraint had greater activation in the left amygdala and the right thalamus than unrestrained co-twins to fattening foods (Schur et al. 2012). Unrestrained eating twins showed stronger responses to non-fattening food images in the medial OFC than did their restrained co-twins. After eating, restrained eaters showed greater decreases in activation to fattening foods in the left amygdala and the occipital cortex, than did their unrestrained co-twins.

Two studies have attempted to capture with neuroimaging, the neurological underpinning of the actual cognitions involved in dietary restraint. To this end Hollman et al. found that fMRI responses in normal weight (n=17) and overweight (n=3) fasting men and women to food pictures when attempting to reduce the desire for tasty food was increased in bilateral OFC, bilateral inferior frontal gyrus / anterior insula, bilateral DLPFC, pre-supplementary motor area and bilateral temporo-parietal junction compared to allowing the desire for tasty foods. On an individual level, there was a positive correlation between TFEQ restraint scores and activation in left DLPFC in cognitive control of tasty foods. The study was underpowered to detect differences between normal weight and overweight individuals (Hollmann et al. 2012). In another PET study, inhibition of the desire to eat when presented with food cues led to decreased activation in the amygdala, hippocampus, insula, orbitofrontal cortex, and striatum in fasting normal weight men, but not women (Wang et al. 2009). Therefore it appears that active dietary restraint may recruit areas of the brain involved in cognitive control such as the DLPFC and potentially reduce neural responses to food in reward areas.

1.7.2.2 Binge eating

In binge eating disorder (BED), discrete episodes of dietary disinhibition or loss of control result in the consumption of a large amount of food (typically at least ½ of the daily

recommended amount of calories), consumed in a short period of time (½ -1 hour), accompanied by a subjective sense of loss of control. In BED, as opposed to bulimia nervosa, binges are not associated with compensatory purging techniques, such as self-induced vomiting in an attempt to rid the body of unwanted calories. Binge eating disorder is associated with overweight and obesity, and occurs in approximately 25-30% of patients seeking bariatric surgery (Kalarchian et al. 2000; Niego et al. 2007; Mathes et al. 2009).

Those with pre-operative binge eating are more likely to develop or retain abnormal eating patterns after surgery, associated with poorer weight loss outcomes (Niego et al. 2007). Post-operative binge eating in BAND surgery has been associated with long term weight regain, revision surgery and poor quality of life (Scholtz et al. 2007).

Binge eating has been postulated to be more closely aligned to other addictions than the heterogeneous condition of obesity. Animal models have demonstrated the importance of μ-opioid receptor (MOR) pathways in the pathogenesis of binge eating, analogous to other addictions, such as alcohol or heroin addiction.

In animal models, a chronic deprivation model to elicit bingeing is used. Using this model, MOR antagonists suppress food bingeing in a number of studies (Mathes et al. 2009). Another model employed is that in which palatable foods only are forbidden for a period, which leads to bingeing specifically on that palatable food and hypophagia of less appealing food (Cottone et al. 2008). Using this model, a MOR antagonist decreased binge behaviour (for the preferred diet of chocolate-flavoured high sucrose), but also increased food intake of the less preferred diet (i.e. chow). Using the same model a specific MOR antagonist (GSK1521498) and naltrexone reduced the propensity to seek (both before and after food

ingestion), and binge eating of, palatable chow. However only GSK1521498 reduced the impact of high hedonic value on ingestion of chocolate, suggesting that the MOR pathway has a specific role to play in conditioned salience in binge eating (Giuliano et al. 2012).

Furthermore direct stimulation of MORs with MOR agonists such as morphine or DAMGO ([D-Ala2, N-Me-Phe4,Gly5-ol5]-enkephalin) within the nucleus accumbens of rats preferentially increases intake of energy rich foods such as fat and sucrose, as well as tasty non-caloric foods such as saccharin and salt (Will et al. 2003; Mathes et al. 2009) and increases or amplified positive affective reactions (i.e. liking reactions) to sucrose taste (Pecina et al. 2000; Pecina et al. 2005).

In human fMRI studies, both obese and lean binge eaters show increased activation in frontal pre-central area of the brain (Geliebter et al. 2006) and the OFC (Schienle et al. 2009) in response to binge food cues. Furthermore, a PET study showed that food presentation, smell and taste was associated with greater increases in dopamine in striatal areas in obese people with BED compared to those without BED, and correlated with binge eating scores (Wang et al. 2011).

A VBM study of women in their 20's found increased OFC volume in patients with BED compared to normal controls. No correction was made for BMI however, so that the higher BMI in the BED group may have been a confounder (Schafer et al. 2010).

1.7.2.3 External eating

External eating is a measure of eating in response to external cues (such as the sight of

appetizing food), as opposed to internal cues (such as hunger). This is thought to be associated with overeating and obesity, although it may be the lack of attention paid to internal cues that is more relevant than the attention paid to external cues (Herman et al. 2008). Lesion studies suggest that frontal lobe damage may increase external eating, and lead to hyperphagia and obesity (Myslobodsky 2003). In fact, relatively few studies have actually confirmed the link between external eating and obesity (Nisbett 1968; Pliner 1973; Braet et al. 1997) although it has been linked to food craving (Burton et al. 2007) and the area remains under-researched (Herman et al. 2008).

Passamonti et al. found correlations with external eating (measured with DEBQ External eating scale) and superior temporal lobe activation to food pictures in 21 normal weight male and female volunteers aged between 19 and 39 years. Increased functional connectivity between the ventral striatum and amygdala and prefrontal motor cortex, but decreased connectivity between the ventral striatum and the dorsal ACC was observed. The authors suggest that increased external eating may therefore be underpinned by specific alterations of those neural pathways involved in the processing of appetitive reward and emotional regulation (Passamonti et al. 2009).

1.7.2.4 Emotional eating

Emotional eating, or eating in response to emotional cues (such as sadness, anxiety or anger), also known as comfort eating, is associated with depression, and a need to escape negative affect. Although emotional eating can refer to eating in response to positive emotions, the most common precipitant is negative emotions, particularly in women. In most studies, emotional eating as measured by the DEBQ emotional eating scale, is positively associated with BMI (Gibson 2012). There are also cross-correlations and

interactions between dietary restraint, external eating, disinhibition and emotional eating. For example, women who scored highly on TFEQ restraint and disinhibition scores, were more likely to eat in response to negative affect, whereas women who scored highly on disinhibition but low on restraint were more likely to overeat in response to positive affect (Yeomans et al. 2009). Emotional eaters and restrained eaters are more likely to eat high calorie or sweet foods in response to stress (Heatherton et al. 1991; Oliver et al. 2000). Emotional eating may also be influenced by the type of stressor, such that ego-threat is likely to increase emotional eating whereas a cognitively demanding task does not (Wallis et al. 2004). Pre-operative emotional eating has been shown to negatively impact on weight loss following bariatric surgery and diet (Canetti et al. 2009).

Neuroimaging studies have demonstrated an interaction of emotional eating and neural activation to food. In a study of 12 normal weight individuals, negative emotional state (induced by sad music and faces) was attenuated by intragastric infusion of fatty acids, with corresponding reduction in BOLD activation in medulla/pons, midbrain, hypothalamus, thalamus, putamen, cerebellum, hippocampus and cingulated cortex, but not the insula or amygdala (Van Oudenhove et al. 2011). In another study, healthy weight women who scored in the highest quartile on the DEBQ emotional eating scale (emotional eaters) were more likely to experience negative affect compared to women who scored in the lowest quartile (non-emotional eaters), when listening to slow sad music (Bohon et al. 2009). They also had greater activation in the caudate and pallidum in response to milkshake receipt and greater activation in response to anticipation of milkshake receipt in the parahippocampal gyrus and ACC, compared to non-emotional eaters, suggesting greater food reward sensitivity.

1.7.2.5 Mood

Depression, and anxiety (and indeed many other mental disorders) are associated with increased adiposity (Hawkins et al. 2012; Lopresti et al. 2013). In depression, at least, that association appears to be bi-directional (Luppino et al. 2010). Negative affect (as measured by the positive and negative affect scale (PANAS)) has also been associated with increasing BMI (Pasco et al. 2013).

Indeed common to both obesity and depression are disruptions in immuno-inflammatory processes (e.g. the kynurenine/tryptophan/serotonin pathway (Breum et al. 2003; Myint 2012), increased oxidative stress, mitochondrial disturbances, hypothalamic-pituitary axis imbalances and neurotransmitter imbalances, all of which offer intriguing possibilities for the investigation of common mediating pathways (Lopresti et al. 2013).

In keeping with Section 1.7.2.4 depression, premenstrual low mood and neuroticism have also been linked to overeating high calorie foods when stressed (Gibson 2006).

Although there have been no studies which specifically examine the effect of clinical depression on food reward processing in the brain, using neuroimaging, fMRI and PET studies have demonstrated altered neural activity in depressed subjects compared to controls both at rest and in task-related activity in various areas including frontal gyri, the DLPFC, cingulate cortex and amygdala (Mitterschiffthaler et al. 2006; Fitzgerald et al. 2006). Neuroimaging studies also found reduced brain volume in depression (Arnone et al. 2012). Negative affect (measured by PANAS) has been associated with greater BOLD activation in the OFC, ACC and insula on viewing high-calorie food pictures (Killgore et al. 2006).

1.7.2.6 Impulsivity and inhibitory control

The issue of overlap between the reward system and inhibitory control system in food intake can lead to some confusion in interpreting study results. It is likely that these systems function in synergy to co-ordinate behavioural approach or inhibition toward food, and may be activated in both. For instance, frontal lobe regions, including superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, medial PFC, DLPFC, VLPFC and OFC all consistently implicated in response inhibition (Liddle et al. 2001; Mostofsky et al. 2003; Aron et al. 2005; Buchsbaum et al. 2005; Simmonds et al. 2008), whereas the OFC has also been implicated in evaluation of food reward (Small et al. 2007). Poor response inhibition (impulsivity) has been implicated in the development of obesity (Ryden et al. 2003; Braet et al. 2007), and poor weight loss during dieting (Jonsson et al. 1986; Nederkoorn et al. 2006) (Weygandt et al. 2013), whereas engagement of areas of inhibitory control in response to food cues may ensure successful weight maintenance (Hare et al. 2009). Better impulse control has been associated with stronger functional connectivity between VMPFC and DLPFC at rest, which predicted greater weight loss during dieting in obese subjects (Weygandt et al. 2013).

Obese people show different activation compared to lean people during tasks designed to elicit self-control or response inhibition. In a task requiring subjects to reduce their craving whilst viewing food pictures, obese women showed more activation of the DLPFC compared to lean women when attempting to reduce compared to increase their craving (Scharmuller et al. 2012), suggesting increased recruitment of this area was required to achieve the same reduction in appetite. On the other hand when asked to increase their craving (compared to passively viewing or decreasing craving), obese women showed less activation in the insula and dorsal striatum compared to lean (Scharmuller et al. 2012). In another study of normal

weight and overweight young women, using a food-specific response inhibition task, those with a higher BMI responded more quickly but less accurately particularly to high-calorie food cues (Batterink et al. 2010). They also had less less activation during response inhibition in the frontal lobes, including superior frontal gyrus, middle frontal gyrus, VLPFC, medial PFC, and OFC and increased activation in right temporal operculum extending to frontal operculum and insula.

1.7.2.7 Gender

Sex based differences in regulation of body weight and eating control have been observed (Rolls et al. 1991; Bates et al. 1999; Beer-Borst et al. 2000; Provencher et al. 2003; Woods et al. 2003; Clegg et al. 2006; Shi et al. 2009).

Cornier et al. found differences in response to food cues after overeating between women and men: women had significantly greater changes in hunger and satiety ratings as well as reduced subsequent energy intake as compared to men (Cornier et al. 2007). In a subsequent study they also found significantly greater activation of DLPFC and parietal cortex in women as compared to men after fasting (Cornier et al. 2010).

Smeets et al. found increased activation in the ventral and dorsal striatum, and parts of the OFC in men in response to chocolate satiation, whereas in women effects were only seen in the ventral striatum. Amygdala activation in women but not men decreased after chocolate satiation and taste activation in the anterior insula increased after satiation in men (Smeets et al. 2006).

Uher et al. found more activation to food picures in women in fusiform gyrus, and more in response to taste in insula and prefrontal cortex compared to men irrespective of whether fasted or fed. (Uher et al. 2006)

PET studies have also shown gender differences in response to hunger (Del Parigi et al. 2002; Wang et al. 2009). Men but not women showed reduction in activation in OFC, amygdala, hippocampus, insula and striatum when stimulated with food cues and asked to inhibit their hunger (Wang et al. 2009). On the other hand a different study found that men tended to have greater activation in DLPFC middle temporal gyrus, and paralimbic areas, including the posterior cingulate and parahippocampal gyrus, compared to women when hungry and greater increases in rCBF in the vicinity of the ventromedial prefrontal cortex to satiation. Women had increased rCBF in DLPFC, precuneus, angular gyrus, and a region including the occipital cortex and some aspects of the posterior temporal lobe than men in satiation (Del Parigi et al. 2002).

1.7.3 Neuroimaging in obesity

1.7.3.1 Genetic obesity

Studies of patients with rare genetic obesity syndromes have revealed both structural and functional brain characteristics in neuroimaging studies which may contribute to abnormal eating behaviour. Prader-willi syndrome (PWS) is a genetic obesity syndrome, associated with neuroendocrine abnormalities, learning disability and behavioural problems with marked hyperphagia developing in childhood, due to loss of expression of paternally expressed imprinted genes on chromosome 15 (15q11–13), leading to, early onset morbid obesity (Goldstone 2006). Patients with PWS have increased activation to food pictures compared to healthy controls following a preload, in the prefrontal region (Miller et al.

2007), OFC, amygdala, insula, hippocampus and parahippocampal areas (Holsen et al. 2006; Holsen et al. 2009). Delay in response to glucose ingestion in reward areas of the brain (prefrontal cortex and insula) (Shapira et al. 2005), cerebellar hypoplasia (Titomanlio et al. 2006), abnormal cortical structure (Miller et al. 2007), and pituitary abnormalities (Miller et al. 2008) have also been demonstrated.

Genetically leptin-deficient individuals also develop early onset obesity. They have increased neural reactivity to food in the nucleus accumbens, caudate, putamen and globus pallidus (Farooqi et al. 2007) with less suppression in these areas after eating than controls (Aotani et al. 2012), which was reversed by leptin administration (Aotani et al. 2012). Leptin administration also reduces BOLD activation to food pictures the insula, parietal and temporal cortex and increases activation in prefrontal cortex (Baicy et al. 2007; Farooqi et al. 2007). However, monogenic disorders leading to obesity such as leptin-deficiency, (as well as melanocortin 4 receptor (*MC4R*), pro-opiomelanocortin (*POMC*) and prohormone convertase 1 (*PCSK1*)) are rare and probably account for less than 5% of obesity.

The genetic influence on obesity is therefore mostly polygenic, and although this may contribute between 45% and 85% of the heritability in BMI, the effect size is likely to be small. For example, the FTO (fat mass and obesity associated gene) allele, one of the most established common gene variants, results only in approximately 0.4 kg/m^2 increase in BMI. The mechanism for the effect of individual genetics on weight is thought to be mostly through increased appetite and eating behaviour (Cecil et al. 2012). Various studies have therefore attempted to link emerging evidence of obesity-associated gene variants, particularly those involved in dopamine pathways, with alterations in the neural circuitry underlying the response to food from an emotional or reward perspective.

For instance, the Taq1A A1 allele of the DRD2 gene has been associated not only with alcoholism, drug abuse, smoking and compulsive gambling, but also with obesity (Comings et al. 2000). Carriers of Taq1A A1 allele have increased impulsivity (White et al. 2008) and increased body weight (Noble 2000). Behavioural studies have shown that especially obese individuals with the allele will work harder in a food reward task for food than those without the allele (Epstein et al. 2007). In addition, healthy weight individuals with the Taq1A A1 allele have reduced dopamine D2 receptors (Jonsson et al. 1999) and lower glucose uptake on FDG PET in prefrontal and striatal (caudate, putamen and nucleus accumbens) areas (Noble et al. 1997), in keeping with the reward deficiency theory of obesity (see Section 1.7.5). Furthermore, 2 fMRI studies of healthy weight participants has shown that those with the Taq1A A1 allele have attenuated activation of the reward circuitry (OFC and prefrontal areas/ thalamus, midbrain) in response to receipt of appetizing food (Felsted et al. 2010; Stice et al. 2010; Stice et al. 2012) and that attenuation of BOLD activation in the putamen and OFC by the Taq1A A1 predicted the risk of future weight gain (Stice et al. 2010). The same genotype appears to moderate the relationship between parental control and emotional eating, so that possession of the allele increased emotional eating in relation to high parental psychological control (van Strien et al. 2010).

Individuals positive for the obesity risk FTO (fat mass and obesity associated gene) allele have reduced brain volume (Ho et al. 2010; Melka et al. 2013) and in one recent study, reduced BOLD in response to food-related images within the hypothalamus, left ventral tegmental area/substantia nigra (VTA/SN), left posterior insula, left globus pallidus, left thalamus, and left hippocampus (Karra et al. 2013).

Table 1.1 fMRI of obesity

Author Year	Design	Subjects	Imaging	Cues	Non-imaging tools	Stats	Brain activation						Comment
	CS/PR WB/ROI	n Gender (M:F) Age (mean) BMI (means) Ethnicity	T Block/ Event Software	Stimulus Fasted (h) Fed (kCal)		Threshold Corrected for MC Corr	Frontal	Striatum	Hipp	Amyg	Insula/ gustatory cortex	Other	
FOOD PICTURES: Children / Adolescents OB vs. NW													
Bruce 2010	CS ROI	n 10 OB / 10 NW M10 : F10 Age 10-17 y BMI N/K Ethnicity N/K	3T Block BV	Food pictures (Food vs. Animals) Fasted 4h Fed 500kCal <10min	Picture recall	<0.0001 or < 0.001 uncorrected	OFC ↑ SFG ↑ MFG ↑ IFG ↑	→	→	→	→		OB had lower reduction in activation after the meal
Davids 2010	CS WB/ROI	n OB-OW /22 NW M17 : F27 Age 9-18 y BMI 16-32 kg/m^2 Ethnicity N/K	1.5 Block SPM-5	Food pictures (Food vs. Pleasant vs. Neutral) Fasted ≥2h	Picture appeal Esteem QN HR monitor	<0.05 Yes	DLPFC ↑	Caudate ↓	↓	→	→	Thalamus↓ Occipital ↓ ACC↓	HR↑ in OB when showed food pictures; no differences between groups in appeal ratings
FOOD PICTURES: Adults OB vs. NW													
Rothemund 2007	CS WB	n 13NW/13OB M0:F26 Age NW25y; OB31y NW21 kg/m^2;OB 36 kg/m^2 Ethnicity: N/K	1.5 Block SPM2	Food pictures (High-calorie vs. Low-calorie vs. Objects) Fasted 1.5h, hungry excluded		<0.001, uncorr, cluster>5 voxels	↑ LvO inf, mid, sup FG	HvO ↑Putamen, Caud HvL ↑Putamen	HvO ↑		HvO ↑	↑ LvO occipital, sup temporal	No correction for menstrual cycle stage

Stoeckel 2008	CS WB/ROI	n 12OB /12NW M0:F24 Age 28y BMI OB 31–41 kg/m^2; NW 20–25 kg/m^2 Ethnicity 50% white	3 Block SPM2	Food pictures (High-calorie vs. Cars) Fasted 8-9hr	VAS hunger PANAS	<0.01 SVC voxels>7 BMI	↑ OFC	↑ NAcc, caudate, putamen	↑	↑	↑	↑ ventral pallidum, ACC	No difference in hunger or appeal rating
Martin 2010	CS WB/ ROI	n 20 M10: F10 Age OB 34y; NW 22y	3 Block BV	Food pictures (Food vs. animals, Food vs. Blurred) Fast 6 h Fed <10min	EI	<0.0001 uncorrected	Fasted FvO ↑ MPFC, mid FC, inf gyrus, cing		Fasted FvO →	Fasted FvO →	Fasted FvO →	Fasted FvO ↑ ACC, Cun, temporal, occipital, cerebellum, brainstem ↓sup temp, planum temporale	Order of scans counterbalanced to correct for order effect. Fasted memory of food pictures better.
							Fasted FvB ↑ mid FC ↓ MPFC, mid/inf gyrus		Fasted FvB ↑	Fasted FvB ↑	Fasted FvB ↓	Fasted FvB ↓ thalamus	
							Fed FvO ↑MPFC, sup gyrus	Fed FvO ↑ caud	Fed FvO ↑				
									Fed FvB ↓ PHG		Fed FvB ↓		
Dimitropoulos 2012	CS WB/ROI	n 22 OB-OW/16 NW M17 : F21 Age 24y BMI OB-OW 32 kg/m^2 ;NW 23 kg/m^2 Ethnicity N/K	4.0 Block BV	Food pictures (High calorie vs. Low calorie) Fast 4h Fed 30min	Picture appeal	<0.05 Yes	Fasted PFC ↑ dlPFC ↓	Fasted →	Fasted ↓	Fasted →	Fasted ↓	Fasted Cingulate↓	ROI statistics invalid, no differences in appeal ratings, many patients did not have breakfast
							Fed OFC/PFC ↑	Fed Caudate ↑	Fed ↑	Fed →	Fed →	Fed ACC/PCC↑	

Frankort 2012	CS WB/ROI	n 29 0M:29F Age OW 24y; NW 23y BMI OW29 kg/m^2 ; NW 21 kg/m^2 Ethnicity100 % white	3 Event BV	Food pictures (Palatable vs. Unpalatable) Fed 60-90min	VAS Restraint scale PANAS	<0.01 (uncorr) Monte Carlo cluster level(>64 mm^3)	↓dmPFC ↓OFC	↓ caudate	↓PHG	↓		↓ ACC ↓ VTA/SN	
Grosshans 2012	CS WB/ROI	n 44 14M:30F Age OB 38y;NW 44y BMI OB 37 kg/m^2; NW22 kg/m^2 Ethnicity N/K	3 Block SPM5	Food pictures (High-calorie vs. Low calorie) Fasted 6h	BDI FTND TFEQ Leptin Food craving	<0.05 FWE	→	→	→	→	→	→	No difference OB vs.NW in BOLD, food craving
Ho 2012	CS WB/ROI	n 21 OB-OW/14 NW M16 : F19 Age OB 24y; NW 25y BMI OB 31 kg/m^2; NW 22 kg/m^2 Ethnicity: 60% white	4.0 Block BV	Food pictures (High-calorie vs. Low calorie vs. Furniture) Fast 4h Fed 30min	Food related problem Q and picture appeal scores	<0.005 or <0.001 Yes Q results	NW → OB dlPFC ↓ impaired satiety when fasted	NW → OB putamen↑ impaired satiety when fed	NW → OB →	NW ↑ impaired satiety when fasted OB ↑ impaired satiety when fed	NW → OB →	NW → OB →	No control for order effects; no direct comparison between groups
Nummenmaa 2012	CS WB/ROI	N OB 14/NW 15 M:F – N/K Age OB 46y; NW 48y BMI OB:44 kg/m^2;NW 24 kg/m^2 Ethnicity: N/K	1.5 Block SPM5	Food pictures (Appetizing vs. Bland) Fasted 12h	Valence ratings	<0.005 No	OFC↓ L SFG↓	caudate ↑	↑	L ↑	L ↓	PCC↑ SSA ↑	Uncorrected statistics, little behavioural data. PET study also performed and showed ↑ FDG uptake in caudate in OB

Sharmuller 2012	CS WB	n 26 M0:F26 Age OB 27y; NW 26y BMI OB 32 kg/m^2; NW 21 kg/m^2 Ethnicity N/K	3 Event SPM8	Food pictures (Food vs. Non-food) Fasted o/n	Appetite ratings	<0.05 Not for all comparisons	dlPFC ↑				insula ↑		Insula higher with passive viewing, dlPFC higher with "cognitive control" in the OB vs. NW, no calorie subgroups examined, no correction for menstrual stage
Jastreboff 2013	CS WB/ROI	n OB 25/NW 50 M31:F19 Age 26y BMI OB 33 kg/m^2; NW 23 kg/m^2 Ethnicity 68% white	3 Block SPM5	Guided imagery (Favourite food vs. Neutral state) Fasted for 2h	Glucose, insulin, HOMA-IR	<0.01 FWE Metabolic markers	Frontal cortex ↑	Putamen ↑	→	↑	↑	→	Participants chose their preferred cues, blood tests done 7 days before imaging, see below for correlations
TASTE ANTICIPATION Adolescents/Adults OB vs. NW													
Stice 2008	CS ROI	n 11NW /15OW /7OB M0:F33 Age 15y BMI 17–39 kg/m^2 Ethnicity 86% white	3 Event SPM5	Taste (Milkshake vs. Water) Fasted 4-6hr		<0.05, corrected, cluster >3voxels BMI	→	→	→	→	↑ROp, FO, insula	↑ Temporal, parietal, ACC	BMI ↑ VLPFC, DLPFC, temporal in food anticipation,↑ insula and FO and ↓ caudate and parietal to food receipt but did not reach statistical significance when corrected for MC
Ng 2011	CS ROI	n 17OB /17NW 0M:38F Age 20y BMI NW 22 kg/m^2; OB 36 kg/m^2 Ethnicity: 77% white	3 Event SPM5	Taste (High-fat milkshake (HF) vs. Low fat milkshake (LF) vs. Water (NT)) Fasted 4-6hr	Restraint Hunger	<0.005, SVC cluster>3voxels FDR in ROI	HFvNT ↑ posting LFvNT ↑ vMPFC HFvLF ↑ inf FG	HFvNT ↑ Caud	LF vNT ↑ HIpp, PHG		LFvNT, HFvNT ↑ ROp HFvLF ↑FO		No difference in restraint or hunger between groups

Frankort 2012	CS WB/ROI	n 29 0M:29F Age OW 24y; NW 23y BMI OW29 kg/m^2 ; NW 21 kg/m^2 Ethnicity100 % white	3 Event BV	Imagined taste (Palatable vs. Unpalatable) Fed 60-90min	VAS Restraint scale PANAS	<0.01 uncorrected Monte Carlo cluster level correction >64mm^3	→	→	→	→	→	↑ ACC	
TASTE RECEIPT: Adults OB vs. NW													
Stice 2008	CS ROI	n 11NW /15OW /7OB M0:F33 Age 15y BMI 17–39 kg/m^2 Ethnicity 86% white	3 Event SPM5	Taste (Milkshake vs. Water) Fasted 4-6hr		<0.05, corrected, cluster >3voxels BMI	→	→	→	→	↑ROp, FO		BMI ↑ VLPFC, DLPFC, temporal in food anticipation, ↑ insula and FO and ↓ caudate and parietal to food receipt but did not reach statistical significance when corrected for MC
Stice 2008	CS/PR ROI	Study 1: n 43 0M:43F Age 20y BMI 29 kg/m^2 NK Study 2: n 33 0M:33F Age 16 BMI 24 kg/m^2	3 Event SPM5	Taste (Milkshake vs.Water)		<0.005, cluster>3 voxels <0.05, FDR in ROI BMI		↓Caud, Putamen					TaqA1 allele amplified the negative BMI correlation. Activation in those without allele predicted future Taq1 weight gain at 1 yr, and negative correlation in those with the allele
Stice 2010	PR ROI/WB	n 26 M0:F26 Age 21y BMI 28 kg/m^2 Ethnicity 77% white	3 Event SPM5	Taste (Milkshake vs. Water) Fasted 4-6hr Weight gain > 2.5% vs. Weight stable	Nil	<0.05 Yes		R caudate ↓					Decreased caudate activation in women who gained weight not seen in weight stable women

Ng 2011	CS ROI	n 17OB /17NW 0M:38F Age 20y BMI NW 22 kg/m^2; OB 36 kg/m^2 Ethnicity: 77% white	3 Event SPM5	Taste (High-fat milkshake (HF) vs. Low fat milkshake (LF) vs. Water (NT)) Fasted 4-6hr	Restraint Hunger	<0.005, SVC cluster>3voxels FDR in ROI	LF>NT ↑ inf FG HF>LF ↑ vMPFC			HF>NT ↑	HF>NT ↑ROp LF>NT ↑ ROp, FO HF>LF ↑ ROp		No difference in restraint or hunger between groups
Stice 2011	CS WB/ROI	n 35HR/25LR M30:F30 Age 15y BMI HR 21 kg/m^2; LR 20 kg/m^2 Ethnicity 85% white	3 Event SPM5	Taste (Milkshake vs. Saliva) Monetary reward task Fasted 4-6hr	Nil	<0.05 FDR, SVC for clusters		HR-OB >LR-OB ↑ Caudate			HR-OB >LR-OB ↑ FO	HR-OB >LR-OB ↑ Parietal	Monetary reward task: HR>LR: Win vs. No-win ↑ Caudate, putamen, insula, thalamus, visual cortex.
Frank 2012	CS WB/ROI	n AN:21/OB:19 / NW:23 Age AN:23y; OB:27y; NW 25y BMI AN:16 kg/m^2; OB:35 kg/m^2; NW 21 kg/m^2 Ethnicity N/K	3.0 Block SPM5	Taste (Sucrose vs. Saliva) Fed 1-2h	Taste ratings Psychological testing	<0.05 or <0.001 Not always	OFC↓ dlPFC ↓	putamen↓	→	→	↓		AN had more activation than NW and OB. No difference in taste ratings. Numerous confounders.
FOOD PICTURES Correlation with adiposity measure													
Killgore 2005	CS ROI	n 13 M0:F13 Age 24y BMI 22 kg/m^2 Ethnicity N/K	1.5 Block SPM99	Food pictures (High-calorie vs. Low-calorie) Fast >90min	Nil	<0.005 SVC BMI	↓ IFG, ↑ MFG	N/A	N/A	N/A	N/A	↓ ACC	Results are correlations with BMI; no control for menstrual periods; no behavioural data; ROI very big

Rothemund 2007	CS WB	n 13NW/13OB M0:F26 Age NW25y; OB31y NW21 kg/m^2;OB36 kg/m^2 Ethnicity: N/K	1.5 Block SPM2	Food pictures (High-calorie vs. Low-calorie vs. Objects) Fasted 1.5h, hungry excluded		<0.001, uncorr, cluster>5 voxels BMI	HvO ↑pos cingulate, OFC	HvO ↑			HvO ↑	HvO ↑ claustrum, globus pallidus	Results are correlations with BMI No correction for menstrual cycle stage
Batterink 2010	PR ROI	n 39 M0:F39 Age 16 y BMI 17–39 kg/m^2 86% white	3T Event SPM-5	Food pictures GNG task (Appetizing vs. Vegetable) Fasted 4-6h	RT during GNG task	<0.001, cluster size 3 voxels FDR BMI	Mid ↓ SFC ↓ VLPFC ↓ OFC ↓	→	n/a	n/a	↑ insula/ FO	Temporal ↑	RT in food task lower and error rate higher with higher BMI
Wallner-Liebmannn 2010	CS WB/ROI	n OB12 /NW12 M12:F12 Age OB 18y; NW 18y BMI OB 34 kg/m^2; NW 21kg/m^2 Ethnicity N/K	3 ? SPM5	Food picture (High-calorie vs. Fixation cross) Fed 1-3h	Behavioural questionnaires	WC			↑				Results are correlations with WC, WC but not BMI correlated with BOLD
Yokum 2011	PR	n 34 M0:F34 Age 16y BMI 17-39 84% European American	3 Event SPM5	Food pictures (Appetising vs. Unappetising food) Fasted 4-6hr	RT to pictures	<0.05 FDR corr BMI	Appetising: OFC ↓ Unappetising: vlPFC ↑				Appetising: ↑ insula/ FO	Appetising: ↓ Pallidum	Results are correlations with BMI RT faster in the OB, weight gain predicted by OFC activation at baseline
FOOD PICTURES Correlation weight gain													
Batterink 2010	PR ROI	n 39 M0:F39 Age 16 y BMI 17–39 kg/m^2 86% white	3T Event SPM-5	Food pictures GNG task (Appetizing vs. Vegetable) Fasted 4-6h	RT during GNG task	1y Δ BMI						Temporal ↓	

Yokum 2011	PR	n 34 M0:F34 Age 16y BMI 17-39 84% white	3 Event SPM5	Food pictures (Appetising vs. Unappetising food) Fasted 4-6hr	RT to pictures	<0.05 FDR corr 1y Δ BMI	Appetising: OFC ↑						RT faster in the OB
TASTE ANTICIPATION Adolescents Correlation weight gain, Taq1A													
Stice 2010	PR ROI	n 39 M0:F29 Age 16y BMI 25 kg/m^2 Ethnicity 84% white	3 ?Block SPM5	Imagined consumption of Palatable vs. Unpalatable foods Fasted 4-6hr	Nil	<0.05 Yes ΔBMI and Taq A1 allele	↓ OFC	putamen↓					Results show hypoactivation in these areas predicting weight gain in the presence of the A1 allele
Stice 2013	CS WB/ROI	n 162 80M: 82F Age 15y BMI 21 kg/m^2 Ethnicity 76% white	3 Event SPM5	Taste (Milkshake vs. Water) Monetary reward Fasted 5hr	Body fat Substance use	<0.001 Yes ↑ OW at 1y						↑ OW at 1yr ↓Precuneus	Taq1 allele had no moderating effect on results. Monetary task Win vs. No-win activation ↓Caudate ↓Putamen predicted ↑ substance misuse at 1yr Monetary reward did not predict ↑ OW at 1yr
TASTE RECEIPT Adolescents Correlation weight gain, Taq1A													
Stice 2013	CS WB/ROI	n 162 80M: 82F Age 15y BMI 21 kg/m^2 Ethnicity 76% white	3 Event SPM5	Taste (Milkshake vs. Water) Monetary reward Fasted 5hr	Body fat Substance use	<0.001 Yes ↑ OW 1y			OW>NW ↑R			OW>NW ↑mid cingulate ↑ OW 1y ↓Mid Temp gyrus	Taq1 allele had no moderating effect on results. Monetary task Win vs. No-win activation ↓Caudate ↓Putamen predicted ↑ substance misuse at 1yr Monetary reward did not predict ↑ OW at 1yr

FOOD PICTURES Adults Weight loss													
Rosenbaum 2008	PR WB	n 6 M2:F4 Age 33-44y BMI 30-60 kg/m^2 Ethnicity N/K	1.5 Block SPM2	Food pictures (Food vs. Non-food) RO: 10% wt loss Fasted N/K	Nil	<0.05 uncorrected	RO: IFG↑/↓ MFG↑/↓	→	RO PHG↑/↓	RO ↓	RO →	OB vs. RO Cingulate gyrus↑/↓ precuneus↑,↓ brainstem↑, hypothalamus↓	RO placebo vs leptin comparison. Small n number, too may pairwise comparisons leading to contradictions, no behavioural data
Cornier 2009	CS WB/ROI	n 19 OB-OW /22NW M21:F20 Age 34 y (25-45) BMI OB-OW 27.4 kg/m^2; NW 21.6 kg/m^2 OB-OW scanned as RO (-8% BW) Ethnicity N/K	3T Block SPM5	Food pictures (High hedonic vs. Neutral hedonic vs. Object) RO: 8% wt loss Fasted-o/night Scanned after 3 days Eucaloric diet (EU)/ 3 days overfeeding (OF)	TFEQ VAS	<0.05 FDR GLM model	→	→	→	→	NW vs. RO EU:↑ NW vs. RO OF:↓	NW vs. RO EU:↑ inf visual cortex NW vs. RO OF:↓ visual cortex and hypothalamus	RO>NW: restraint and disinhibition scores VAS premeal hunger and food intake corr changes Insula activation in OF
FOOD PICTURES Successful dieters													
McCafferey 2009	CS WB/ROI	n 18NW/ 16OB/ 17SWL 6M:45F Age OB 49; SWL 49; NW 44y; BMI NW 22 kg/m^2 ; OB 35 kg/m^2 ; SWL 24 kg/m^2 Ethnicity: N/K	3 Block AFNS	Food pictures (Food vs. Non-food) Fasted 4h SWL: >13.6kg wt loss for 3y		ROI <0.001 Cluster>300mm^3	SWL vs. OB /NW ↑	→	→	→	→	SWL vs. NW temporal ↑ OB vs. SWL precentral ↑	WB exploratory only <0.01 uncorrected, so not included

Murdaugh 2012	PR WB/ROI	n 29 OB-OW/ 13 NW 11M:27F Age OB-OW 48y; NW 45y BMI OB-OW 33 kg/m^2; NW 23 kg/m^2 Ethnicity N/K	3 Block SPM8	Food pictures (High-calorie vs. Low-calorie) Fasted 8h 12wk wt loss programme	EDDS	<0.05 FDR and FWE % wt loss at 12 wks	↑MFG	↑NAcc			↑insula, FO	↑ACC, mid cingulate, sup parietal, cerebellum, temporal	
						% wt change 9month	↑IFG	↑putamen, VTA	↑		↑	↑temporal, fusiform gyrus, inf parietal, cerebellum	
BED/FOOD ADDICTION													
Gearhardt 2011	CS ROI	n 48 0M:48F Age 21y BMI 28 kg/m^2 Ethnicity N/K	3 Event SPM5	Taste (milkshake): anticipation & receipt Fasted 4-6h	YFAS DEBQ	<0.001 FDR & Bonferroni YFAS	↑OFC	↑ACC		↑	→		
						<0.001 FDR & Bonferroni High v Low FA: anticipation	↑	↑ Caud					
						<0.001 FDR & Bonferroni High v Low FA: receipt	↓OFC	→		→	→		
Martin 2010	CS WB/ ROI	n 20 M10: F10 Age OB:34y; NW: 22y	3 Block BV	Food pictures (Food vs. animals, Food vs. Blurred) Fast 6 h	EI	<0.0001 uncorrected	Fasted EI ↑MPFC	→	→	→	→	Fasted EI ↓ ACC	
EFFECT LEPTIN													

Grosshans 2012	CS WB/ROI	n 44 14M:30F Age OB 38y;NW 44y BMI OB 37 kg/m^2; NW22 kg/m^2 Ethnicity N/K	3 Block SPM5	Food pictures (High-calorie vs. Low calorie) Fasted 6h	BDI FTND TFEQ Leptin Food craving	<0.05 FWE Leptin		↑					
Rosenbaum 2008	PR WB	n 6 M2:F4 Age 33-44y BMI 30-60 kg/m^2 Ethnicity N/K	1.5 Block SPM2	Food pictures (Food vs. Non-food) Leptin vs. Placebo after RO: 10% wt loss Fasted N/K	Nil	<0.05 uncorrected	RO Lpt vs. Pl IFG↑/↓, MFG↑/↓ SFG ↓	RO Lpt vs. Pl Putamen ↑	RO Lpt vs. Pl ↓PHG	RO Lpt vs. Pl →	RO Lpt vs. Pl ↓	RO Lpt vs. Pl Brainstem ↓ Cingulate gyrus ↑/↓ Hypothalamus ↑ Lingual gyrus ↑/↓ Mid occipital ↓ Mid temp ↑/↓ Sup temp ↓ Postcentral↑	Small n number, too many pairwise comparisons leading to contradictions, no behavioural data
CORRELATION GLUCOSE/INSULIN													
Matsuda 1999	CS WB/ROI	n OB:10/NW:10 M10: F:10 Age OB:34y; NW:32y BMI OB:34 kg/m^2 ; NW:22 kg/m^2 Ethnicity: N/K	1.9 Resting state MEDX	Cue was an OGTT Fasted 12h	Glucose, insulin	<0.05 No Metabolic markers	N/A	N/A	N/A	N/A	N/A	Slower and ↓ inhibitory response in the VMH and PVN	No correction for multiple comparisons, hypothalamus too small and close to arteries/sinuses making interpretation of the results problematic, no behavioural testing
Jastreboff 2013	CS WB/ROI	n OB:25/NW:50 M31:F19 Age 26y BMI OB:33 kg/m^2; NW:23 kg/m^2 Ethnicity 68% white	3 Block SPM5	Guided imagery Favourite food Fasted 2h	HOMA-IR, Insulin, Glucose	HOMA-IR Insulin	OB (food) →	OB (food) Putamen ↑	OB (food) ↑	OB (food) →	OB (food) ↑	OB (food) thalamus ↑	Correlations in OB but not NW. Similar regions for stress and relaxed imagery. Participants chose their preferred cues, blood tests done 7 days before imaging
						Fasting glucose	OB (food) →	OB (food) Putamen ↑	OB (food) →	OB (food) →	OB (food) ↑		

Wallner-Liebmannn 2010	CS WB/ROI	n OB12 /NW12 M12:F12 Age OB 18y; NW 18y BMI OB 34 kg/m^2; NW 21kg/m^2 Ethnicity N/K	3 ? SPM5	Food picture (High-calorie vs. Fixation cross) Fed 1-3h	Behavioural questionnaires	<0.001 Unclear Fasting insulin	↓		↑		↑	↓thalamus	WC but not BMI correlated with activation

Abbreviations:

AFNS: Analysis of Functional Neuroimage Software, **ACC:** Anterior cingulated cortex, **Amyg:** Amygdala, **AN**: anorexia nervosa, **BDI II**: Beck's depression inventory Version 2, **BED:** Binge Eating Disorder, **BMI:** Body mass index, **BPND**: Binding potential, **BW:** body weight, **BV:** BrainVoyager QX, **CES-D:** Center for Epidemiological Studies Depression Scale, **corr:** correlation with, **CS:** Cross sectional, **D2**: Dopamine type 2 receptor, **D3**: Dopamine type 3 receptor, **DEBQ:** Dutch Eating Behaviour Questionnaire, **DLPFC:** dorsolateral prefrontal cortex, **EFS:** external food sensitivity (DEBQ external), **EI**: Stunkard's Eating Inventory, **F:** female, **^{18}F:** radioligand Fallypride, **FA**: Food addiction, **FDR**: False discovery rate, **FFA**: free fatty acids, **FG**: frontal gyrus, **FO:** Frontal operculum, **FTND**: Fagerstrom Test for Nicotine Dependence, **FvB**: Food vs. Blurred, **FvO**: Food vs. Non-food, **FWE:** Family wise error, **gluc**: glucose, **HF**: High fat, **Hipp:** Hippocampus/perihippocampus, **HR:** heart rate, **HR-OB:** High-risk for obesity, **HvL**: High-calorie vs. Low-calorie, **IFG:** inferior frontal gyrus; **LF**: Low fat, **Lpt**: Leptin, **LR-OB:** Low-risk for obesity, **M:** male, **MFG:** middle frontal gyrus, **n/a**: not applicable, **N/K:** not known, **NT**: No taste, **NW:** normal weight, **OB:** obese, **OFC:** orbitofrontal cortex, **o/n**: overnight, **OW:** overweight, **PANAS**: Positive and Negative Affect Scale, **PCC**: posterior cingulate cortex, **PET**: positron emission tomography, **Pl**: Placebo, **PR:** prospective, **QN:** questionnaire, **rCBF**: regional cerebral blood flow, **RO:** obese reduced weight, **ROI:** Region of interest study, **ROp**: Rolandic operculum, **RT:** reaction time, **RYGB**: Roux-en-Y gastric bypass, **SFG:** superior frontal gyrus, **SPECT**: Single photon emission computer tomography, **SPM:** Statistical Parametric Mapping, **SSA:** somatosensory area, **SSTAI:** Spielberger State and Trait Anxiety Inventory, **T:** Tesla, **TFEQ**: Three factor Eating Questionnaire, **VAS**: visual analogue scale, **VMPFC**: Ventromedial prefrontal cortex, **VSG**: vertical sleeve gastrectomy, **WB:** whole brain, **wks:** weeks, **wt:** weight, **y**: years, **YFAS**: Yale Food Addiction Scale

ARROWS represent comparison of OB vs NW, or positive, negative or no correlation with BMI

Table 1.2 PET studies of obesity

Author Year	Design	Subjects	Imaging	Cues	Non-imaging tools	Stats	Binding						Comment
	CS/PR WB/ROI	N Groups Gender (M:F) Age (mean) BMI (mean) Ethnicity	Method Tracer	Fasted-h Fed-kCal		Threshold Corrected for MC Corr	Frontal	Striatum	Hipp	Amyg	Insula/ Gustatory cortex	Other	
PET rCBF OB vs. NW													
Gautier 2000	CS WB	n 11OB/11NW M22:F0 Age OB 27y; NW 35y BMI N/K Ethnicity N/K	PET rCBF/^{15}O-water	Fed liquid meal after 36h fast	Insulin Gut hormones FFA	<0.0005 uncorrected Metabolites	↑DLPFC↑VLPFC ↓OFC		↓ hipp, PHG		↓	↓Cerebellum ↓Temporal pole	Results are changes in activation upon satiation; correlated precuneus rCBF ↓ with changes in insulin
Gautier 2001	CS WB	n 12OB/10NW M0:F22 Age OB 30y; NW 32y BMI OB 41 kg/m^2 ; NW 23 kg/m^2 Ethnicity N/K	PET rCBF/^{15}O-water	Fed liquid meal after 36h fast	Insulin Gut hormones FFA	<0.0005 uncorrected Metabolites	↑VMPFC ↓IFG	↓caudate	↓PHG		↑FO ↓insula	↓Temporal	
Del Parigi 2004	CS ROI	n 21OB/21NW M20:F21 Age OB 28.3y; NW 33y BMI N/K Ethnicity N/K	PET rCBF/^{15}O-water SPM99	Taste liquid meal Fasted 36h	TFEQ Gluc, Insulin FFA, VAS	<0.05 ?SVC	↓OFC, ↓post cing				↑ mid insula	↑midbrain ↓temporal	Correlations: TFEQ disinhibition, glucose and body fat with ↑post cing and insula rCBF
Le 2006	CS WB	n 9OB /9NW M18:F0 Age OB 33y; NW 32y BMI OB 39 kg/m^2; NW22 kg/m^2 Ethnicity N/K	PET rCBF/^{15}O-water SPM99	Fed fixed and then satiating liquid meal after	VAS Glucose Insulin FFA	<0.001, uncorrected Random effects analysis	↓DLPFC	→	→	→	→		Obese had less activation in DLPFC to meal regardess of size of meal, (ie. Fixed or satiating), but no difference in hunger ratings between groups

				36h fast									
Le 2007	CS WB/ROI	N OB9 /NW10/ RO9 M0:F27 Age OB 31y; NW 33y; RO 39y BMI: N/K Ethnicity N/K	PET rCBF/^{15}O-water SPM5	Fed liquid meal after 36h fast	Glucose Insulin FFA	<0.001 or 0.05 SVC	↓ DLPFC	→	→	↓	→	↑ACC	Results in response to meal, good control for nutritional state, no metabolic results shown
PET rCBF Successful dieters													
Del Parigi 2004	CS WB	n 11RO/23OB/21NW M:F N/K Age RO 40y; OB 29y; NW 33y BMI RO24kg/m^2; OB 40kg/m^2; NW 23kg/m^2 Ethnicity N/K	PET rCBF/^{15}O-water	Taste and satiation Liquid meal after 36h fast	VAS Glucose insulin FFA	<0.05 Corr? Age, VAS, gluc, insulin, FFA	Post cing: OB taste ↑, satiation↓		RO/OB satiation↓	OB satiation↓	RO/OB taste ↑		rCBF no corr with any measures
Del Parigi 2007	CS WB/ROI	n 9 dieters/ 20 non-dieters M0:F29 Age RO 38y; OB 31y BMI N/K N/K	PET rCBF/^{15}O-water	Satiation Liquid meal after 36h fast RO: wt loss from >35 to < 25kg/m^2	Metabolic VAS TFEQ	<0.001 No TFEQ, Metabolic	DLPFC ↑ OFC↓	Putamen ↑	→	→	→		Results shown of dieters vs non-dieters. Dieters had higher restraint
Le 2007	CS WB/ROI	N OB9 /NW10/ RO9 M0:F27 Age OB 31y; NW 33y; RO 39y BMI: N/K Ethnicity N/K	PET rCBF/^{15}O-water SPM5	Fed liquid meal after 36h fast RO: wt loss from >35 to < 25kg/m^2	Glucose Insulin FFA	<0.001 or 0.05 No or SVC No	RO ↑ DLPFC ↓OFC	→	→	→	→	RO ↓occipital gyrus	Results in response to meal, good control for nutritional state, no metabolic results shown

Abbreviations:

AFNS: Analysis of Functional Neuroimage Software, **ACC:** Anterior cingulated cortex, **Amyg:** Amygdala, **AN**: anorexia nervosa, **BDI II**: Beck's depression inventory Version 2, **BED:** Binge Eating Disorder, **BMI:** Body mass index, **BPND**: Binding potential, **BW:** body weight, **BV:** BrainVoyager QX, **CES-D:** Center for Epidemiological Studies Depression Scale, **corr:** correlation with, **CS:** Cross sectional, **D2**: Dopamine type 2 receptor, **D3**: Dopamine type 3 receptor, **DEBQ:** Dutch Eating Behaviour Questionnaire, **DLPFC:** dorsolateral prefrontal cortex, **EFS:** external food sensitivity (DEBQ external), **EI**: Stunkard's Eating Inventory, **F:** female, **^{18}F:** radioligand Fallypride, **FA**: Food addiction, **FDR**: False discovery rate, **FFA**: free fatty acids, **FG**: frontal gyrus, **FO:** Frontal operculum, **FTND**: Fagerstrom Test for Nicotine Dependence, **FvB**: Food vs. Blurred, **FvO**: Food vs. Non-food, **FWE:** Family wise error, **gluc**: glucose, **HF**: High fat, **Hipp:** Hippocampus/perihippocampus, **HR:** heart rate, **HR-OB:** High-risk for obesity, **HvL**: High-calorie vs. Low-calorie, **IFG:** inferior frontal gyrus; **LF**: Low fat, **Lpt**: Leptin, **LR-OB:** Low-risk for obesity, **M:** male, **MFG:** middle frontal gyrus, **n/a**: not applicable, **N/K:** not known, **NT**: No taste, **NW:** normal weight, **OB:** obese, **o/n**: overnight, **OFC:** orbitofrontal cortex, **OW:** overweight, **PANAS**: Positive and Negative Affect Scale, **PCC**: posterior cingulate cortex, **PET**: positron emission tomography, **Pl**: Placebo, **PR:** prospective, **QN:** questionnaire, **rCBF**: regional cerebral blood flow, **RO:** obese reduced weight, **ROI:** Region of interest study, **ROp**: Rolandic operculum, **RT:** reaction time, **RYGB**: Roux-en-Y gastric bypass, **SFG:** superior frontal gyrus, **SPECT**: Single photon emission computer tomography, **SPM:** Statistical Parametric Mapping, **SSA:** somatosensory area, **SSTAI:** Spielberger State and Trait Anxiety Inventory, **T:** Tesla, **TFEQ**: Three factor Eating Questionnaire, **VAS**: visual analogue scale, **VMPFC**: Ventromedial prefrontal cortex, **VSG**: vertical sleeve gastrectomy, **WB:** whole brain, **wks:** weeks, **wt:** weight, **y**: years, **YFAS**: Yale Food Addiction Scale

ARROWS represent comparison of OB vs NW, or positive, negative or no correlation with BMI

1.7.3.2 Obese compared to lean (see Table 1.1 and 1.2)

Based on the behavioural studies which tend to indicate that obese have increased reward responsivity to food, and reduced ability to control their response to food stimuli, one might expect neuroimaging studies of obese compared to lean individuals to find increased neural activation to food cues in many areas of the brain associated with modulating dopamine release (VTA, nucleus accumbens, caudate, putamen), reward or saliency interpretation (OFC, ACC), integration of sensory information relating to food (insula, primary gustatory cortex), motivation or drive to seek reward (OFC), emotional response and regulation (amygdala), learning and conditioning (hippocampus), and potentially less activation for inhibitory control areas (DLPFC) (Volkow et al. 2011). In fact, results from studies examining this are surprisingly inconsistent with this hypothesis (Ziauddeen et al. 2012)(see also Table 1.1 and 1.2).

This may be largely due to the fact that obesity is a heterogeneous condition and although most studies use BMI as a marker of obesity, a raised BMI may be the end result of a combination of any number of etiological pathways and influences, all of which may differentially affect or be affected by the neurological response to food (Berthoud 2012). In other words, although obesity is largely the end product of eating in excess of an individual's energy requirements, eating behaviour itself is complex. The interplay of individual psychological, genetic and metabolic factors with an obesogenic environment affect how an individual's brain reacts to the sight, smell or taste of food, on a conscious and unconscious level, governing eating behaviour in different situations. For instance, individual personality traits (such as impulsivity and reward responsivity), different cognitive styles (such as rigid dietary restraint or self-control), and behaviour indicative of possible underlying deficits in affect regulation (such as emotional eating, binge eating and disinhibition) may all be

expressed to varying degrees in the obese population and may also increase with BMI (see Section 1.7.2). Each of these will have their own, possibly diverse, effect on the reward response to food cues as well as cognitive and executive control network functioning in response to food cues (Hollmann et al. 2013). A better understanding the effects of how these factors affect neural reactivity to food in different parts of the brain, linking this to observed eating behaviour and BMI could significantly improve our interpretation and analysis of data from neuroimaging studies (Smeets et al. 2012).

As evident from Table 1.1 and Table 1.2, there also exists a great deal of variability in study paradigms, which makes comparison, and summary of results across studies difficult. The type of food stimulus used may be important and may elicit different responses in different parts of the brain depending on whether food pictures (Stoeckel et al. 2008; Dimitropoulos et al. 2012; Grosshans et al. 2012; Ho et al. 2012; Scharmuller et al. 2012), guided imagery (Stice et al. 2010; Jastreboff et al. 2013), imagining taste (Frankort et al. 2012), anticipation of taste (Stice et al. 2008; Ng et al. 2011) or actual taste receipt (Stice et al. 2008; Stice et al. 2008; Stice et al. 2013), was employed as the stimulus (see Table 1.1 for examples). Even the type of food pictures used may vary considerably across studies with the use of pictures of high-calorie foods (Rothemund et al. 2007; Stoeckel et al. 2008; Dimitropoulos et al. 2012; Grosshans et al. 2012; Ho et al. 2012), appetising foods (Nummenmaa et al. 2012) and palatable foods (Frankort et al. 2012) all interchangeably used. Some studies use pictures of foods that subjects have themselves chosen as palatable, introducing a further variability factor (Karhunen et al. 2000; Jastreboff et al. 2013). However, even within studies that make use of the same types of stimuli there is variability in the paradigms, for instance whether scanning took place when subjects were fasted (Stoeckel et al. 2008; Grosshans et al. 2012; Nummenmaa et al. 2012) or fed (Frankort et al. 2012) or both on different occasions (Martin et al. 2010; Dimitropoulos et al. 2012). Even duration of fasting varies considerably, e.g. 2

hours (Jastreboff et al. 2013) to overnight (Scharmuller et al. 2012) in fMRI studies and up to 36 hours in some PET studies (Gautier et al. 2000; Gautier et al. 2001; Le et al. 2006; Le et al. 2007).

A further problem is the fact that subject numbers are often limited by technical difficulties in this population (Botkin et al. 2007) as well as the expense of neuroimaging food studies. In a population with such heterogeneity such as in obesity, this can be a significant problem, particularly when there is variability across study design. Furthermore, very large subjects may not be able to fit into conventional scanners, so that studies generally do not include subject with a BMI of more than 50kg/m^2, potentially excluding patients where large effect sizes might have been found.

The variability across studies also limits the degree to which meta-analyses can be used. A recent meta-analysis was only able to include 7 of the over 40 studies carried out in this area (Brooks et al. 2013). Of the 126 subjects included in the 7 studies examining whole brain response to food images, obese in comparison to healthy weight subjects had increased activation in the left dorsomedial prefrontal cortex, right parahippocampal gyrus, right precentral gyrus and right ACC, and reduced activation in the left DLPFC and left insula.

There are also other factors concerning individual variability that are not accounted for in all studies, for example, stage of menstrual cycle in women, which is known to affect visual response to food cues (Frank et al. 2010) may not be taken into account (Rothemund et al. 2007; Scharmuller et al. 2012). Other factors varying between groups, such as age, gender, ethnicity, and level of education may also affect BOLD response to food.

Furthermore there exists variability in methods of statistical analysis of neuroimaging studies, with the use of different analysis software (e.g. FSL, SPM-5, BrainVoyager), different methods of determining significance levels (including making correction for multiple comparisons, or not), and different methods of pre-processing and processing data (See Chapter 2 for more details).

As evident from Table 1.1 and Table 1.2 neuroimaging techniques such as fMRI and PET although both used to investigate brain responses to food, make use of very different techniques to do so. As such they are measuring different biological aspects of this response, e.g blood flow changes measured by changes in magnetic property of blood in fMRI vs. blood flow changes measured by changes in the degradation of radio-actively tracer attached to water molecules (^{15}O-water PET). Comparison between different modalities is therefore not necessarily possible or advisable, but the techniques should be seen as complimentary.

The use of standardized protocols, including standardized food picture databases, and data sharing between units to allow pooling of data to improve statistical robustness (as has been successful in other areas of neuroscience) will improve advances in this field (Smeets et al. 2012).

In an attempt to summarise the existing studies in this area, taking into account the above variability, Table 1.1 groups studies according to the food stimulus used, and whether the studies were carried out in children/adolescents or adults. There are two studies comparing

obese and lean adolescents' responses to food pictures. Increased activation in frontal regions (OFC, inferior and middle frontal gyrus) (Bruce et al. 2010) and in the DLPFC (Davids et al. 2010) was seen in obese compared to lean adolescents when viewing food pictures. However less activation in the caudate, hippocampus and ACC was seen in obese compared to lean adolescents in the latter study. In the first study, obese adolescents had less reduction in frontal area activation than lean adolescents when scanned again after a 500kcal meal.

In the adult studies employing the same food stimulus (food pictures) the results are not consistent. Even when excluding results from those studies that did not make correction for multiple comparisons (Rothemund et al. 2007; Martin et al. 2010; Frankort et al. 2012; Nummenmaa et al. 2012), inconsistency remains. For instance, out of the 6 studies where robust statistical analysis was employed, greater BOLD activation to high-calorie or any food pictures in obese compared to normal weight individuals, was seen in the OFC in 2 studies (Stoeckel et al. 2008; Jastreboff et al. 2013), the DLPFC in 2 studies (Ho et al. 2012; Scharmuller et al. 2012), the putamen and amygdala in 3 studies (Stoeckel et al. 2008; Ho et al. 2012; Jastreboff et al. 2013), and the insula in 3 (Stoeckel et al. 2008; Scharmuller et al. 2012; Jastreboff et al. 2013). Greater caudate, hippocampus and ACC activation to food pictures in the obese compared to lean subjects was also observed in two of the studies (Stoeckel et al. 2008; Dimitropoulos et al. 2012), although in the latter, this was only observed in the fed, not fasted state. For the rest, obese people either had no difference in or lower (Dimitropoulos et al. 2012; Grosshans et al. 2012; Ho et al. 2012; Jastreboff et al. 2013) BOLD activation to food pictures in all or some of the above areas compared to normal-weight individuals in the fasted state.

In those studies employing anticipated receipt of milkshake or imagination of receiving food, 3 studies included data in which correction for multiple comparisons was made. Of these, 2 out of 3 studies showed increased ACC activation (Stice et al. 2008; Frankort et al. 2012), and 2 increased gustatory cortex (Rolandic operculum and/or insula and/or frontal operculum) activation to taste anticipation in obese compared to lean individuals.

Two of these studies also found similar gustatory cortex responses in obese subjects when measuring BOLD response to actual receipt of the milkshake (Stice et al. 2008; Ng et al. 2011). However, later studies by the same group did not replicate these findings and instead found reduced caudate activation in response to milkshake receipt in obese subjects compared to healthy weight (Stice et al. 2008; Stice et al. 2010). Importantly, these 2 studies also found that the lower activation in the caudate in response to milkshake receipt was associated with future weight gain, which was moderated by presence of the Taq A1 allele.

PET studies using ^{15}O-water tracers to measure regional cerebral blood flow (rCBF) in obese compared to lean subjects are again inconsistent in their results, although fairly consistent in their paradigms. Subjects were subjected to prolonged fasting of 36 hours before being given a liquid meal, and differences in rCBF in response to either a taste (DelParigi et al. 2005) or satiation (Gautier et al. 2000; Gautier et al. 2001; Le et al. 2006; Le et al. 2007) with the meal were measured in obese and lean subjects.

Satiation resulted in reduced DLPFC rCBF in obese compared to lean men (Le et al. 2006) and women (Le et al. 2007) in two studies using this paradigm, but another study utilizing an almost identical paradigm found *increased* activation of DLPFC in obese compared to lean

men (Gautier et al. 2000). Similarly satiation resulted in greater reduction of rCBF in the ventrolateral and ventromedial prefrontal cortex, gustatory cortex, temporal and hippocampal areas in obese compared to lean men (Gautier et al. 2000) and women (Gautier et al. 2001) in the two earlier studies. These results were not replicated in the later studies, which found no significant differences in rCBF in response to satiation in these areas between lean and obese (Le et al. 2006; Le et al. 2007). The differences in these studies may however be due to differences in statistical thresholding of results.

Taste by delivery of 2ml of liquid meal in fasted subjects, resulted in greater gustatory cortex and less posterior cingulate, OFC and temporal rCBF in obese compared to lean subjects (DelParigi et al. 2005).

1.7.3.3 Correlation with BMI

fMRI studies and PET studies including obese subjects have found correlations with BMI or other measures of adiposity and neural reactivity to food. Again results are variable. In the fMRI studies lean and obese adult subjects using food pictures, BMI was positively correlated with BOLD activation to high-calorie food compared to neutral pictures in the caudate, putamen, anterior insula, PCC, globus pallidus and OFC in one study (Rothemund et al. 2007) and negatively with BOLD activation in inferior frontal gyrus and ACC in another study (Killgore et al. 2003). The first study also found positive correlations with BMI and BOLD activation to food pictures in the globus pallidus and OFC in an obese-only group.

In the three studies of adolescents, BMI positively correlated with BOLD activation to high-calorie food or appetizing food pictures in the hippocampus in one study (Wallner-Liebmann

et al.), and positively in the insula, and negatively in the OFC in another study (Yokum et al. 2011). Similarly BOLD activation in response to exercising cognitive control in response to appetizing food pictures, correlated positively in the insula and negatively in the OFC with BMI (Batterink et al. 2010).

One PET study has shown correlation with body fat and increased PCC and insula rCBF in response to taste of a liquid meal.

1.7.3.4 Correlation with weight gain

In one study of adolescent girls, increase in BMI after 1 year was predicted by increased baseline OFC activation to pictures of appetizing foods (Yokum et al. 2011) and in another by less temporal activation when exercising cognitive control in response to appetizing food pictures (Batterink et al. 2010). In the same portfolio of studies, utilizing taste rather than pictures as the stimulus, *lower* activation in the OFC and putamen in response to imagining eating palatable foods also predicted increased BMI after a year (Stice et al. 2010), and *lower* precuneus activation in anticipation of milkshake taste delivery and *lower* temporal activation to actual delivery of the milkshake taste predicted increase in progression to overweight in adolescents.

Increased activation in the nucleus accumbens to viewing food pictures also predicted weight gain 6 months later in a study of 58 normal weight college students. Unfortunately, however this study did not make correction for multiple comparisons when comparing activation in voxels across the ROI or whole brain, thereby reducing the reliability of their results, although the whole brain and ROI analysis did give consistent results (Demos et al.

2012).

1.7.3.5 Correlation with weight loss in the obese

In a longitudinal study of 6 obese people, 10% weight loss has been associated with decreased BOLD activation to food pictures in the ACC and amygdala, increased activation in the brainstem and both increased and decreased activation in the inferior and middle frontal gyrus and hippocampal areas (Rosenbaum et al. 2008). These changes were reversible by administration of leptin in some but not all areas.

Compared to normal weight individuals, obese people who had lost 8% body weight had less BOLD activation to food pictures in the insula and visual cortex (Cornier et al. 2009). They also had less attenuation of BOLD signal in the same areas after overfeeding on a 3-day hypercaloric diet compared to the normal weight participants.

1.7.3.6 Correlation with successful weight loss/weight loss maintenance

Two PET studies found reduced rCBF in the OFC and increased rCBF in the DLPFC in response to satiation with a liquid meal after a 36 hour fast in people who had successfully lost weight (DelParigi et al. 2007; Le et al. 2007). These results suggest that successful dieters may have preferential engagement of areas of inhibitory control (DLPFC) in response to food cues that may ensure successful weight maintenance (Hare et al. 2009) as well as reduction in the salience attributed to food (processed in the OFC).

The first study compared obese to reduced obese subjects, and the second normal/overweight dieters to non-dieters. The latter study also found increased rCBF in the putamen in response to satiation in dieters compared to non-dieters. An earlier cross-

sectional PET study found no difference however between weight stable, and 10% reduced obese participants in rCBF in response to taste or satiation of a liquid meal after a 36 hour fast (DelParigi et al. 2004).

In a cross-sectional fMRI study, obese people who sustained an average of 13kg weight loss over 3 years had more BOLD activation to food pictures in the precentral gyrus and frontal regions compared to weight-stable obese patients, areas which have also been implicated in conscious control of food intake and dietary restraint (McCaffery et al. 2009).

In another study of overweight and obese participants in a 12 week weight loss programme, the least successful dieters had higher activation to high-calorie food pictures in the nucleus accumbens, ACC, middle frontal gyrus and insula, whereas successful maintenance of weight loss correlated with lower activation in the putamen, insula, inferior frontal gyrus and hippocampus (Murdaugh et al. 2012).

1.7.3.7 Dopamine hypo- or hyper-function in obesity

Two PET and one SPECT study have shown that obese people have fewer D2 receptors availability in striatal areas, compared with lean people (Wang et al. 2001; Volkow et al. 2008; de Weijer et al. 2011) and that this is correlated with BMI, although two other studies have not replicated these findings (Haltia et al. 2008; Dunn et al. 2012). The findings of the first three studies support a dopamine hypofunction etiological theory of obesity. This theory suggests that dopamine hypofunction leads to impaired reward signaling in obese individuals, so that more food or food of higher reward valence is needed to reach affective satiety in reward deficient obese people. This theory is derived from the reward deficiency

theory of drug addiction. It has not been clearly established however whether this serves as a predisposing factor to obesity or is a result of overeating. In addition, since dopamine is a flux system in the brain, low dopamine receptor availability may also be a result of *increased* dopamine release triggered by food anticipation or receipt leading to eventual down-regulation of receptors. This scenario would be more in keeping with a dopamine hyperfunction theory of obesity.

A unified theory of the above, is that receipt of food may induce attenuated activation of non-homeostatic food control areas over time, so that in established obesity less deactivation in striatal areas (caudate and putamen) is seen following receipt of food (Stice et al. 2009). On the other hand, adolescents at high risk of obesity, but still normal weight showed *increased* caudate activation in response to milkshake receipt compared to those at low risk (Stice et al. 2011), suggesting they differ in this respect to the already obese. A potential mechanism is demonstrated in animal models where a repeated intake of sweet and fatty foods resulted in down-regulation of post-synaptic D2 receptors, increased D1 receptor binding, and decreased D2 sensitivity and μ-opioid receptor binding (Colantuoni et al. 2001; Bello et al. 2010), paralleling neural response to chronic use of drugs that increase dopamine signaling.

1.7.3.8 Functional connectivity in obesity

Altered functional connectivity between reward areas has also been seen in obesity both at rest (Garcia-Garcia et al. 2012; Kullmann et al. 2012; Nummenmaa et al. 2012) and when viewing food pictures (Stoeckel et al. 2009; Kullmann et al. 2012; Nummenmaa et al. 2012).

Increased connectivity (increased influence) between OFC on the nucleus accumbens and reduced modulation of the amygdala on both the OFC and nucleus accumbens was demonstrated in one study (Stoeckel et al. 2009) whilst increased influence of the amygdala and insula on caudate was found in another study (Nummenmaa et al. 2012) in obese people compared to normal weight. Other studies have also demonstrated increased connectivity in salience networks (Garcia-Garcia et al. 2012; Kullmann et al. 2012; Kullmann et al. 2012) and increased connectivity in the precuneus and decreased connectivity in right ACC in the default mode network, and decreased connectivity in the insula in the temporal lobe network in obese compared to lean individuals when at rest (Kullmann et al. 2012). Taken together, these studies suggest a dysfunction in assessing and adapting to reward value of food cues in obese individuals. On the basis of these findings, it has been suggested that an imbalance between cognitive and emotional processing of food cues is present in obese individuals which drives eating behaviour in the absence of physiological hunger (Kullmann et al. 2012).

1.8 Structural brain changes in obesity

1.8.1 Grey matter density and volume (VBM) (Table 1.3)

In general, most studies utilizing VBM, show an apparent negative association of increased BMI with grey matter density/volume in various areas of the brain associated with the processing of reward, although results are inconsistent (see Table 1.3). However, caution should be exercised in interpreting the results since age may play an important role in the interaction of BMI and grey matter volume.

In adolescents, obesity has been associated with lower total grey matter volume (Yokum et al. 2012), and lower grey matter volume in the OFC (Maayan et al. 2011) in obese compared to normal-weight teenagers. In the first of these studies, a trend for lower grey matter

volume in frontal regions (superior and medial frontal gyri) was associated with weight gain at 1-year follow up, as was higher white matter volumes in the dorsal striatum (caudate, putamen) and hippocampus (Yokum et al. 2012). Another adolescent study found increased grey matter volume in the hippocampus of obese compared to normal weight teenagers (Moreno-Lopez et al. 2012).

In studies including adults <70 years old, again frontal and striatal regions as well the gustatory cortex and amygdala emerged as holding differences between obese and normal weight people, although the direction of the association is inconsistent between studies. Increased grey matter volume in the OFC was associated with increased BMI in one study (Horstmann et al. 2011), whereas another found no correlation (Orsi et al. 2011) and yet another, a negative association with waist circumference, although not BMI (Kurth et al. 2012). Grey matter volume in the inferior frontal gyrus has also been shown to be increased in obesity (Pannacciulli et al. 2006), and positively associated with BMI in normal weight men (but not women) (Taki et al. 2008).

BMI correlated positively with grey matter volume in the dorsal (caudate and putamen), and ventral striatum (nucleus accumbens), in normal weight individuals in two studies, (Taki et al. 2008; Horstmann et al. 2011), although another study found a negative correlation with grey matter volume in the caudate (Kurth et al. 2012). BMI also positively correlated with amygdala grey matter volume in one study (Orsi et al. 2011). Reduced grey matter volume in the gustatory cortex (frontal operculum or insula) has been associated with increased BMI (Kurth et al. 2012) and obesity (Pannacciulli et al. 2006).

In older adults (>70 years) however, obesity appears more clearly to be associated with

reduced grey matter volume in frontal, striatal (putamen) (Raji et al. 2010), peri-hippocampal (Raji et al. 2010), gustatory cortex (Walther et al. 2010)and amygdala (Ho et al. 2010) regions. However this apparent association may be confounded by the effect of age as there may be an interaction with BMI and age on reducing grey matter volume. In addition, not all studies included age as a covariate in their analyses (Ho et al. 2010; Raji et al. 2010; Brooks et al. 2012). There may also be other confounders affecting these results since many of these studies were originally investigating the effect of dementia on grey and white matter volume, and this may have a further interaction with BMI. As Driscoll et al. point out, the effect of BMI or obesity may be overestimated in studies of grey matter volume which include older adults, even if non-demented at the time, since a subset of these will go on to develop dementia and sub-clinical brain volume effects may already be present (Driscoll et al. 2012). In their study of patients with average age of 69 years, they found an association of age with reduced grey matter volumes over one year in frontal, cingulate and hippocampal areas. Midlife obesity emerged as a modifier of brain atrophy associated with dementia, but not in non-demented subjects. Therefore, excluding patients who went on to develop dementia abolished the association with reduced grey matter volume in these areas.

Gender (Horstmann et al. 2011; Kurth et al. 2012; Taki et al. 2012), hypertension (Walther et al. 2010) or other metabolic diseases in cross-sectional studies may be important confounders, which are not always taken into account or corrected for.

For instance, in a their study of women of average age 70 years, with normal cognitive functioning, correcting for hypertension decreased the number of areas in which grey matter volume negatively correlated with BMI, and increased the areas in which white

matter volume was positively associated with BMI (Walther et al. 2010).

Studies investigating grey matter volume in patients with genetic obesity syndromes are also summarized in Table 1.3. PWS has been associated with reduced grey matter volume in the cerebellum (Miller et al. 2009; Ogura et al. 2011), caudate and OFC (Ogura et al. 2011). Leptin replacement therapy in leptin deficiency syndrome was associated in 3 patients with increased frontal, ACC and cerebellar volume over a six month period (Matochik et al. 2005).

The FTO gene has also shown association with reduced total brain volume (Melka et al. 2013), and reduced grey matter volume in frontal regions (Ho et al. 2010). Obesity-prone individuals (defined by having first degree obese relatives, and experiencing significant difficulty losing weight and maintaining a steady weight), had lower grey matter volume in the OFC and gustatory cortex compared to those defined as obesity-resistant (Smucny et al. 2012).

Three studies have linked eating behaviour to regional grey matter differences, particularly in the OFC. In one study, binge eating young women had increased OFC and ACC grey matter volume compared to non-eating disordered normal weight individuals (Schafer et al. 2010). Although BMI was adjusted for, the groups were significantly different in their BMI which may have confounded the results. Higher disinhibition scores on the TFEQ has also been linked to increased OFC grey matter volume in adolescents (Maayan et al. 2011). Another study linked anatomical brain differences in obese compared to normal weight subjects with food choice; in particular choice of healthier foods was associated with increased OFC grey matter volume in obese/overweight subjects, but not lean (Cohen et al. 2011).

Furthermore hormones that control appetitive behaviour centrally, such as leptin and PYY have also been shown to affect grey and white matter volumes. In leptin deficient patients, leptin replacement increased grey matter volume in frontal regions, ACC and cerebellum (Matochik et al. 2005). Plasma leptin levels in a group of obese and normal weight individuals (average age 32years) were associated with reduced frontal operculum and putamen grey matter volume and increased cerebellar and temporal grey matter volume after adjusting for age, body fat and insulin levels, although these associations did not survive correction for multiple comparisons (Pannacciulli et al. 2007). In normal weight elderly subjects (average age 65years), plasma leptin levels were associated with increased cerebellar and hippocampus grey matter volume after adjusting for age, sex, BMI, and waist-hip ratio (Narita et al. 2009). In another study, post-prandial PYY was positively associated grey matter volume in the caudate (Weise et al. 2012).

Table 1.3 Grey matter volume and density in obesity

Author Year	Design	Subjects	Adjustment made	Correlation with	Total brain GM	Grey matter density/volume						Comment
	CS/PR	**N** **Gender (M:F or F%)** **Age (mean)** **BMI (means)** **Ethnicity/Country origin**				**Frontal**	**Striatum**	**Hipp**	**Amyg**	**Insula /gustatory cortex**	**Other**	
OB vs. NW Adolescents												
Maayan 2011	CS	N OB45/ NW 36F OB 63%; NW 56% Age 17y BMI OB 40kg/m^2; NW 22kg/m^2 USA	Age, ICV		n/a	↓OFC	n/a	n/a	n/a	n/a		Not clear if correction for multiple comparison
			SBP, HOMA-IR, ICV		n/a	↓OFC	n/a	n/a	n/a	n/a		
Yokum 2011	PR	N OB17/ OW36/ NW31 F 100% Age 18y BMI N/K USA (mixed ethnicity)			↑	n/a	n/a	n/a	n/a	n/a		Total WM: OB vs. NW→, OB vs. OW→, OW vs. NW ↑
Moreno-Lopez 2012	CS	N OB20/ OW16/ NW16 F 67% Age 14y BMI OB 32kg/m^2; OW25kg/m^2; NW 20kg/m^2 Spain	Total GMV, gender		**n/a**	→	→	↑	→	→		
OB vs. NW Adults <70y												
Pannacciulli 2006	CS	n OB 24/NW 36 F:OB 55%; NW 31% Age OB 32y; NW 33y BMI OB 39kg/m^2; NW 23kg/m^2 USA	Age, sex, handedness & GTD		n/a	↑ IFG ↓ MFG	↓ putamen	→	→	↓FO	↓ postcentral gyrus, cerebellum ↑ cuneus, occipital	↑ WM striatum

Haltia 2007	CS/PR	N OB 30/ NW 16 F OB 75%; OW 60%; NW 50% Age 37y BMI OB 33kg/m^2; NW 22kg/m^2 Finland	Sex, ICV		→	→	→	→	→	→	→	WM: ↑PHG, fusiform gyrus, parietal, brainstem, cerebellum Significant difference in M:F ratio between OB and NW
Gunstad 2008	CS	N OB21/ OW63/ NW117 F OB 57%; OW 41%; NW 51% Age OB 45y; OW 42y; NW 33y BMI OB35kg/m^2; OW 27kg/m^2; NW 22kg/m^2 USA	Age		↓	→	→	→	→	→	→	Trend for lower GM in parietal and temporal lobes in OB. TBV lower in OB vs. OW and NW. Significant difference in age between OB and NW
OB vs. NW Age>70y												
Raji 2010	CS	N OB14/ OW51/ NW53 F OB 64%; OW 53%; NW 48% Age OB 77y; OW 77y; NW 76y BMI *OB35kg/m^2*; OW28kg/m^2; NW22kg/m^2 USA	Age, sex, race, DM		→	↓	→	↓	→	→	↓ ACC, basal ganglia	Age>70y, OW<NW: basal ganglia, parietal
Brooks 2012	CS	N OB59/ NW 97 F OB 58%; NW 54% Age 70-75y BMI OB34kg/m^2; NW 23kg/m^2 Swedish white	Sex, TBV, Edu, DM		↓TBV	↓DLPFC, MPFC, IFG	→	→	→	→	↓ SMA, postcentral gyrus	Age>70y; WB results uncorrected for multiple comparisons. No correlations GM with cognitive test (TMT)
Correlation adiposity measures Adolescents												
Yokum 2011	CS/PR	N OB17/ OW36/ NW31 F 100% Age 18y BMI N/K USA (mixed ethnicity)	Total GMV	BMI	OB vs. NW ↓	↓	→	→	n/a	n/a	↑occipital	WM ↑caudate, putamen, fusiform gyrus, temporal, hippocampus, occipital
				ΔBMI 1 yr		↓SFG, MFG	→	→	n/a	n/a		

Moreno-Lopez 2012	CS	N OB20/ OW16/ NW16 F 67% Age 14y BMI OB 32kg/m^2; OW25kg/m^2; NW 20kg/m^2 Spain	Total GMV, gender	BMI	n/a	→	→	→	→	→	↓postcentral gyrus	Positive correlation inhibition score Stroop DLPFC in NW only
Correlation adiposity measures Adults<70y												
Ward 2005	CS	N OB 21/OW 42/ NW 51 M 41:F73 Age 54y BMI 26kg/m^2 USA	Age, sex, BMI FHx AD, APOE, chol, BP as covariates.	BMI	↓ TBV^{norm} =TBV/ICV	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**		Cohort was half HR for dementia
Pannacciulli 2006	CS	n OB 24/NW 36 M36:F24 Age OB 32y; NW 33y BMI OB 39 kg/m^2; NW 23 kg/m^2 USA	Age, sex, handedness & GTD	BMI		→	→	→	→	→	↓ postcentral gyrus	↑ WM striatum. No correlation GM with glucose, insulin levels.
Haltia 2007	CS/PR	N OB 30/ NW 16 M23: F23 Age 37y BMI OB 33 kg/m^2; NW 22 kg/m^2 Finnish	Sex, ICV	WHR	→ GMV	→	→	→	→	→	→	WM: ↑ temporal, occipital brainstem, cerebellum
Gunstad 2008	CS	N OB21/ OW63/ NW117 F OB 57%; OW 41%; NW 51% Age OB 45y; OW 42y; NW 33y BMI OB35 kg/m^2; OW 27 kg/m^2; NW 22 kg/m^2 USA	Age	BMI	↓	→	→	→	→	→	→	ROI analysis no correlation with BMI and GM. BMI negatively correlated with TBV. Significant difference in age between OB and NW

Taki 2008	CS	N 1428 M690:F738 Age 45y BMI 23 Japan	Age, sex, alc intake, HT, DM	BMI	M only: ↓	M only: ↑ SFG, IFG ↓ SFG, IFG	M only: ↑caudate	→	→	→	M only: ↓ Precuneus, fusiform gyrus, temporal, cerebellum, midbrain ↑cerebellum, thalamus	Results for male only, no correlation in female. Main effect of BMI on GM, and effect of interaction BMI and gender on GM
Cazettes 2011	CS	N 44OB / 19NW F OB 48%; NW 58% Age OB 59y; NW 58y BMI OB31kg/m^2; NW 24kg/m^2 USA	Age, HT, WHR, lipid, gluc	Fibrinogen		OB ↓OFC	n/a	→	n/a	n/a		Fibrinogen used as a marker of obesity-related inflammation. DTI results: ADC (GM): fibrinogen positively correlated with OFC in OB
Horstmann 2011	CS	n 122 M61: F61 Age 25y BMI 27kg/m^2 German	Age, TBV	BMI		↑	↑putamen, NAcc	→	→	→	↑ hypothalamus	Significant interaction between BMI, gender and plasma leptin. Obese women, but not men scored worse on Iowa gambling task.
				Leptin		↑DLPFC (F only), OFC	↑putamen (F only), NAcc	→	→	→	↑ hypothalamus (M only)	
Orsi 2011	CS	N 92 F 57% Age 23y BMI 22kg/m^2 Hungary	ICV	BMI		→OFC	**n/a**	**n/a**	↑ (M only)	**n/a**		Not clear if correction for multiple comparison
Kurth 2012	CS	N OB11/ OW31/ NW 73 F 53% Age 45y BMI 25kg/m^2 USA	Age, Sex, TBV	BMI		↓frontal lobe, SFG, IFG, MFG	↓caudate	↓		↓	↓mid cingulate, parietal, postcentral gyrus, temporal, hypothalamus, cerebellum	Significant interaction of gender with BMI and WC
				WC		↓OFC, PFC, SFG, MFG, IFG		↓		↓	↓parietal, fusiform gyrus, temporal, hypothalamus, cerebellum, lingual gyrus, globus pallidus, occipital, cuneus	
Weise 2013	CS	N 76 F 32% Age 32y BMI 30 USA (mixed)	Age, sex, handedness	Fat free mass index		↓VMPFC, OFC				↓	↓temporal	Fat mass index similar (OFC and temporal) but less extensive correlations No correlation with fat mass and %body fat

Correlation adiposity measures Adults>70y												
Raji 2010	CS	N OB14/OW51/NW53 F OB 64%; OW 53%; NW 48% Age OB 77y; OW 77y; NW 76y BMI OB 35kg/m^2; OW 28kg/m^2; NW 22kg/m^2 USA	None	BMI	→	↓OFC	↓ putamen	↓	→	→		Age>70y. BMI correlated negatively with total WM but not GM
			None	Insulin	↓	↓OFC	→	→	→	→	↓ globus pallidus, thalamus	Insulin correlated negatively with total WM and total GM.
				DM	→	↓PFC, frontal gyri	↓putamen, caudate	→	→	→	↓ basal ganglia, globus pallidus, parietal, mid cingulate, cerebellum, occipital, cuneus, lingual gyrus	
Walther 2010	CS	N OB20 /OW22 / NW53 F 100% Age OB 70y; OW 70y; NW 71y BMI OB35kg/m^2; OW 28kg/m^2; NW 22kg/m^2 USA	Age, ICV	BMI		↓ OFC, MFG, IFG,	↓	→	→	↓ FO	↓ brainstem, cerebellum, occipital, cuneus, lingual gyrus, parietal, fusiform gyrus, postcentral gyrus,	Age>70y; female only, NW and OW/OB differed in education years and hypertension. WM: BMI positively correlated with OFC, IFG, MFG, SFG, parietal
Ho 2012	CS	N OB16/ OW64/ NW 82 F 45% Age 75y BMI 25kg/m^2 USA	None	BMI		→	→	→	↓	→		Alzheimers disease inclusion criteria
RO vs. OB												
Haltia 2007	PR	N OB 16/ RO 16 M23: F23 Age 37y BMI OB 33kg/m^2; RO 30kg/m^2(6 wk diet) Finnish	Sex, ICV		**n/a**	→	→	→	→	→	→	WM: ↓ PHG, fusiform gyrus, temporal DTI results: FA ↓ cingulate, cerebral peduncle, temporal. ADC (GM) ↑ temporal, PFC, parietal
GENETIC OBESITY												

Matochik 2005	PR	N 3 leptin deficiency M1:F2 Age 27-40y BMI 47-55kg/m^2 Turkey	GMV	Leptin replacement therapy		Δ6m ↑MFG, IFG					Δ6m ↑ACC, parietal, cerebellum Δ18m↑ACC, parietal, cerebellum	
Miller 2009	CS	N OB 12/NW15 F 50% Age 9-12 BMI OB 36kg/m^2; NW 19kg/m^2 USA			→	→	→	→	→	→		Small study early onset obesity vs. sibling cohorts. Subset analysis: PWS vs. NW ↓cerebellar volume
Melkaye 2012	CS	N 598 F 51% Age 15y BMI N/K Canadian French	Age, sex	FTO	↓TBV	n/a	n/a	n/a	n/a	n/a	n/a	
Ogura 2011	CS	N 12PWS/ 13NW F PWS 50%; NW 53% Age PWS 25y; NW 24y BMI PWS 34kg/m^2; NW 20kg/m^2 Japan			↓	↓ OFC	↓caudate	→	→	→	↓postcentral gyrus, precuneus, temporal, cerebellum	↓WM
			Age, GMV			↓ OFC						Adjusting for GMV, or GMV/sex did not change results
HR-OB vs. LR-OB												
Ho 2010	CS	N HR-OB 128/ LR-OB 78 F HR-OB 44%; LR-OB 48% Age HR-OB 76; LR-OB 76 BMI HR-OB 27kg/m^2 ; LR-OB 26kg/m^2 USA white	Age, sex	BMI		↓frontal lobe gyri					↓parietal, temporal, brainstem, cerebellum, occipital	HR defined by SNP genotyping
			Age, sex, BMI	FTO		↓frontal					↓occipital	
Smucny 2012	CS	N HR-OB28/ LR-OB 23 F 50% Age HR-OB 30y; LR-OB 31y BMI HR-OB 26kg/m^2 ; LR-OB 21kg/m^2	Age, sex, body fat mass, TBV			↓OFC	→	→	→	↓	↓cerebellum	HR defined as 1st degree relative, difficulty losing wt, wt fluctuation
DM-OB vs. non DM-OB												

Yau 2010	CS	n OB18 / DM 18 M:F N/K Age OB 17y; DM 16y BMI OB 37kg/m^2; DM 38kg/m^2 USA	Age		→	→	n/a	n/a	n/a	n/a	n/a	WM: ↓total, ↓ OFC
Correlations other												
Cohen 2011	CS	n OB-OW 41 / NW 98 F OB-OW 48%; NW 48% Age OW-OB 59y; NW 61y BMI OW-OB 32kg/m^2; NW 24kg/m^2 USA	Ed, IQ, ICV	Healthy food choice		↑OFC	n/a	n/a	n/a	n/a	n/a	
Maayan 2011	CS	N OB45/ NW 36 F OB 63%; NW 56% Age 17y BMI OB 40kg/m^2; NW 22kg/m^2 USA	Age, ICV	TFEQ disinhibition		↑OFC	n/a	n/a	n/a	n/a	n/a	OB higher TFEQ disinhibition, hunger and restraint scores. Stroop scores and disinhibition positively correlated with BMI
Taki 2012	CS	N 381 Age 58y BMI 23kg/m^2 Japan	Sex, ICV, SBP, BMI	Age		↓OFC ↑DLPFC, VLPFC	↑caudate	↑		↑	↓cingulate, cerebellum ↑parietal, temporal, cerebellum	F stronger correlation
Driscoll 2012	PR	N 152 F 41% Age 69y BMI 25kg/m^2 USA	Sex, ethnicity, education, smoking status, ICV	Age	Δ1y↓	Δ1y↓	n/a	Δ1y↓	n/a	n/a	Δ1y↓cingulate, parietal, temporal	Half of cohort had dementia, results shown did not survive Bonferroni correction for multiple comparisons
Smucny 2012	CS	N HR-OB28/ LR-OB 23 F 50% Age HR-OB 30y; LR-OB 31y BMI HR-OB 26kg/m^2 ; LR-OB 21kg/m^2	Age, sex, body fat mass, TBV	Leptin	→	→	→	→	→	↓		
Schafer 2012	CS	N BED17/ BN 14/ NW 19 F 100% Age BED 26y; BN 23y; NW 22y BMI BED 32kg/m^2; BN 22kg/m^2; NW 21kg/m^2 German	BMI	BED>NW		↑OFC	→	n/a	n/a	n/a	↑ACC	

Abbreviations:

alc intake: Alcohol intake, **ACC:** Anterior cingulated cortex, **Amyg:** Amygdala, **BED:** Binge Eating Disorder, **BMI:** Body mass index, **BN:** Bulimia nervosa, **BW:** body weight, **corr:** correlation with, **CS:** Cross sectional, **DEBQ:** Dutch Eating Behaviour Questionnaire, **DM:** Type 2 Diabetes Mellitus, **DLPFC:** dorsolateral prefrontal cortex, **Ed:** Years of education, **F:** female, **FFMI:** Fat free mass index, **FG**: frontal gyrus, **FMI:** Fat mass index, **FO:** Frontal operculum, **FTND**: Fagerstrom Test for Nicotine Dependence, **FvB**: Food vs. Blurred, **FvO**: Food vs. Non-food, **FWE:** Family wise error, **gluc**: glucose, **GM:** grey matter, **GMV:** Grey matter volume, **GTD:** Global Tissue Density, **HF**: High fat, **Hipp:** Hippocampus/perihippocampus, **HOMA-IR**: Homeostatic Model Assessment - Insulin Resistance **HR:** heart rate, **HR-OB:** High-risk for obesity, **HT:** Hypertension, **HvL**: High-calorie vs. Low-calorie, **ICV:** Intracranial volume, **IFG:** inferior frontal gyrus**, LR-OB:** Low-risk for obesity, **m:** months, **M:** male, **MFG:** middle frontal gyrus, **n/a**: not applicable, **NAcc:** Nucleus Accumbens **N/K:** not known, **NT**: No taste, **NW:** normal weight, **OB:** obese, **OFC:** orbitofrontal cortex, **o/n**: overnight, **OW:** overweight, **PANAS**: Positive and Negative Affect Scale, **PCC**: posterior cingulate cortex, **PET**: positron emission tomography, **Pl**: Placebo, **PR:** prospective, **PWS:** Prader-Willi Syndrome **QN:** questionnaire, **rCBF**: regional cerebral blood flow, **RO:** obese reduced weight, **ROI:** Region of interest study, **ROp**: Rolandic operculum, **RT:** reaction time, **RYGB**: Roux-en-Y gastric bypass, **SFG:** superior frontal gyrus, **SMA:** Supplementary Motor Area, **SPECT**: Single photon emission computer tomography, **SPM:** Statistical Parametric Mapping, **SSA:** somatosensory area, **SSTAI:** Spielberger State and Trait Anxiety Inventory, **T:** Tesla, **TBV:** Total brain volume, **TFEQ**: Three factor Eating Questionnaire, **TMT:** Trail making task, **VAS**: visual analogue scale, **VMPFC**: Ventromedial prefrontal cortex, **VSG**: vertical sleeve gastrectomy, **WB:** whole brain, **WC:** Waist Circumference, **WHR:** Waist-Hip Ratio, **wks:** weeks, **WM:** white matter, **wt:** weight, **y**: years, **YFAS**: Yale Food Addiction Scale, **Δ:** change

ARROWS represent comparison of OB vs NW, or positive, negative or no correlation with BMI

1.8.2 White matter structural integrity (DTI)

There have been a few studies examining white matter microsctructural integrity using DTI in obesity. All studies thus far have found evidence of reduced structural integrity of white matter with increased body weight. Whereas FA appears to be consistently negatively correlated with BMI, mean diffusivity results are not so straightforward. Mean diffusivity is not easy to interpret since it represents the average resistance to water flow in all directions within a voxel. Furthermore both intra- and extra-cellular diffusion is represented, further complicating matters. However, it is generally accepted that increased diffusivity is usually the result of loss of cell membrane integrity resulting in increased displacement of water molecules. Chronic inflammation and disease leads to increased mean diffusivity. On the other hand, acute injury, such as ischaemia, results initially in reduced mean diffusivity, followed by gradual increases.

Verstynen et al. examined a group of adults with BMs ranging from 19.6 to 45.7kg/m^2 and found that BMI negatively correlated with FA in 63% of white matter voxels in the brain. Clusters that were negatively correlated with BMI included the middle and superior cerebellar peduncles, areas of the midbrain, internal capsule, cingulum and peri-hippocampal tracts. Mean diffusivity also correlated negatively with BMI, and the authors explored the possibility that FA correlations were explained by mean diffusivity. However, 8% of the relationship between BMI and FA was accounted for. Interestingly, in many cases, within the same ROI there were voxels that correlated positively, and others that correlated negatively with BMI. In most cases, negatively correlated voxels outnumbered positive ones, so that the overall effect across the brain was of negative correlation with BMI (Verstynen et al. 2012). Stanek et al. also used a ROI-based approach to show how the FA of voxels in the corpus callosum and fornix decreased with higher BMI (Stanek et al. 2011). Mueller et al.

focused primarily on the corpus callosum and found similar results using tract-based and ROI analyses. They also found sex differences in white matter integrity; in women FA was negatively correlated with BMI, but in men this relationship was absent or weaker, depending on the type of analysis used (Mueller et al. 2011).

Yau et al. examined obese adolescents, focusing on the effect of metabolic co-morbidities of obesity. In the first study, obese adolescents with and without T2DM were compared (18 in each group). Those with T2DM were found to have decreased FA in several areas of the brain including the frontal, temporal and cingulate areas, indicative of reduced white matter microintegrity in these areas. They also had higher mean diffusivity in temporal, prefrontal and parietal cortices (Yau et al. 2010). In a second study comparing 49 adolescents with metabolic syndrome (abdominal obesity, insulin resistance, low high density lipoprotein (HDL)-cholesterol, hypertriglyceridaemia and hypertension) with 62 healthy controls, they found reduced FA in 14 clusters (including corpus callosum, right optic radiation, left parietal, left medial longitudinal fasciculus, left external capsule, left internal capsule, medial genu, left cerebral peduncle, right middle cerebellar peduncle). The metabolic syndrome group had lower academic achievement and intelligence quotient (IQ) scores, and lower hippocampal volumes (Yau et al. 2012). The same group also found a negative correlation between cholesterol levels and frontal/prefrontal FA in obese/overweight adults (Cohen et al. 2011).

VBM studies found obesity positively correlates with white matter volume, in striatal (caudate, putamen) (Pannacciulli et al. 2006; Haltia et al. 2007; Yokum et al. 2012), parahippocampal and temporal regions (Haltia et al. 2007). Haltia et al. also found that dieting reduced white matter volume in the these areas in obese patients (Haltia et al.

2007). Together results from these two different approaches suggest that white matter is affected by obesity (or increasing BMI) in such a way as to increase volume and reduce tract integrity in specific areas, although the mechanism for this is not known.

1.9 Gut hormone effects on food reward systems in the brain

Several key peptides have been implicated in regulating food intake, and in many cases, their action in homeostatic appetite centres have been well-researched. Increasingly their effect on non-homeostatic reward systems regulating food intake has generated interest.

Ghrelin receptors are located in the VTA and ghrelin acts within the dopaminergic system to increase reward to natural and non-natural rewards (Yeomans et al. 1993). Studies by our group and others using identical or similar fMRI paradigms to Chapter 3 and 4 have shown that ghrelin mimics the effect of fasting in mediating the reward response to food pictures in the OFC, amygdala, caudate, VTA, hippocampus and insula (Malik et al. 2008; Goldstone et al. 2010). In animal studies, intra-VTA injection of ghrelin increases the intake of palatable food (Egecioglu et al. 2010), whereas peripheral administration of ghrelin receptor antagonists reduce the preference of both high fat and high sugar foods (Perello et al. 2010; Skibicka et al. 2011).

Leptin administration into the VTA reduces food intake, reduces the work rats will do to obtain a rewarding food in a progressive ratio task (Bruijnzeel et al. 2011) and causes rats to no longer prefer an area they have been trained to associate with palatable food (Figlewicz et al. 2001). This effect is not seen in rats fed a high-fat diet suggesting that leptin resistance seen in obesity and applicable to homeostatic appetite centres, may apply to reward

circuitry in the brain too.

Leptin deficient humans have increased neural reactivity to food in the nucleus accumbens, caudate, putamen and globus pallidus (Farooqi et al. 2007) with less suppression in these areas after eating than controls (Aotani et al. 2012). This is reversed by leptin administration (Aotani et al. 2012). Leptin administration in these patients also reduces BOLD activation to food pictures the insula, parietal and temporal cortex and increases activation in prefrontal cortex (Baicy et al. 2007; Farooqi et al. 2007). Leptin administration to obese patients who have lost weight has also been shown to reverse some of the changes in BOLD activation to food pictures seen with weight loss (Rosenbaum et al. 2008).

Insulin also normally reduces appetite centrally in hypothalamic centres, and affects dopamine release in the rat striatum. At low concentrations, insulin increases dopamine release but inhibits release at higher concentrations (Potter et al. 1999). As with leptin, central administration of insulin can reduce sucrose intake in rats (Figlewicz et al. 2006) and decreases preference to a place associated with food reward (Figlewicz et al. 2009).

However as with leptin, insulin resistance seen peripherally in obesity may also be present in the brain, and may alter reward processing. For instance, exposure to a high-energy diet increases sucrose self-administration and prevents the ability of centrally administered insulin to reduce sucrose intake (Figlewicz et al. 2006; Cheah et al. 2012). In humans, insulin resistance is associated with attenuated striatal and prefrontal brain glucose metabolism following insulin infusion (Anthony et al. 2006). Altered resting state functional connectivity in the OFC and putamen is influenced by insulin resistance (Kullmann et al. 2012). Moreover,

although intranasal insulin augments post-prandial satiety and reduces food intake in normal weight individuals, this effect is not observed in obese individuals (Tschritter et al. 2006; Hallschmid et al. 2008).

Evidence of their role of PYY and GLP-1 in the success of RYGB for weight loss have provided renewed support for investigation of the mediation of these hormones on the gut-brain axis controlling food intake. However it has become increasingly apparent that these and other hormones may act not only on homeostatic hypothalamic appetite centres, but also non-homeostatic systems which control ingestive behaviour as is evidenced by both animal and human studies (Egecioglu et al. 2011).

To date, there have been no animal studies investigating PYY action on non-homeostatic brain areas. Human subjects given a PYY infusion compared to saline, showed activation of the parabrachial nucleus, the VTA, limbic regions, the ventral striatum and certain frontal cortical regions as assessed by BOLD imaging (Batterham et al. 2007). The substantia nigra, parabrachial nucleus and hypothalamic BOLD response correlated with PYY levels, whereas and OFC activation predicted food intake and correlated negatively with hedonic ratings of food when PYY was given (Batterham et al. 2007).

GLP-1 receptors have been identified in the nucleus accumbens and VTA, and activation of these receptors with GLP-1 agonists intracerebral infusions increased c-fos expression in the nucleus accumbens, decreased intake of especially highly-palatable foods, and reduced body weight in rats (Dossat et al. 2011; Alhadeff et al. 2012). Moreover, blockade of these in the VTA and nucleus accumbens core resulted in a significant increase in food intake. Food-

reward behaviour is also reduced in rats by administration of a GLP-1 agonist, as rats no longer prefer an environment previously paired to chocolate pellets. The peripheral administration of a GLP-1 agonist also decreased motivated behaviour for sucrose in a progressive ratio task (Dickson et al. 2012; Skibicka et al. 2012).

A combination of PYY and GLP-1 infusion reduced average BOLD activation to food pictures in combined reward regions (amygdala, caudate, insula, nucleus accumbens, OFC, and putamen) compared to saline and to GLP-1 infusion alone (De Silva et al. 2011).

1.10 Neuroimaging of reward systems following bariatric surgery

Currently the only successful treatment for obesity is bariatric surgery, and gastric bypass surgery (RYGB) is the most effective of the various procedures producing weight loss of around 25% and significant reduction of obesity related illness (Sjostrom et al. 2007; Kashyap et al. 2010; O'Brien 2010; Rubino et al. 2010). The success of RYGB surgery over other weight loss methods, including BAND surgery, may be at least in part due its ability to influence hedonic food responses in obese people (de Castro 2010; Shin et al. 2011), and preliminary longitudinal studies are supportive of this possible mechanism (Ochner et al. 2011; Ochner et al. 2012; Ochner et al. 2012). However direct comparisons between different procedures have not been performed.

In an fMRI study, 10 (Ochner et al. 2011) (and in a follow-up article, 14)(Ochner et al. 2012) obese patients were scanned 1 month before and 1 month after RYGB. After surgery patients had reduced activation to high-calorie pictures and words (compared to neutral cues) especially in the lentiform nucleus, putamen and frontal gyri (DLPFC) (n=14). Pre- to

post-RYGB reduction in activation was greater for high-calorie than for low-calorie food cues and the difference was greatest in DLPFC, precuneus, dorsal cingulate, ventral striatum, lentiform nucleus, superior temporal gyrus, inferior parietal lobule and precuneus (n=10) (Ochner et al. 2011). A greater reduction in the desire to eat following exposure to high-calorie food cues compared to low-calorie food cues and "liking" of high-calorie foods compared to low-calorie foods, was seen after RYGB compared to before, mirroring brain activation patterns. The reduction in activation in DLPFC, dorsal striatum, anterior cingulate, thalamus, inferior parietal areas correlated with the reduction in desire to eat high-calorie compared to low-calorie foods (n=14) (Ochner et al. 2012).

The same study was repeated in the same patients in the fed state, but interestingly, no differences were seen between pre- and post- operative neural responsivity to food cues in these regions when patients were fed (Ochner et al. 2012). A longitudinal study of 10 BAND patients before and 12 weeks after surgery, showed decreased activation in parahippocampus, medial prefrontal cortex, insula, and inferior frontal gyrus and increased activation in anterior prefrontal cortex to food compared to non-food pictures, as well as increased dietary restraint (Bruce et al. 2011). Problems with these studies are the small numbers, lack of control group for weight loss or order effect of scanning, lack of rigorous statistical thresholding (whole brain data uncorrected for multiple comparisons), and the possibility that scanning so shortly after surgery in the RYGB patients, led to liquid diet constraints and acute weight loss being potential confounders.

PET studies of changes in dopamine receptor availability after RYGB have produced conflicting results. Since D2/3 receptor availability is reduced in obesity, and assuming that this is due to down-regulation of receptors from resistance, then it is hypothesized that this

should be corrected by weight loss. In a small study of 5 obese women who underwent RYGB aged in their 30's, ^{11}C-raclopride (antagonist radioligand of D2 and D3 receptors) PET studies were carried out 6 weeks pre- and post-operatively. The analysis was limited to striatum (anterior and posterior putamen, and anterior and posterior caudate), and found the predicted increases in D2/D3 receptor binding after RYGB (Steele et al. 2010). By contrast a study of 5 women in their 40's with similar mean BMI to previous study, pre- and 7 (6-11) weeks post- RYGB and VSG using PET ^{18}F-Fallypride, to measure D2 receptor availability, found decreased D2 receptor availability after surgery in the substantia nigra, caudate, putamen, ventral striatum, hypothalamus, medial thalamus and amygdala (Dunn et al. 2010).

The authors note that the discrepancies between the studies may be related to lower age and changes in pre- to post-operative depression scores, in the subjects taking part in Steele's study. Out of the 5 patients scanned in that study, 4 had reduction in depression scores, which may have been a confounder. In the second study, there were no differences in depression scores. In the first study one of the patients had opposite results to the rest. In the second study, the group was also heterogenous as one patient underwent VSG, not RYGB. In addition, in both studies, scanning took place in the acute weight loss phase following surgery, which may have independent effects on dopamine receptor availability. In the first study, the authors begin with the assumption that pre-operatively, obese patients have low D2/D3 receptor binding, but in fact this was no different to the lean controls matched for age and sex. In addition, small subject numbers limits interpretation of both studies.

1.11 Summary

In summary, although BAND and RYGB surgery are often collectively referred to and seen as similar operations, the mechanisms governing weight loss may be significantly different between the operations. This may be due to their distinct physiological manipulations of gut anatomy. Weight loss in BAND surgery appears to be primarily a function of gastric restriction. In RYGB surgery however, the anatomical rearrangement of the gut as well as possibly accelerated gastric emptying, leads to alterations in bile and nutrient delivery to the lower gut which in turn causes profound changes in post-prandial release of specific gut hormones known to affect appetite via the gut-brain axis. In this respect particularly PYY and GLP1 have been shown to significantly affect eating behaviour in pre-clinical and clinical studies and to have direct effects on not only homeostatic but also brain reward systems. The role of other hormones such as ghrelin, CCK, leptin, and other adipokines seems less clear, and may be a result of, rather than cause of weight loss in RYGB surgery. In addition, it is possible that some weight loss may be accounted for by alterations in resting energy expenditure and malabsorption in RYGB surgery, but these effects do not appear to be large. Observed differences in food preference and dietary habits between RYGB and restrictive surgeries have not been fully explored or explained and studies in this area suffer from limitations of recall bias and measurement difficulties.

Akin to addictive behaviours, alterations in dopaminergic and opioid pathways, involved in the expectancy, appraisal and receipt of food reward appear to be important in the development and maintenance of obesity. Several components of the reward system, including the striatal nucleus accumbens and caudate nucleus (key to dopaminergic reward conditioning, expectancy and motivation), amygdala (emotional responses to rewarding stimuli), anterior insula (integrating gustatory and other sensory information) and OFC

(reward value appraisal, cognitive control and attention) have been implicated. Activation in these areas to food cues not only predicts food consumption and choice, and prospective weight gain, but may be altered in obesity, predict the success of weight loss strategies, changes with successful weight loss, including surgical treatments, and is altered in specific eating behaviour psychopathology such as dietary restraint, dietary disinhibition, binge eating and hyperphagia in genetic obesity. Interestingly modulation of activation of these reward systems both at rest and in response to food stimuli by gut hormones has been described.

Functional MRI offers a validated method of testing the effects of different bariatric surgeries on brain reward and cognitive control systems. Preliminary data from longitudinal fMRI studies suggest that RYGB may have beneficial effects on food hedonics including brain food reward systems, which may favour increased weight loss over BAND surgery. The differential effects of RYGB and BAND on brain food reward systems has not been tested, nor the relationship of these with behavioural and metabolic phenotypes (including exaggerated gut hormone release in RYGB) found in these two surgeries for obesity. The differential effects of RYGB and BAND surgery on brain structure including grey matter volume and white matter tract integrity has also not been examined. Understanding how different surgeries differentially affect eating behaviour and food reward on a functional and anatomical level in the brain may help establish the mechanism by which RYGB achieves greater success in treating obesity. This not only highlights the importance of gut-brain food hedonics in the treatment of obesity, including the development of novel, non-surgical treatments, but also raises the potential of more personalized approaches to surgical obesity treatments, according to relevant clinical, behavioural and metabolic phenotypes.

1.12 Hypothesis

Obese patients after RYGB will have healthier brain reward responses to food compared to after BAND procedures, and hence healthier eating behaviour, which may explain the greater weight loss seen after RYGB. These differences in food hedonics will not be explicable by differences in hunger levels or psychological traits, but will be associated with increased plasma GLP-1, PYY, bile acids and post-ingestive dumping symptoms, indicating potential mediators. Therefore, lowering plasma anorexigenic gut hormones PYY and GLP-1 in fed obese patients will increase hunger, reward system activation and hedonic responses to food in RYGB but not BAND patients. Differences in food hedonics between RYGB and BAND will be associated with differences in grey matter volume and density in corresponding reward areas of the brain.

Increased grey matter volume in the reward areas of the brain have been reported in obese adults under the age of 70 years. Therefore, based on the assumption that obesity-related changes in brain structure are reversible with weight loss it is hypothesized that grey matter volume in these regions would be lower in operated patients (RYGB and/or BAND), compared to BMI-matched unoperated controls.

Increased BMI and behavioural traits linked to obesity such as reward sensitivity, have been associated with reduced white matter integrity in frontostriatal, corpus callosum and peri-hippocampal tracts. It is therefore hypothesized that white matter integrity in these tracts would be greater in operated patients (RYGB and/or BAND), compared with BMI-matched unoperated controls, and perhaps also greater in the RYGB compared to the BAND group, if RYGB subjects had healthier food hedonic reponses.

1.13 Aims

1. To compare BOLD activation in brain reward systems whilst undertaking a food evaluation task between BMI- matched patients after RYGB and BAND, with BMI-matched unoperated controls that had not lost weight, using fMRI.

2. To compare food appeal, preference and palatability, as well as eating behaviour measures and actual food intake between BMI- matched patients after RYGB and BAND using questionnaires, visual analogue scales, test meals and food diaries.

3. In order to confirm known potential mediators for differences between the two surgical groups in food hedonics in this cohort, measurement of fasting and post-prandial plasma gut hormones, glucose and bile acids, as well as retrospective early post-operative and current dumping symptoms.

4. To investigate the role of exaggerated anorexigenic post-prandial plasma gut hormone (PYY and GLP-1) on brain reward systems, food appeal, food intake and palatability using fMRI, visual analogue scales and test meals, whilst suppressing post-prandial release of PYY and GLP-1 using subcutaneous Octreotide in BMI-matched patients after RYGB and BAND.

5. To compare grey matter density and volume and white matter integrity in areas of the brain associated with emotional and cognitive processing of food reward and eating behaviour, between subjects who underwent bariatric surgery (RYGB and BAND) and unoperated BMI-matched controls and between RYGB compared to BAND surgery subjects, using VBM, subcortical volumetric analysis and DTI.

6. To determine whether grey matter density and white matter integrity correlates with BMI, independently of group.

CHAPTER 2: MATERIALS AND METHODS

2.1 Participants

Patients who had previously undergone gastric bypass (RYGB) or gastric banding (BAND) surgery were recruited from Imperial Weight Centre, Charing Cross Hospital at follow up clinics or through invitation letters. A control BMI-matched unoperated control group (BMI-M) was recruited from the clinic or by public advertisement. The study was approved by the Local Research Ethics Committee (REC 08/H0707/139) and was performed in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent. Patient information sheets and consent forms are contained in Appendices 1-2.

2.1.1 Exclusion and inclusion criteria

Inclusion criteria for the study were for surgical groups were: (i) loss of more than 8% of their total body weight since surgery, (ii) surgery more than 2 months ago. All surgical procedures had been performed by one of two surgeons, with RYGB as previously described (Olbers et al. 2003).

Exclusion criteria for the study were: (i) smoking, (ii) pregnancy or breast feeding, (iii) significant neurological, psychiatric or cardiovascular disease including addiction, stroke and epilepsy, other than previous depression, (iv) commencement of antidepressants less than 6 months ago, and (v) type 2 diabetes mellitus (T2DM) treated with agents other than metformin alone, and (vi) type I diabetes mellitus.

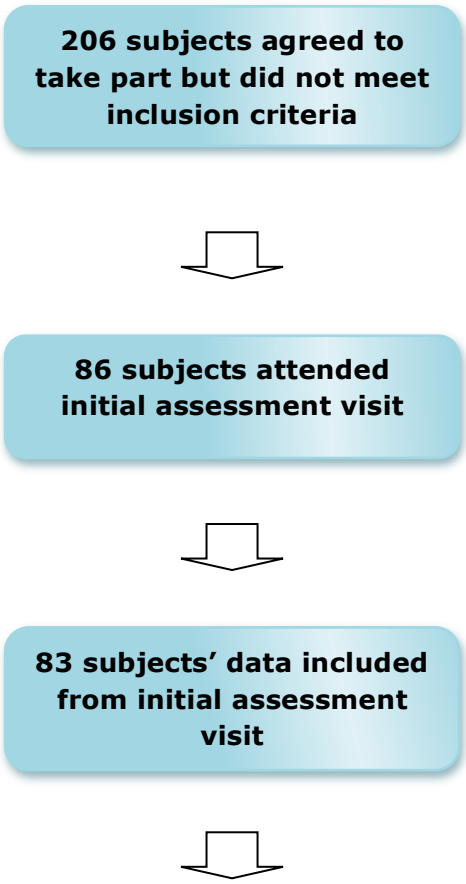
Exclusion criteria for the scanning visits were: (i) inability to use right-handed button keypad, (ii) claustrophobia, (iii) shoulder width >58cm (inability to fit in scanner bore), (iv) metal

implants which would preclude safe MRI scanning, (v) vegetarianism or veganism, (vi) reported gluten or lactose intolerance and (vii) non-Western diet assessed by dietary record.

Eligible subjects attended an initial assessment visit during which they completed a medical history, physical examination, questionnaires to assess mood, psychological traits and eating behavior and a computer-based task to measure food preference and choice (Leeds Food Preference Questionnaire, LFPQ) (Finlayson et al. 2007). Medical notes were examined to ascertain pre-operative clinical information including body weight, presence of T2DM, and binge eating disorder (BED) from review by the clinic psychiatrist or psychologist, and calculation of obesity comorbidity score using the Kings criteria (Aylwin et al. 2008).

Of the 86 patients who attended the initial assessment visit, data from 83 participants (30 RYGB, 28 BAND patients and 25 BMI-M controls) were included in the study. 3 patients were excluded on the basis of psychiatric or medical conditions identified during screening, which would significantly affect their questionnaire scores or ability to complete the study.

Figure 2.1 Flow diagram of patient entry into studies



	Cohort Initial visit	Study 1 Fasted	Study 2 Fed visits	Study 3 VBM	Study 3 DTI
RYGB	30	21	10	19	17
BAND	28	20	9	19	12
BMI-M	25	20	n/a	20	17

In line with standard policy of the obesity clinic, patients in this study had chosen themselves which surgical procedure to undergo and were not guided by medical professionals as to which patients had which surgery, as there were no evidence based guidelines to inform bariatric procedure selection. However, in practice, patients with T2DM tended to choose

RYGB more often due to its more beneficial effects on glycaemic control and T2DM resolution (Kashyap et al. 2010; Pournaras et al. 2012). There was therefore a significantly greater prevalence of T2DM and thus obesity co-morbidity score in the RYGB group pre-operatively, but no significant difference in post-operative T2DM prevalence or other characteristics between surgical groups (see Table 3.2).

2.2 Psychological and eating behaviour questionnaires (Appendices 3-9)

The following questionnaires were completed at screening:

1. Dutch Eating Behaviour Questionnaire (DEBQ) to measure dietary restraint, emotional (e.g. stress-induced eating) and external (e.g. food palatability) influences on eating behavior (Wardle 1987). These factors have been shown to influence fMRI responses to food cues. For instance, increased DEBQ restraint scores have been associated with increased activation in nucleus accumbens and amygdala in response to food pictures (Demos et al. 2011; Schur et al. 2012); increased DEBQ external eating scores have been associated with increased superior temporal activation to food pictures (Passamonti et al. 2009) and increased DEBQ emotional eating scores with increased activation in parahippocampal gyrus and ACC in response to milkshake anticipation and in caudate and pallidum in response to milkshake receipt (Bohon et al. 2012).
2. Eating Disorder Examination Questionnaire (EDE-Q) to measure dietary restraint, preoccupation with weight and shape and binge eating (Fairburn et al. 1994). Binge eating has been associated with increased striatal dopamine release in response to food presentation, smell and taste(Wang et al. 2011), and with increased OFC activation to food pictures (Schienle et al. 2009).
3. Positive and Negative Affect Schedule (PANAS) to measure symptoms of positive and negative affect over the previous week which have previously been correlated with fMRI

responses to food pictures (Watson et al. 1988; Killgore et al. 2006).

4. Beck Depression Inventory (BDI-II) to identify symptoms of depression (Beck et al. 1996). Although there have been no studies which specifically examine the effect of clinical depression on food reward processing in the brain, using neuroimaging, fMRI and PET studies have demonstrated altered neural activity in depressed subjects compared to controls both at rest and in non-food task-related activity in various areas including frontal gyri, the DLPFC, cingulated cortex and amygdala (Mitterschiffthaler et al. 2006) (Fitzgerald et al. 2006).
5. Barratt Impulsivity Scale to measure impulsivity, which has been linked to overeating (Patton et al. 1995; Yeomans et al. 2008). Impulsivity has also been linked to DLPFC functioning (Hare et al. 2009) and better impulse control has been associated with stronger functional connectivity between VMPFC and DLPFC at rest (Weygandt et al. 2013).
6. Eysenck Personality Questionnaire (EPQ-R) to measure extraversion, psychoticism, neuroticism and tendency to lying (Eysenck 1985). Extraversion includes traits such as sociability, dominance and sensation seeking; neuroticism includes anxiety, obsessiveness, tension, guilt and low self-esteem; psychoticism includes aggression, egocentricity and being tough-minded.
7. Behavioural Activation / Behavioural Inhibition Scales (BAS/BIS) to measure punishment and reward sensitivity. BIS/BAS (reward responsiveness) scores have previously been correlated with fMRI responses to food pictures (Carver et al. 1994; Beaver et al. 2006).

2.3 Leeds Food Preference Questionnaire (LFPQ)

LFPQ is a self-administered computerized paradigm that assesses food reward by measuring explicit and implicit components of food choices and ratings. Subjects are presented with food pictures and asked to rate their 'explicit liking' ("How pleasant would you find the taste of this food right now?") and 'explicit wanting' ("How much do you want to eat this food right now?") on a visual analog scale (100 mm). In addition foods are presented in randomized pairs and subjects are asked to choose a food ("select the food that you most want to eat right now") as quickly and accurately as possible. During the latter, both frequency of preferred choice (relative food preference) and reaction time were measured. Because participants were not informed about the measurement of their reaction time for each choice and were unable to monitor their responses, this measure provided a nonverbal, implicit assay of their motivation (implicit wanting). Food pictures contained foods that varied in fat (high or low) and taste (savoury or sweet). These 4 categories were matched on energy density, fat content, and type of food. There were 8 pictures of each category; "high fat sweet" (HFSW) e.g. cream éclair, ice cream, "high fat savoury" (HFSA) e.g. crisps, pizza, "low fat sweet" (LFSW) e.g. marshmallows, dried apricots, and "low fat savoury" (LFSA) e.g. boiled potatoes, rice crackers with cream cheese, presented in 2 runs in a random order.

The task has been validated in other populations including obese and binge eaters (Finlayson et al. 2007; Griffioen-Roose et al. 2012). Hunger has been associated with an exaggerated bias toward high fat foods and 'wanting' HFSA but 'liking' HFSW, whereas satiation with a bland savoury meal is associated with a reduction in explicit wanting and liking of all food categories, and increased implicit wanting and relative preference of sweet foods, demonstrating a dissociation between liking and implicit wanting following satiation

(Finlayson et al. 2008). In binge eating, dietary disinhibition on TFEQ and compensatory eating after exercise greater implicit wanting of HFSW foods is observed and in binge eaters, greater 'liking' of all foods compared to non-bingers (Finlayson et al. 2009; Finlayson et al. 2012). A dissociation of liking and particularly implicit wanting is believed to be an important contributor to overeating (Finlayson et al. 2012)

In our adaptation of the LFPQ, pictures of foods that would have been difficult for BAND patients to swallow were substituted for pictures similar in calorie content and visual appeal. To further ensure equipoise, subjects were shown all the food pictures prior to completing the task and if they reported never eating a particular food, a substitute was shown instead. There were two occasions where a picture of bread was substituted for crackers on account of the fact that BAND subjects were unable to eat bread due to its consistency.

As this task was added later on in the study only 13 RYGB and 12 BAND patients of the 83 screened participants completed the LFPQ. Data are presented in the following format: scores for liking, wanting, choice and implicit wanting of each category foods (HFSW, HFSA, LFSW, LFSA). Additionally, a "fat bias" "liking", "wanting" and "implicit wanting" score was calculated using Mean (HFSA, HFSW) – Mean (LFSA, LFSW).

2.4 Study visit participants

For Study 1 (Chapter 3), 61 (21 RYGB, 20 BAND patients and 20 BMI-M controls) of the screened subjects attended a study visit, which included fMRI scanning, metabolic and hormonal phenotyping and a test meal (Fasted-Saline) (Study protocol: Fig. 2.1).

Demographics of Study 1 participants are contained in Table 3.1. Due to excess motion or poor image quality, the scans of 2 patients (Subject 1: RYGB, 44years, female, BMI 27.9kg/m^2, Subject 2: BMI-M, 41years, female, BMI 42.6kg/m^2) from Study 1 were excluded from functional imaging analysis.

For Study 2 (Chapter 4), 19 of the subjects from Study 1 (10 RYGB, 9 BAND) attended for 2 further study visits identical to the first visit except that they received a standardized milkshake breakfast (1 sachet Complan® plus 200ml whole milk giving 385kCal) at t = 0 mins, 60 mins prior to scanning. They were randomized to receive, 15 min before breakfast (t = -15 mins), either a subcutaneous injection of saline (Fed-Saline) or Octreotide (100 mcg) (Fed-Octreotide) plus weight adjusted dosage of short-acting insulin to correct for somatostatin analogue-induced suppression of insulin which would otherwise lead to hyperglycaemia (see Section 2.7). Of these, 2 RYGB subjects did not undergo scanning, but completed the rest of the protocol, and a further 3 subjects' visits had to be excluded from analysis due to insufficient sleep the night before scanning, feeling unwell on the study visit day and excessive head motion (Subject 1: RYGB, 33years, female, BMI 23.19kg/m^2, Subject 2: RYGB, 59years, female, BMI 23.69kg/m^2, Subject 3: BAND, 59years, female, BMI 37.39kg/m^2). Demographics of Study 2 participants are contained in Table 4.2.

For Study 3 (Chapter 5), structural T1-weighted and diffusion weighted scans of 19 RYGB, 19 BAND and 20 BMI-M were used to calculate voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) data of 17 RYGB, 12 BAND and 17 BMI-M subjects acquired during Study 1 and 2 were analyzed. Due to poor image quality, scans from 2 patients from VBM analysis and 1 patient from DTI analysis were excluded (Subject 1: RYGB, 58years, female, BMI 31.89kg/m^2, Subject 2: RYGB, 38years, female, BMI 33.79kg/m^2). An additional patient

was excluded from VBM analysis due to a history of premature birth (<32 weeks gestation)(BAND, 57years, female, BMI 33.99kg/m^2). Demographics of Study 3 participants are contained in Table 5.1 and 5.7.

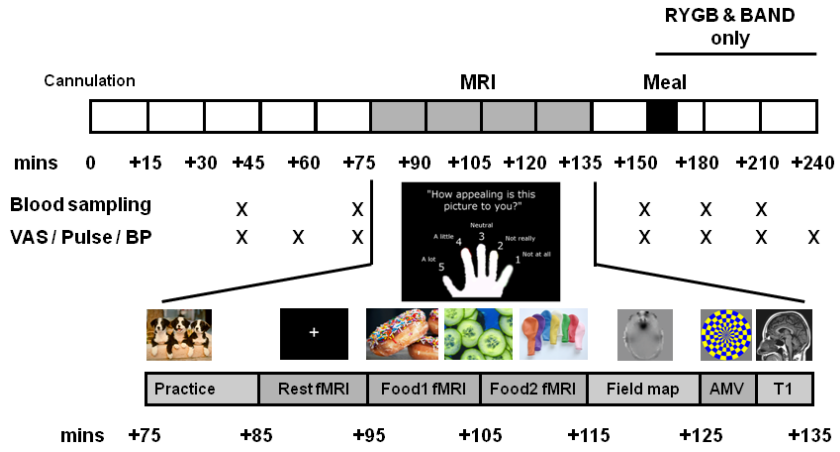
2.5 Scanning visit protocol

On the day before each scanning visit, subjects were instructed to avoid exercise and alcohol intake, to eat their usual supper at 8.00pm, and then attend the Sir John McMichael Centre Clinical Investigation Unit in the morning having eaten or drunk nothing since supper the evening before other than water. Subjects had measurements of height, weight, % body fat by bio-electrical impedance analysis (Bodystat 1500, Isle of Man, UK), and completed the Positive and Negative Affect Schedule (PANAS) to measure mood over the preceding week. Visual analogue scales were used to measure appetite ratings, lunch palatability and other confounding symptoms (see Appendix 10).

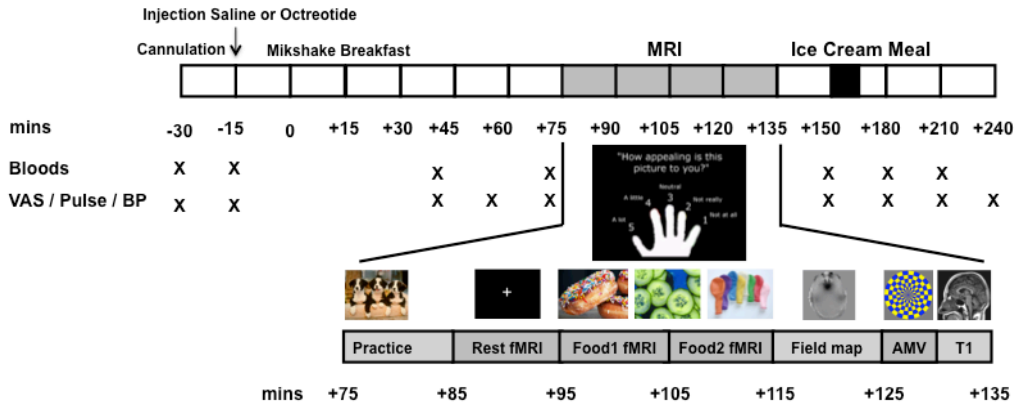
The visit protocol is illustrated in Fig.2.1A and B. Area under the curve (AUC) for VAS ratings were calculated from +40 to +150 min to cover the period over the MRI scan in all three groups; and post-prandial changes in VAS ratings were calculated as delta AUC from baseline at +150 to +240 mins in the two surgical groups.

Figure 2.2 Study protocol

A



B



Abbreviations: AMV: audio-motor-visual task, BAND: gastric banding, BP: blood pressure, fMRI: functional magnetic resonance imaging, RYGB: gastric bypass, VAS: visual analogue scales

2.6 fMRI protocol

Patients were asked to refrain from strenuous exercise and alcohol the day before and day of the study visits. Patients were scanned for 1 hour starting between 11am and noon (Goldstone et al. 2009). Female participants were scanned in first half phase of menstrual

cycle (apart from one BMI-matched control subject (age 43years, BMI 29.7 9kg/m^2) in Study 1 who was scanned on day 16 of her cycle) to avoid variations in reward responses including food over the menstrual cycle (Frank et al. 2010). Pregnancy was excluded at each visit using a urinary hCG test.

2.7 Octreotide injection

Octreotide acetate (Sandostatin, Novartis Pharmaceuticals) is a somatostatin analogue and therefore mimics its action. Somatostatin suppresses the release of a number of gastrointestinal hormones including GLP-1, PYY, gastrin, cholecystokinin (CCK), secretin, motilin, vasoactive intestinal peptide (VIP), gastric inhibitory polypeptide (GIP) and enteroglucagon. It also has various other effects within the gastro-intestinal system including decreasing the rate of gastric emptying, reducing smooth muscle contractions and blood flow within the intestine, suppression of the release of pancreatic hormones, including insulin, and inhibiting the release of glucagon. Octreotide is a more potent inhibitor of growth hormone, glucagon and insulin than naturally occurring somatostatin, and has a longer half-life (90 minutes). It is poorly absorbed from the gut and so is usually administered subcutaneously. Octreotide is safely used to treat disorders associated with high levels of gut hormones such as tumours of the pancreas and intestine (Modlin et al. 2010). Octreotide is an ideal compound to use to lower gut hormones after obesity surgery because its properties, dosage and side effect profile are well understood, and its effects only last a few hours.

In Study 2, 15 min before the milkshake breakfast (t = -15 mins), 100mcg of Octreotide was administered subcutaneously in a randomized double-blinded manner, together with

Actrapid (short-acting insulin, Novo Nordisk, 0.075-0.10 units/kg) to prevent hyperglycaemia, or placebo saline injections (which were also given at Fasted-Saline visits in Study 1 to all groups). The Octreotide/insulin injections were only given to the post-operative RYGB and BAND groups.

2.8 Milkshake breakfast

In Study 2, subjects received a standardized milkshake breakfast (1 sachet Complan® plus 200ml whole milk) containing 385 kCal, 60 mins prior to scanning at t = 0 mins. They were asked to consume the whole amount.

2.9 fMRI confounding variables

At each scanning visit, BMI, % body fat, time since last meal, sleep duration the night before the visit (Sterpenich et al. 2007), or positive or negative affect (PANAS) (Killgore et al. 2006) were recorded. Head motion during the food evaluation or auditory-motor-visual fMRI tasks was also recorded to measure factors that could independently affect BOLD signal in order to determine if these were equal between groups.

2.10 fMRI acquisition

Whole-brain fMRI data were acquired on a 3T Philips Achieva MRI scanner (Robert Steiner MRI Unit, Hammersmith Hospital, London, UK) with T2* weighted gradient-echo echoplanar imaging with an automated higher-order shim procedure: 44 ascending contiguous 3.25 mm thick slices, 2 x 2 mm voxels; SENSE factor 2 repetition time (TR) 3000 ms; echo time (TE) 30

ms; 90º flip angle; FOV 190x219, matrix 112x112, slice acquisition angle -30º from AC-PC line to reduce frontal lobe signal drop out (Deichmann et al. 2003).

Field maps were used to correct for geometric distortions caused by inhomogeneities in the magnetic field as follows: TR 29 ms; TE 3.6ms, 30º flip angle; FOV 190 x 219, 44 ascending contiguous 3.25mm thick slices, 2 x 2 mm voxels, ðTE 0 and 2.5.

High-resolution T1-weighted turbo field echo structural scans were also collected (TE 4.6 ms; TR 9.7 ms; flip angle 8°; FOV 240 mm; voxel dimensions, 0.94 x 0.94 x 1.2 mm), along with DTI brain scans (32 directions, b factor 1000, TR 13951 ms, TE 59 ms, 73 slices, SENSE 2.5, FOV 224 x 224, voxel size 1.75 x 1.75 x 2mm, slice acquisition parallel to the AC - PC line).

2.11 Food picture evaluation fMRI paradigm

During the fMRI food picture paradigm, four types of color photographs were presented in a block design split across two 9 minute, 192 volume runs: (1) 60 high-calorie foods (e.g. pizza, cakes and chocolate), (2) 60 low-calorie foods (e.g. salads, vegetables, fish), (3) 60 non-food related household objects (e.g. furniture, clothing) and (4) 180 Gaussian blurred images of the other pictures (as a low-level baseline), similar to those used previously (Goldstone et al. 2009). Food images were selected to represent familiar foods that are typical to the modern Western diet. Pictures were obtained from freely available websites and the International Affective Picture System (IAPS, NIMH Center for the Study of Emotion and Attention, University of Florida, Gainesville, FL, USA). Food and object pictures were of similar luminosity and resolution.

Each run contained different pictures in 5 blocks each of high-calorie and low-calorie foods and objects interleaved with 31 blocks of blurred pictures (6 pictures per 18 secs) using one of four pseudorandom block orders with a randomized picture order within each block. Every image was displayed for 2500 ms, followed by a 500 ms inter-stimulus interval of a fixation cross. Each high-calorie food block consisted of equal numbers of foods containing chocolate, non-chocolate sweet and savory non-sweet foods (2 of each).

The total caloric load, caloric density and macronutrient composition of the food pictures used in the fMRI task were assessed using Dietplan6 (Foresfield Software Ltd, West Sussex, UK) - high-calorie foods: 834 ± 100 kCal, 321 ± 13 kCal/100g, 42 ± 2 % fat, 48 ± 1 % carbohydrate, 10 ± 1 % protein; low-calorie foods: 157 ± 18 kCal, 64 ± 5 kCal/100g, 35 ± 3 % fat, 35 ± 3 % carbohydrate, 29 ± 3 % protein; high-calorie vs. low-calorie foods: $P<0.001$ for energy content, density, % protein and % carbohydrate; and $P=0.03$ for % fat.

Images were viewed via a mirror mounted above an 8 channel RF head coil which displayed images from a projector using the IFIS image presentation system (In Vivo, Wurzburg, Germany) and ePrime 2 software (Psychology Software Tools Inc., Pittsburgh, PA, USA). Whilst each image was on display to subjects in the scanner, they were asked to immediately and simultaneously rate how 'appealing' each picture was to them using a 5 button hand-held keypad (1=not at all, 2=not really, 3=neutral, 4=a little, 5=a lot) (Goldstone et al. 2009). The appeal rating was thus made and recorded simultaneously with the stimulus presentation used for fMRI activation.

2.12 Auditory-motor-visual control fMRI paradigm

A 6 min, 114 volume auditory-motor-visual (AMV) control task was performed. Over nine 33 second blocks, subjects performed two of each of the following tasks simultaneously: (i) listening to a story, (ii) tapping their right index finger once every second, or (iii) watching a 4Hz color (yellow/blue) flashing checkerboard, with each task performed in 6 blocks, and instructions about whether to start or stop the motor task displayed for 3 seconds prior to each block.

2.13 FMRI analysis

FMRI data processing used the FMRI Expert Analysis Tool v5.98 (www.fmrib.ox.ac.uk/fsl).

The first 6 scans were discarded to allow for the BOLD signal to stabilize. The following preprocessing was applied: motion correction using MCFLIRT; field map-based EPI unwarping using PRELUDE+FUGUE non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 6.0mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=100.0s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction including picture onsets, temporal derivative and motion parameters as covariates. Two subjects (1 gastric bypass, 1 BMI-matched control) were excluded from fMRI analysis as their average relative motion over the food evaluation or control AMV fMRI tasks was greater than 0.5 mm/TR.

Registration to high resolution T1 structural and/or standard space images was carried out using FLIRT. Registration from high resolution structural to standard space was then further refined using FNIRT non-linear registration.

GLM analysis was used to measure BOLD signal activation to (i) any food (high-calorie or low-calorie), (ii) only high-calorie or (iii) only low-calorie foods (compared to objects) in the food evaluation task, and for (iv) auditory, motor or visual tasks in the control paradigm.

In study 1, whole brain mixed effects analysis compared BOLD signal between surgical groups and then between each surgical group and the BMI-matched controls, using unpaired t-tests with cluster threshold $Z>2.1$, corrected $P<0.05$ including age, gender and BMI as covariates.

For the food pictures, higher level analysis was carried out using a fixed effect model to combine the two runs, by forcing the random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann et al. 2003; Woolrich et al. 2004) to determine activation for the following contrasts: food > objects (high-calorie or low-calorie food), high-calorie food only > objects and low-calorie food only > objects

2.14 MRI regions of interest

Functional regions of interest (fROIs) for the following areas: bilateral OFC, amygdala, nucleus accumbens, anterior insula and caudate nucleus were determined from a separate cohort of 24 overweight/obese subjects who underwent an identical protocol fasting overnight. Characteristics of these subjects are contained in Table 2.1.

Table 2.1 Characteristics of separate cohort of overweight/obese subjects used to create functional regions of interest in brain activation analysis.

n	24
Age (years)	29.0 [26.0 - 38.5] (20.0 - 48.0)
Gender (Male : Female)	6:18
Ethnicity: European Caucasians, n (%)	14 (58%)
Current BMI (kg/m^2)	30.7 [26.3 - 32.8] (25.4 - 42.7)
Current body fat (%)	36.3 ± 2.0 (17.1 - 54.5)
Current DM, n (%)	0 (0%)
Current obesity co-morbidity score	0.0 [0.0 - 0.0] (0.0 - 8.0)
Duration fasting (hours)	15.9 [15.4 - 16.8] (13.7 - 19.7)

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range). Abbreviations: BMI: body mass index, DM: type 2 diabetes mellitus.

Higher level whole brain analysis was carried out with mixed effects analysis to identify those voxels which were significantly more activated at the group level, with voxel-based correction for multiple comparisons made using false discovery rate (FDR) at $P<0.05$ for the food>objects contrast (high-calorie or low-calorie food minus objects) (Fig. 2.3, Table 2.2). Similar functional localizers were made from this separate cohort for the control auditory,

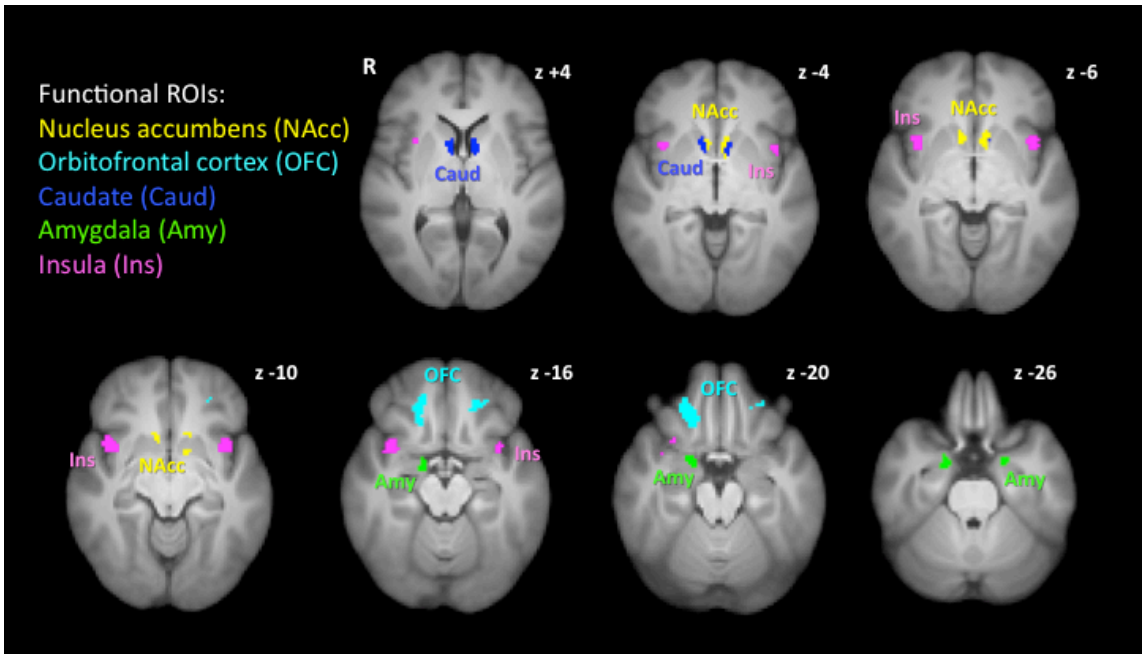
motor and visual tasks for bilateral superior posterior temporal gyrus (auditory), left pre-central gyrus (motor), bilateral lingual gyrus (visual) (Figure 2.3, Table 2.2).

Table 2.2 Spatial coordinates of functional regions of interest in brain activation analysis.

Functional region of interest	**Hemisphere**	**Number of voxels**	**Z statistic**	**x**	**y**	**z**
Food vs. Object contrast						
Anterior orbitofrontal cortex	Right	170	3.81	18	36	-18
	Left	63	3.60	-20	38	-14
Amygdala	Right	110	3.85	18	0	-26
	Left	16	3.99	-18	0	-26
Nucleus Accumbens	Right	62	3.45	8	14	-4
	Left	91	4.11	-6	10	-2
Anterior Insula	Right	188	5.08	40	8	-14
	Left	116	4.43	-38	8	-12
Caudate	Right	129	3.88	8	6	2
	Left	74	4.18	-6	-6	0
Auditory task						
Posterior division of superior temporal gyrus	Right	1109	5.56	64	-14	4
	Left	1108	5.39	-62	-22	2
Motor task						
Precentral gyrus	Left	873	5.78	-36	-24	56
Visual task						
Lingual gyrus	Bilateral	1412	5.59	6	-90	-10

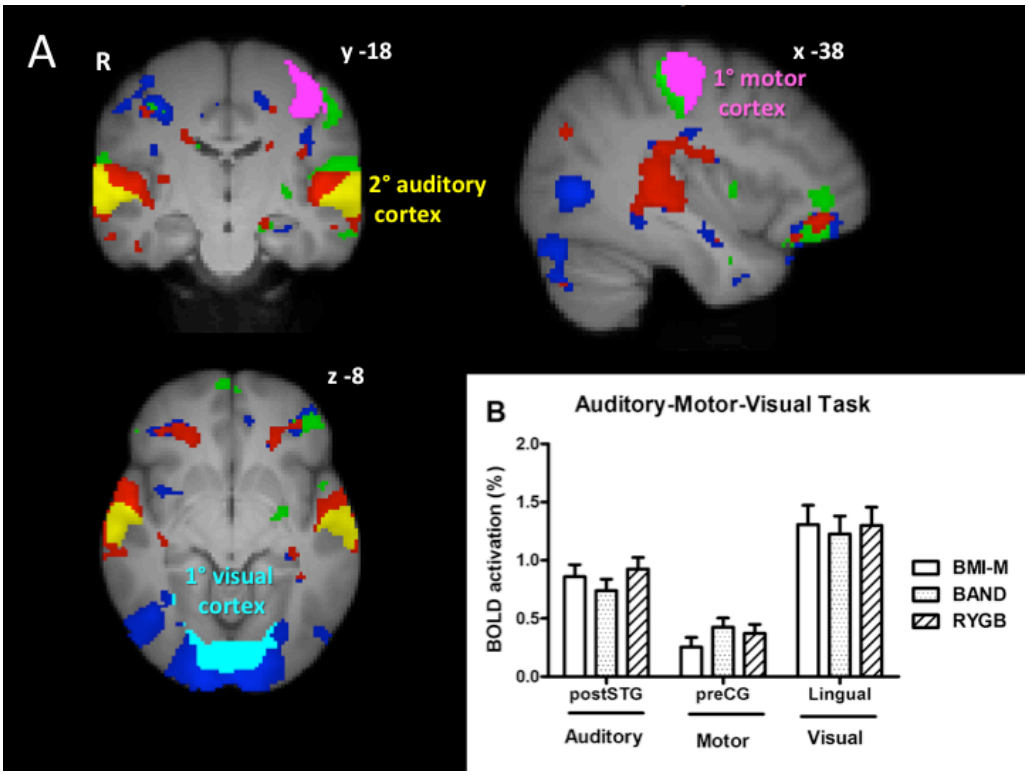
Stereotactic coordinates (x, y, z) for peak voxel of group activation, adjusting for age, gender and BMI, thresholded at FDR P<0.05 (n=23), given in standard MNI space.

Figure 2.3 *A priori* functional regions of interest for reward system activation during food evaluation task



Group activation in separate cohort of obese/overweight patients for any food (high-calorie or low-calorie) vs. object picture contrast. Activation is thresholded at voxel-corrected FDR P<0.05, overlaid onto the average T1 scan for all subjects (n=24). *A priori* functional regions of interest (ROIs) are indicated: nucleus accumbens (nucleus accumbens, yellow), orbitofrontal cortex (OFC, light blue), caudate (Caud, dark blue), amygdala (Amy, green), anterior insula (Ins, magenta). Co-ordinates are given in standard MNI space.

Figure 2.4 *A priori* functional regions of interest for auditory, motor and visual cortex activation during control task



(A) Group activation maps of separate cohort of overweight/obese subjects overlaid with *a priori* anatomical regions of interest for control auditory-motor-visual task: auditory (red: listening to story) with bilateral posterior division of superior temporal gyrus (overlaid in yellow), motor task (green: button press) with left pre-central gyrus (overlaid in magenta), and visual (dark blue: flashing checkerboard) with lingual gyrus (overlaid in light blue). Activation is thresholded at voxel-corrected FDR $P<0.05$, overlaid onto the average T1 scan for all subjects (n=24). Co-ordinates are given in standard MNI space.

(B) Comparison of BOLD signal for auditory, motor and visual control task in *a priori* functional regions of interest between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery, adjusting for age, gender and BMI. Data are presented as mean ± SEM. n=19-20 per group.

The functional anatomically constrained ROIs were obtained by masking these group activation maps with the a priori anatomical ROI. These were defined by the relevant bilateral ROIs from the cortical and subcortical structural Harvard FSL atlases thresholded at 10% probability. The OFC fROI included regions in the OFC and frontal pole with $y > 22$ and $z < -6$, since analysis of functional activation in this region demonstrated distinct bilateral clusters overlapping the anatomical Harvard atlas regions (Figure S2). The insula mask was subdivided into the anterior insula ($y > 4$) (Chang et al. 2012).

The average (median) magnitude of bilateral BOLD activation within each a priori fROI was then extracted for each individual subject separately for any food, high-calorie food and low-calorie food (> object) contrasts using featquery in FSL, to measure the differences in activation between groups for the different picture categories, or different control auditory-motor-visual tasks. Average BOLD activation for each of these contrasts within each ROI was then compared between groups outside FSL, adjusting for age, gender and BMI.

In Study 1 and 2, activation in these *a priori* fROIs was compared between the three groups for the food evaluation task, as well as the average BOLD activation in all 5 fROIs representing brain food reward systems (De Silva et al. 2011).

Similar time-series statistical analysis was performed for the single run AMV paradigm including the onsets of each task (auditory, motor and visual), with temporal derivative and motion parameters as covariates, to contrast activation during performance of each task with that when the other tasks were being performed. fROIs for the control paradigm were

bilateral superior posterior temporal gyrus (auditory), left pre-central gyrus (motor), and bilateral lingual gyrus (visual) (Fig. 2.3).

2.15 VBM analysis

Structural changes in grey matter volume were analysed using FSL-VBM v3.1.8 to perform voxel-based morphometry (Good et al. 2001). Inter-subject spatial normalisation was achieved through brain extraction of each T1 image using BET (Smith, 2002a), which segmented the brain from non-brain tissue. The next step involved tissue-type segmentation using FAST4 (Zhang et al. 2001) in order to separate grey matter, white matter and cerebrospinal fluid to enable individual analyses. The next process required the construction of a template on which all images would be non-linearly registered. Using the FLIRT (Jenkinson et al. 2001; Jenkinson et al. 2002) and FNIRT tools (Anderson et al. 2007; Anderson et al. 2007) the template was created from the subject list using equal numbers of RYGB, BAND and BMI-M participants (n=19 in each group to avoid bias in template construction towards a group) to produce a custom template. The final stage of preparing the T1 images involved non-linear re-registration onto the template, which was then modulated in order to correct for regional increases and decreases through dividing by the Jacobian of the warp field, after which the adjusted images were then smoothed using an isotropic Gaussian kernel set at sigma 4mm (full width at half maximum) which was chosen for its greater accuracy and higher t-values.

Then a voxel-wise general linear model (GLM) was employed using permutation-based non-parametric testing (randomise) which corrected for multiple comparisons using threshold free cluster enhancement (TFCE) at corrected $P<0.05$ (Nichols et al. 2002). The whole brain

VBM structural data were analysed using a GLM which included age, gender and BMI as covariates in order to determine the potential differences in grey matter density between the three groups (RYGB, BAND, BMI-M). Subsequent VBM anatomical ROIs were selected based on the examination of previous research including nucleus accumbens, amygdala, caudate nucleus, hippocampus, insula, pallidum and the putamen, as well as the precentral gyrus as a control region.

Multiple regression analysis using a GLM analysis with age and gender as covariates, examined the effect of BMI*group, BMI and group in each ROI.

2.1.5.1 Sub-cortical segmentation volumetrics (Smith et al. 2002)

In order to determine subcortical volumes of the amygdala, nucleus accumbens, caudate nucleus, hippocampus, pallidum, putamen and thalamus the FSL tool FIRST was used on the T1 images (Patenaude et al. 2011). FIRST is a model-based segmentation/registration tool. 3D T1 images were transformed into standard space (MNI152) and a subcortical mask was used with a threshold set at 10% applied to identify the subcortical regions of interest. The shape/appearance models used in FIRST were constructed from manually segmented images provided by the Center for Morphometric Analysis (CMA), MGH, Boston. Based on learned models, using voxel boundary correction to ensure correct identification of each subcortical region, FIRST searched through linear combinations of shape modes of variation for the most probable shape instance given the observed intensities in the T1 image. Volumetric analysis was then carried out by determining the label number of the structure of interest and using fslstats to measure the volume. SPSS was then used to analyse the volume measurement for each area, correcting for age, gender, BMI and intracranial volume (ICV).

In order to calculate ICV, brain tissue volume, (un-normalised or normalized for subject head size), was calculated using the FSL tool SIENAX (Smith et al. 2001; Smith et al. 2002). SIENAX starts by extracting brain and skull images from the single whole-head 3D T1 image input data (Smith et al. 2002)[Smith 2002b]. The brain image is then affine-registered to MNI152 space (Jenkinson et al. 2001; Jenkinson et al. 2002)(using the skull image to determine the registration scaling); this is primarily in order to obtain the volumetric scaling factor, to be used as a normalisation for head size. Next, tissue-type segmentation with partial volume estimation was carried out in order to calculate total volume of brain tissue (including separate estimates of volumes of grey matter, white matter, peripheral grey matter and ventricular CSF) (Zhang et al. 2001)[Zhang 2001]. Intracranial volume was then calculated by adding white matter, grey matter and ventricular CSF. The un-normalised ICV was then used as a covariate in statistical analyses.

2.16 DTI analysis

Voxelwise statistical analysis of the FA and MD data was carried out using Tract-Based Spatial Statistics (TBSS) (Smith et al. 2006), part of FSL. First, FA and MD images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using Brain Extraction technique (BET) (Smith 2002). All subjects' FA and MD data were then aligned into a common space using the nonlinear registration tool FNIRT (Anderson et al. 2007; Anderson et al. 2007), which uses a b-spline representation of the registration warp field. Next, the group mean FA image was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group. Each subject's aligned FA and MD data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics.

A voxel wise general linear model analyses evaluated the between group differences of measured FA and MD across the whole FA skeleton, including age, gender and BMI as covariates. Three methods of permutation-based non-parametric testing (randomise), were employed to detect differences between the groups: threshold free cluster enhancement (TFCE) at corrected P<0.05, voxel-based thresholding at FDR corrected P<0.05, and cluster-based thresholding (cluster size >1.5mm) FDR corrected P<0.05 to calculate the *t*-statistics maps between the three groups (RYGB, BAND and BMI-M) (Nichols et al. 2002).

Subsequent white matter tract regions of interest (ROI) were selected based on the examination of previous research (Hellyer et al. 2012) and included anterior thalamic tract, cingulate cingulum, cingulate hippocampus, corticospinal tract, inferior fronto-occipital tract, inferior longitudinal fasciculus, superior longitudinal fasciculus, superior longitudinal frontal tract, uncinate fasciculus, body, genu and splenium of corpus callosum, forceps major and forceps minor. The ROIs were created by masking the mean FA skeleton with individual tracts from the JHU white matter tract atlas thresholded at 10%.

Multiple regression analysis using a GLM stepwise regression with age and gender as covariates, examined the effect of BMI*group, the effect of BMI and the effect of group in each ROI.

2.17 Appetite and food palatability

Visual analogue scale (VAS) ratings (0-10 cm) of appetite and other symptoms were recorded at serial time points to measure hunger, pleasantness to eat, volume of food

wanting to eat, fullness, sickness, sleepiness, anxiety and stress (Flint et al. 2000; Blundell et al. 2010). The questions asked (and anchors) were as follows: “How hungry/full do you feel right now?” (Not at all, Extremely); “How pleasant would it be to eat right now?” (Not at all, Extremely); “How much do you think you could eat right now?” (Nothing, A large amount); “How sick/sleepy/anxious/stressed do you feel right now?” (Not at all, Extremely) (Appendix 10).

Scanning was followed by an *ad libitum* Hagen Daz™ vanilla or pralines and cream flavored ice cream meal, , given to subjects in the operated groups in 50ml (43g) portions every 5 minutes, starting at t = +150 mins as per previous experiments (le Roux et al. 2007). Ice cream was chosen as both BAND and RYGB subjects were able to consume without difficulty Subjects were asked to eat until comfortably full. Upon completion, they were asked to rate by VAS the sweetness, tastiness (“How tasty was the meal?”) and pleasantness (“How pleasant to eat was the meal?”) of the ice cream test meal.

2.18 Dietary habits

Caloric intake and diet macronutrient composition was assessed using 3-day self-reported dietary records in the two surgical groups and analyzed using Dietplan® (Foresfield Software Ltd., West Sussex, UK) (Appendix 11).

2.19 Metabolic, hormone and bile acid assays

Serial blood samples before and after scanning were collected for measurement of plasma glucose, insulin, gut hormones (PYY, GLP-1 and acyl ghrelin) and bile acids (Fig.2.2).

Blood samples for gut hormone analysis were collected into chilled lithium heparin polypropylene tubes, containing 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (A8456 Sigma-Aldrich) and aprotinin (Nordic Phama UK) protease inhibitor to give final concentration of 1 mg/mL and 200 kIU/mL whole blood respectively. Blood samples were centrifuged at 4ºC, 4000 rpm for 10 min. Aliquots of separated plasma were immediately mixed with HCl (final concentration of 0.05M) for subsequent assay of acyl ghrelin, and separate unacidified aliquots for assay of other gut hormones (GLP-1 and PYY). All plasma samples were stored at -80°C until assay. Other metabolic and hormonal assays were done on plain serum or fluoride oxalate plasma samples sent immediately to the routine clinical laboratory.

Plasma glucose and serum insulin were measured in the Department of Clinical Biochemistry, Imperial College Healthcare NHS Trust using either an Abbott Architect ci8200 analyzer (Abbott Diagnostics, Maidenhead, UK) or an Axsym analyzer (Abbott Diagnostics, Maidenhead, UK). Intra-assay coefficients of variation of all measurements were 1.0–5.0%. Plasma GLP-1 (GLP-1 $_{1\text{-}36}$ amide, GLP-1 $_{7\text{-}36}$ amide and GLP-1 $_{9\text{-}36}$ amide) and PYY (total PYY $_{1\text{-}36}$ and PYY $_{3\text{-}36}$) were assayed using established in-house radio-immunoassays (Allen et al. 1984; Kreymann et al. 1987). Plasma acyl ghrelin was measured by a two-site sandwich ELISA in a single run (Liu et al. 2008). Intra-assay coefficients of variation (CV) for gut hormones were <10%.

Extraction of bile acids (BA) from plasma was performed as described previously (Tagliacozzi et al. 2003). BA fractions were analysed using high-performance liquid chromatography

(Jasco, Essex, UK) tandem mass spectrometry (Applied Biosystems, Cheshire, UK). The method was linear between 0.1 and 10 μmol/L for all BAs and their conjugates with CV of 1.5-6.8% at the lower limit of quantitation (0.1 μmol/L). The inter-assay CV was 3.6-8.0%.

Area under the curve (AUC) for metabolites and hormones were calculated from +40 to +150 mins, and for bile acids from +70 to +150 mins, to cover the period before and during the MRI scan in all three groups. In the two surgical groups post-prandial changes in metabolites, hormones and bile acids were calculated as delta AUC from baseline at +150 to +210 mins per kCal ice cream eaten at lunch.

2.20 Dumping symptoms

The presence of symptoms of possible 'dumping syndrome' was assessed using change in nausea and sleepiness from before lunch to 1.5 hours after lunch (ΔAUC +150 to +240 mins), and change in physiological markers indicative of dumping syndrome, pulse and blood pressure, from before lunch to one hour after lunch (difference +150 to +210 min) (Ukleja 2005) (Appendix 12).

In addition patients retrospectively completed two validated questionnaires to assess post-prandial symptoms of dumping (e.g. fainting, breathlessness, sleepiness, palpitations, headaches and nausea) in the 3 months following surgery (Sigstad 1970; Arts et al. 2009).

2.21 Statistical analysis

Results are presented as mean ± SEM or median [interquartile range] for data that was not normally distributed. Comparisons of averages between groups used unpaired t-tests or one way ANOVA with *post-hoc* Fisher's LSD test or, if not normally disturbed, Mann Whitney U test or Friedman ANOVA on Ranks with *post-hoc* Dunn's test. Comparison of prevalence between groups used chi-squared test. Comparisons between groups for fMRI activation and eating behavior and psychological questionnaires were adjusted for age, gender and BMI. To further investigate the link between brain responses to food cues, food hedonics and potential mediators, correlations between BOLD activation (adjusted for age, gender and BMI) and ice-cream palatability or gut hormones/bile acids/dumping syndrome scores were performed to determine Pearson, or if not normally distributed Spearman, correlation coefficients.

Comparison of ROI VBM and FIRST volumetric results between groups were analysed adjusting for age, gender, BMI and un-normalised ICV. Multiple linear regression was used to analyse the potential relationship between grey matter density corrected for age, gender and ICV and the effect of group*BMI, and if this was non-significant, group and BMI.

Comparison of ROI FA and MD results between groups were analysed adjusting for age, gender and BMI. Significance was taken as $P<0.05$. Statistical analysis was performed using IBM SPSS statistics programme v19.0.0 and Prism v5.01.

CHAPTER 3: OBESE PATIENTS AFTER GASTRIC BYPASS SURGERY HAVE LOWER BRAIN HEDONIC RESPONSES TO FOOD THAN AFTER GASTRIC BANDING

3.1 Introduction

Bariatric surgery is currently the most effective long-term treatment for obesity and its associated co-morbidities (Sjostrom et al. 2012). Over 20 years, Roux-en-Y gastric bypass (RYGB) surgery achieves on average 25% weight loss, compared with 14% with gastric banding (BAND) surgery, whereas traditional weight loss measures such as behavioural therapy, dieting and exercise, achieve at best weight stability (Sjostrom et al. 2012). The specific anatomical manipulations of the gut in each procedure have very different physiological effects and these may result in differing gut-brain hedonic responses and hence eating behaviour. This, in turn, may provide an explanation for the greater weight loss seen in RYGB (Stefater et al. 2012).

In RYGB, the formation of a small gastric pouch, enables food to have earlier contact with the mid and distal small bowel. Food bypasses the stomach and proximal small bowel, but undiluted bile has contact with the proximal small bowel. Vagal fibers across the stomach may be disrupted (Dixon et al. 2012). Although gastric volume is restricted in RYGB and BAND surgery, gastric restriction alone is not thought to explain weight loss achieved in RYGB. On the other hand, reduced hunger and increased satiety after RYGB are increasingly attributed, at least in part, to early and exaggerated post-prandial responses of anorexigenic intestinal hormones, such as peptide YY (PYY) and glucagon-like polypeptide-1 (GLP-1) (le Roux et al. 2006; Bryant et al. 2012). These hormones form part of the gut-brain axis regulating ingestive behaviour and act on both brainstem-hypothalamic (Parkinson et al. 2009) as well as reward systems (Batterham et al. 2007; De Silva et al. 2011) in the brain. These gut hormone changes are absent after BAND surgery, where the adjustable band around the proximal stomach reduces hunger through increased intraluminal pressure on vagal afferent mechanoreceptor (Burton et al. 2010). Factors such as malabsorption of

macronutrients, changes in gastric emptying, altered vagal tone, altered plasma levels of the stomach-derived orexigenic hormone ghrelin and the fat-derived anorexigenic hormone leptin have all been investigated as potential mediators of the additional weight loss seen in RYGB, especially when compared with BAND surgery, but thus far have not shown the same promising results as the exaggerated PYY and GLP-1 response as a potential mediator of RYGB successful weight loss, and as a future target for weight loss treatments (Halmi et al. 1981; Furnes et al. 2009). The role of bile acids, dumping syndrome and gut microbiota also offer promise as mechanisms for weight loss following RYGB and are worthy of further investigation.

Human eating behaviour is affected by hunger, but also by the reward value of food (Shin et al. 2011). An advantageous shift away from consumption of high-fat and sweet food after RYGB surgery has been reported in animal and human studies (le Roux et al. 2011; Shin et al. 2011; Miras et al. 2012). However differences in food hedonics between RYGB and BAND, the two most commonly performed procedures around the world, and their underlying neural basis, have not been explored.

Functional MRI allows study of brain reward-cognitive systems related to eating behaviour by measuring regional changes in the blood oxygen level dependent (BOLD) signal to food stimuli, a marker of neuronal activation (Carnell et al. 2012). These include dopaminergic and opioid corticolimbic networks with several specific structures implicated in the processing of food reward. The striatal nucleus accumbens and caudate nucleus are thought to encode reward conditioning, expectancy, motivation as well as habitual behaviour (Schultz 2001; Vanderschuren et al. 2005; Lowe et al. 2009). The amygdala processes emotional responses to rewarding stimuli (Murray 2007), and the anterior insula integrates

sensory information about rewarding stimuli, including taste, forms part of the gustatory cortex and acts as an interface between feeling, cognition and behaviour (Chang et al. 2012). The orbitofrontal cortex (OFC) is an important area involved in encoding reward value and salience, and decision making (Small et al. 2007; Small 2009; Stice et al. 2009).

3.2 Aims

1. To compare BOLD activation in brain reward systems whilst undertaking a food evaluation task between BMI- matched patients after RYGB and BAND, with BMI-matched unoperated controls that had not lost weight, using fMRI.

2. To compare food appeal, preference and palatability, as well as eating behaviour measures and actual food intake between BMI- matched patients after RYGB and BAND using questionnaires, visual analogue scales, test meals and food diaries.

3. In order to confirm known potential mediators for differences between the two surgical groups in food hedonics in this cohort, measurement of fasting and post-prandial plasma gut hormones, glucose and bile acids, as well as retrospective early post-operative and current dumping symptoms.

3.3 Hypothesis

1. Obese patients after RYGB have healthier brain reward responses to food compared to after BAND procedures, and hence healthier eating behaviour, which may explain the greater weight loss seen after RYGB.

2. These differences in food hedonics are not explicable by differences in hunger levels or psychological traits.

3. Plasma GLP-1, PYY, bile acids and post-ingestive dumping symptoms will be higher after RYGB than BAND, indicating potential mediators for the observed differences in food hedonics between the groups.

3.4 Results

3.4.1 Participant characteristics

There were no significant differences between the three groups [BMI-matched unoperated (BMI-M), gastric bypass (RYGB), gastric banding (BAND)] in age, gender ratio, ethnic background distribution, current BMI, percentage body fat, prevalence of type 2 diabetes mellitus (T2DM) or binge eating disorder (BED), for both the scanned participants only (Table 3.1) and the whole cohort (Appendix 13). The two surgical groups had similar pre-operative BMI and prevalence of BED. The RYGB group had more obesity-associated co-morbidities pre-operatively, including T2DM, and a higher obesity co-morbidity compared to the BAND group but were not different in post-operative obesity co-morbidity scores or prevalence of T2DM. RYGB patients had also lost significantly more weight than the BAND patients, although their BMI at the time of scanning was similar to BAND patients. In the whole cohort the time from surgery to study attendance was longer in RYGB than BAND patients, but in the scanned cohort, there was no difference between surgical groups.

Table 3.1. Characteristics of scanned subjects

	BMI-M	BAND	RYGB	P values [a]
n	20	20	21	
Age (years)	39.1 ± 2.3 (20.0 - 55.0)	40.9 ± 2.5 (22.0 - 59.0)	43.5 ± 2.0 (23.0 - 59.0)	0.38
Gender (Male : Female)	3:17	1:19	2:19	0.57
Ethnicity: European Caucasians, n (%)	10 (50%)	15 (75%)	16 (76%)	0.14
Pre-operative BMI (kg/m^2)	n/a	44.8 [41.9 - 49.2] (36.5 - 57.0)	48.4 [40.7 - 58.0] (34.7 - 74.6)	0.23
Current BMI (kg/m^2)	35.4 ± 1.9 (24.7 - 55.6)	35.1 ± 1.4 (25.3 - 49.2)	35.3 ± 1.7 (22.6 - 52.4)	0.99
Current Height (m)	1.64 ± 0.02 (1.49 - 1.78)	1.66 ± 0.02 (1.53 - 1.79)	1.66 ± 0.02 (1.52 - 1.85)	0.64
Current Weight (kg)	97.0 ± 3.1 (73.9 - 119.8)	97.0 ± 3.1 (73.9 - 119.8)	98.1 ± 4.9 (63.7 - 137.9)	0.97
Current body fat (%)	42.1 ± 2.2 (26.0 - 58.2)	41.9 ± 1.8 (23.3 - 54.7)	41.3 ± 1.9 (28.4 - 56.0)	0.96
Weight loss (% of pre-operative weight)	n/a	23.1 [14.5 - 29.3] (9.7 - 52.4)	29.9 [23.4 - 36.5] (16.3 - 40.4)	0.018 RYGB > BAND
Time since surgery (months)	n/a	9.1 [5.2 - 19.2] (3.6 - 64.6)	8.1 [5.9 - 11.5] (2.6 - 26.2)	0.25
Pre-operative DM, n (%)	n/a	2 (10%)	10 (48%)	0.02 RYGB > BAND
Current DM, n (%)	2 (10%)	0 (0%)	3 (14%)	0.23
Pre-operative obesity co-morbidity score	n/a	6.0 [4.5 - 6.0] (1.0 - 10.0)	10.0 [6.6 - 11.5] (3.0 - 19.0)	<0.001 RYGB > BAND
Current obesity co-morbidity score	0.0 [0.0 - 5.0] (0.0 - 18.0)	0.0 [0.5 - 2.0] (0.0 - 9.0)	1.0 [0.8 - 3.0] (0.0 - 10.0)	0.85
Pre-operative BED, n (%)	n/a	4 (25%)	4 (19%)	1.00
Current BED, n (%)	2 (10%)	2 (10%)	1 (5%)	0.78

Data included only for those participants who had fMRI scanning. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).

[a] P value for overall comparison between groups.

Abbreviations: BAND: gastric banding, BED: binge eating disorder, BMI: body mass index, BMI-M: BMI-matched, DM: type 2 diabetes mellitus, n/a not applicable, RYGB: gastric bypass.

3.4.2 Psychological Trait Questionnaires

There were no differences between the three groups in any psychological questionnaire measures (Table 3.3). Specifically there was no difference in depression or mood as measured by the Beck Depression Inventory (BDI-II) and Positive and Negative Affect Scale (PANAS). There was no difference in impulsivity as measured by Barratt's Impulsivity Scale between the three groups, nor in reward sensitivity (reward responsiveness, reward drive, fun-seeking, behavioural inhibition) as measured by the Behavioural activation and inhibition scale (BIS/BAS). There was also no difference between the groups in personality traits of extraversion, psychoticism, neuroticism and lying as measured by Eysenck's Personality Inventory (EPQ-R) (Table 3.2).

Table 3.2 Psychological questionnaires

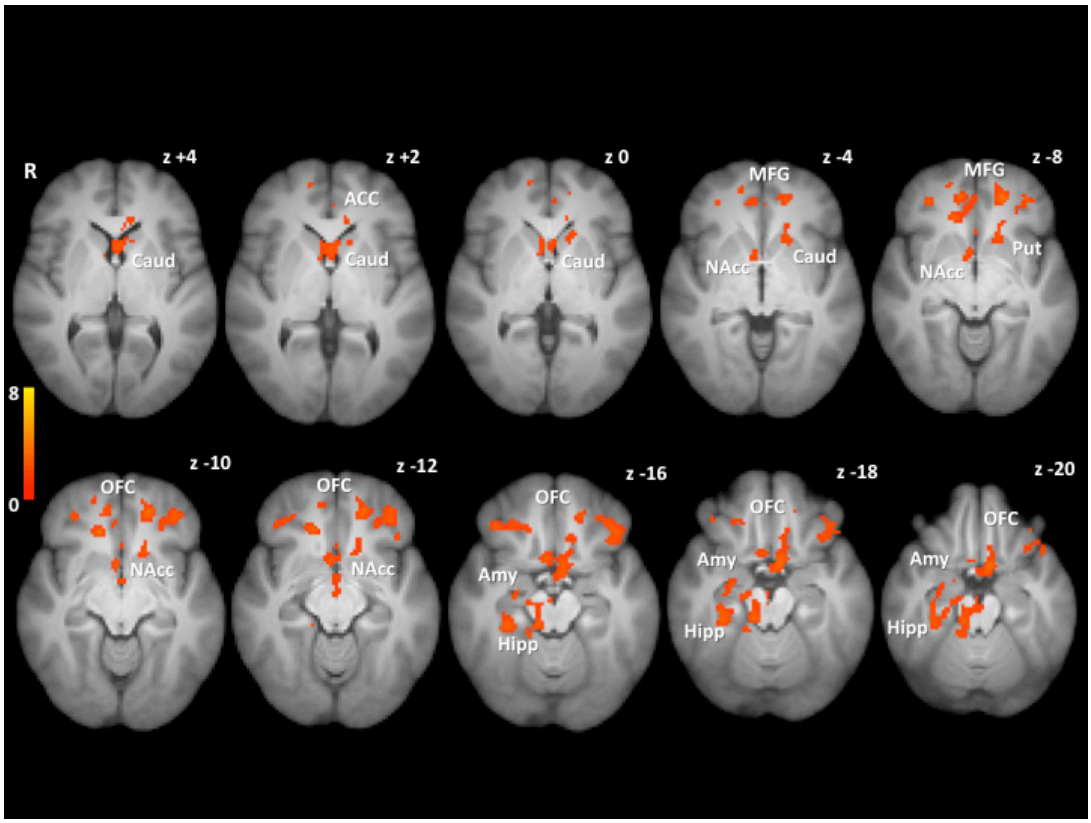
	BMI-M	BAND	RYGB	P[a]
n	25	28	30	
Beck Depression Inventory II (score/63)	8.0 [2.0 - 14.0] (1.0 - 44.0)	6.0 [3.0 -14.5] (1.0 - 38.0)	4.5 [2.0 - 11.0] (0.0 - 32.0)	0.99
Moderate-severe depression (>15), n (%)	5 (20%)	7 (25%)	7 (23%)	0.22
On antidepressants treatment, n (%)	3 (12%)	5 (18%)	8 (27%)	0.38
PANAS				
Negative affect (score /50)	18.0 [12.5 - 24.3] (10.0 - 43.0)	15.0 [13.0 - 20.5] (9.0 - 33.0)	15.0 [12.0 - 18.0] (10.0 - 35.0)	0.67
Positive affect (score /50)	32.3 ± 1.7 (18.0 - 49.0)	30.6 ± 2.0 (15.0 - 49.0)	32.8 ± 1.7 (12.0 - 47.0)	0.63
Behavioural activation and inhibition scale				
BAS drive (score /16)	11.0 [9.0 - 13.0] (7.0 -15.0)	10.0 [8.5 - 11.5] (5.0 - 15.0)	10.0 [7.0 - 12.0] (4.0 - 16.0)	0.35
BAS reward responsiveness (score /20)	18.0 [15.8 - 19.0] (9.0 -20.0)	17.0 [15.0 - 19.5] (8.0 - 20.0)	17.0 [14.0 - 19.0] (11.0 - 20.0)	1.00
BAS fun-seeking (score /16)	12.1 ± 0.4 (8.0 - 16.0)	11.6 ± 0.4 (7.0 - 16.0)	11.0 ± 0.5 (5.0 - 16.0)	0.32
BIS (score /28)	21 [17.8 -24.0] (11.0 - 28.0)	21.5 [19.0 - 22.5] (11.0 - 28.0)	20.0 [18.0 - 21.0] (12.0 -28.0)	0.87
Impulsivity				
Barratt impulsivity scale (score /120)	60.5 ± 2.4 (30.0 -77.0)	66.6 ± 2.6 (45.0 - 99.0)	63.2 ± 2.4 (25.0 - 93.0)	0.20
EPQ-R				
Extraversion (score /23)	14.9 ± 0.9 (2.0 - 22.0)	14.2 ± 1.0 (5.0 - 23.0)	13.7 ± 1.0 (4.0 - 23.0)	0.49
Psychoticism (score /32)	6.4 ± 0.6 (0.0 - 13.0)	6.6 ± 0.5 (2.0 - 13.0)	5.4 ± 0.6 (1..0 - 13.0)	0.35
Neuroticism (score /24)	12.9 ± 0.9 (6.0 - 23.0)	11.9 ± 1.3 (1.0 - 24.0)	12.6 ± 1.0 (2.0 - 24.0)	0.73
Lying (score /21)	8.7 ± 1.0 (1.0 - 17.0)	9.6 ± 0.7 (3.0 - 17.0)	9.8 ± 0.8 (0.0 - 18.0)	0.83

Data included for the whole cohort. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range), adjusted for age gender and BMI. [a] P value for overall comparison of averages or prevalence between groups.
Note that similar results were obtained when limiting the analysis to the scanned subjects only (data not shown). Abbreviations: BAND: gastric banding, BAS/BIS: Behavioural Activation and Inhibition Scale, BMI-M: body mass index matched, EPQ-R: Eysenck Personality Questionnaire, PANAS: Positive and Negative Affect Schedule, RYGB: gastric bypass.

3.4.3 Brain activation to food pictures

In whole brain analysis, there was significantly lower BOLD activation in the RYGB compared with the BAND group when viewing *high-calorie* foods in clusters within the OFC, subcallosal cortex, putamen, caudate, nucleus accumbens, hippocampus, cingulate and paracingulate gyri (Fig.3.1, Table 3.3). BOLD activation when viewing *low-calorie* foods was also lower in the OFC and subcallosal cortex in RYGB than BAND (Table 3.4). By contrast, there were no clusters with *greater* BOLD activation in the RYGB compared to the BAND group when viewing high-calorie or low-calorie foods.

Figure 3.1 Whole brain comparison of activation to high-calorie foods between obese patients after gastric bypass and gastric banding.



Whole brain group level comparison for high-calorie vs. object picture contrast to demonstrate clusters in which BOLD signal was lower in patients after gastric bypass (RYGB) compared with gastric banding (BAND) surgery, adjusting for age, gender and body mass index. No clusters showed greater activation in RYGB than BAND groups. Color bar indicates Z values. Cluster activation thresholded at Z>2.1, cluster-corrected FWE P<0.05, overlaid onto the average T1 scan for all subjects (n=20 per group). Co-ordinates given in standard MNI space. Abbreviations: ACC; anterior cingulate cortex, Amy: amygdala, Caud: caudate, NAcc: nucleus accumbens, Hipp: hippocampus, MFG: middle frontal gyrus, OFC: orbitofrontal cortex, Put: putamen.

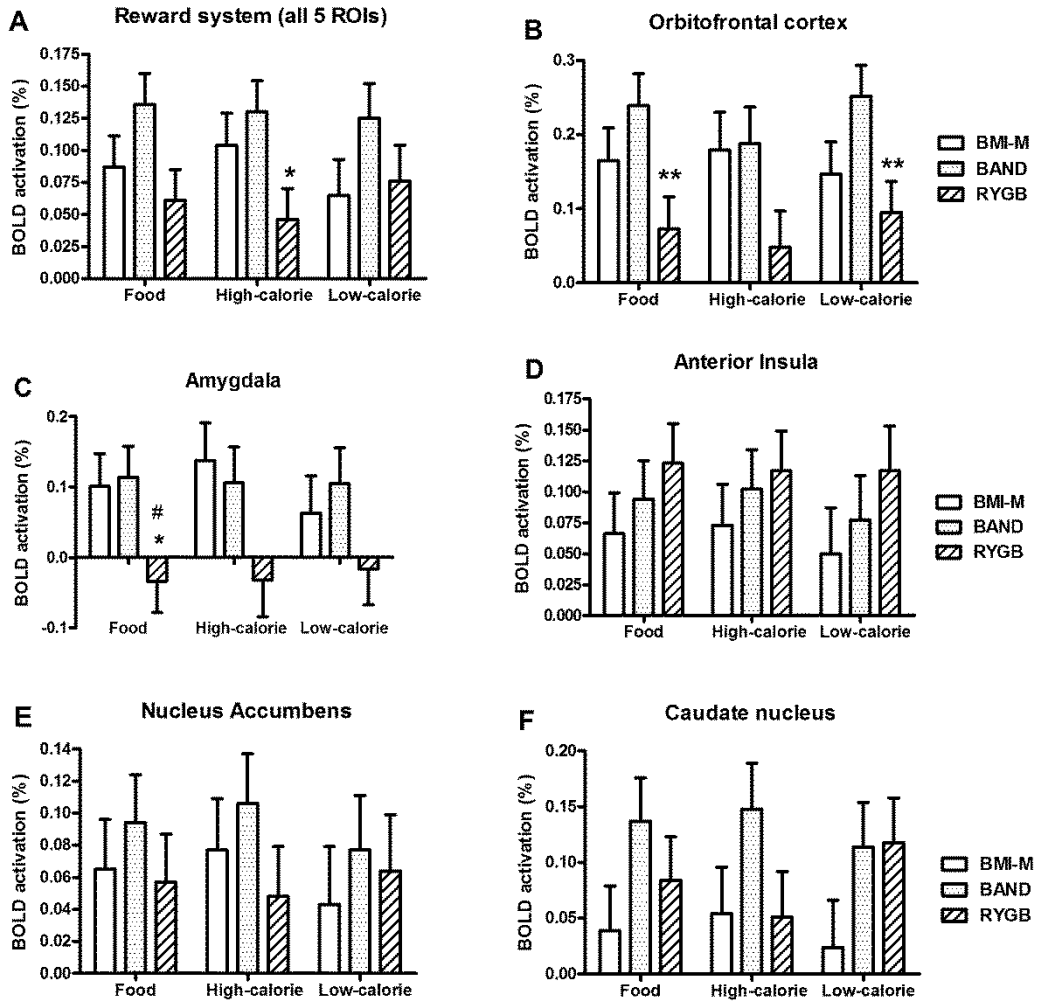
In the fROI analysis (Fig 3.2 A, Table 3.4), BOLD activation within the whole reward system (average activation in the OFC, amygdala, anterior insula, nucleus accumbens and caudate) was *lower* in the RYGB compared with the BAND when viewing high-calorie, but not low-calorie, foods.

When examining individual fROIs, BOLD activation in the OFC was lower in the RYGB compared with the BAND and/or control groups when viewing any food or low-calorie foods, with a trend for high-calorie food (Fig. 3.2 B, C, Table 3.4). BOLD activation in the amygdala was also significantly lower in the RYGB compared with the BAND and control groups for any food, with a trend for high-calorie, but not low-calorie, foods (Fig. 3.2 C, Table 3.4).

There were no differences in BOLD activation of the other fROIs in the food evaluation task, although there was a tendency in each fROI for a similar pattern i.e. BOLD signal change was generally lower in response to any food/high-calorie food in those that had undergone RYGB compared to BAND and/or BMI-matched unoperated controls (Fig. 3.2 D-F, Table 3.4).

There was no difference in BOLD activation in auditory, motor or visual cortices for the control fMRI task between the three groups in either the whole brain or fROI analysis (Fig. 3.2 B, Table 3.4).

Figure 3.2. Region of interest activation to food in obese patients after gastric bypass and gastric banding and unoperated controls.



Comparison of BOLD signal to any food, only high-calorie or only low-calorie food (vs. objects) in *a priori* functional regions of interest (fROI) between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery, adjusting for age, gender and BMI. (A) Average in all five fROIs, (B) orbitofrontal cortex (OFC), (C) amygdala, (D) anterior insula, (E) nucleus accumbens, (F) caudate. Data are presented as mean ± SEM. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.005 vs. BMI-M; *P<0.05, **P<0.01, ***P<0.005 vs. BAND; n=19-20 per group.

Table 3.3 Spatial coordinates of whole brain comparison of activation to food between surgical groups.

Contrast	Number of voxels	Z statistic	x	y	z	Brain region
GASTRIC BANDING > GASTRIC BYPASS						
Any food (high-calorie or low-calorie)	Cluster 1 - 1470	4.12	16	30	-12	Right orbitofrontal cortex
		3.69	-18	44	-8	Left orbitofrontal cortex
		3.61	-6	8	-20	Left orbitofrontal cortex
		3.45	-16	40	-14	Left orbitofrontal cortex
		3.42	16	16	-18	Right orbitofrontal cortex
		3.20	0	22	-8	Right orbitofrontal cortex
		3.18	4	10	-14	Right subcallosal cortex
		2.93	38	34	-16	Right orbitofrontal cortex / subcallosal cortex
		2.89	-8	18	-20	Left orbitofrontal cortex / subcallosal cortex
		2.83	-16	18	-8	Left putamen / caudate / nucleus accumbens
High-calorie food	Cluster 1 - 980	4.05	-38	18	-30	Left temporal cortex
		3.55	-18	44	-10	Left orbitofrontal cortex
		3.51	16	30	-10	Right orbitofrontal cortex
		3.21	-42	26	-14	Left orbitofrontal cortex
		3.17	40	34	-14	Right orbitofrontal cortex
		3.12	-36	38	-12	Right orbitofrontal cortex
		3.04	32	42	-8	Right orbitofrontal cortex / frontal pole
		3.03	-42	30	-16	Left orbitofrontal cortex / frontal pole
		3.00	10	46	-8	Right cingulate/paracingulate gyrus
		2.92	-34	44	-8	Left frontal pole
	Cluster 2 - 1232	3.54	-6	6	-18	Left subcallosal cortex
		3.28	10	-32	-18	Right brainstem
		3.22	4	10	-14	Right subcallosal cortex

		3.21	32	-32	-18	Right hippocampus
		3.05	10	-22	-24	Right brainstem
		3.04	2	-22	-22	Right brainstem
		2.89	-16	18	-8	Left putamen / caudate / nucleus accumbens
		2.88	12	-40	-22	Left brainstem
Low-calorie food	Cluster 1 - 1041	3.95	14	30	-12	Right orbitofrontal cortex
		3.46	-16	40	-14	Left orbitofrontal cortex
		3.43	4	22	-8	Right subcallosal cortex
		3.32	-4	8	-18	Left subcallosal cortex
		3.25	16	16	-18	Left orbitofrontal cortex
		3.20	-16	46	-6	Left orbitofrontal cortex
		3.17	12	8	-18	Right orbitofrontal cortex / subcallosal cortex
		3.02	-6	18	-18	Left subcallosal cortex
		3.01	-18	42	-20	Left orbitofrontal cortex / frontal pole
		2.94	-8	12	-22	Left orbitofrontal cortex / subcallosal cortex
GASTRIC BYPASS > GASTRIC BANDING						
Any food (high-calorie or low-calorie)						Nil significant
High-calorie food						Nil significant
Low-calorie food						Nil significant

Stereotactic coordinates (x, y, z) for peak voxel of group activation for food category vs. objects, adjusted for age, gender and BMI, cluster thresholded at $Z>2.1$, $P<0.05$ (n=20 per group), given in standard MNI space.

Table 3.4 Region of interest activation during food evaluation and auditory-motor-visual control task

Region of interest	Contrast	BMI–M	BAND	RYGB	P value [a]
n		19	20	20	
FOOD EVALUATION TASK					
Reward system (all 5 ROIs)	**Food**	0.082 ± 0.029 (-0.127 to 0.335)	0.138 ± 0.020 (0.005 to 0.340)	0.064 ± 0.021 (-0.101 to 0.225)	0.08 BAND > RYGB 0.03
	High-calorie	0.100 ± 0.027 (-0.152 to 0.294)	0.131 ± 0.022 (-0.012 to 0.372)	0.049 ± 0.023 (-0.176 to 0.235)	0.05 BAND > RYGB 0.02
	Low-calorie	0.060 ± 0.033 (-0.150 to 0.348)	0.128 ± 0.026 (-0.042 to 0.472)	0.078 ± 0.022 (-0.060 to 0.253)	0.28
Orbitofrontal cortex	**Food**	0.177 ± 0.050 (-0.064 to 0.878)	0.235 ± 0.040 (-0.121 to 0.543)	0.066 ± 0.040 (-0.459 to 0.306)	0.029 BAND > RYGB 0.008
	High-calorie	0.191 ± 0.060 (-0.099 to 0.853)	0.182 ± 0.044 (-0.285 to 0.474)	0.043 ± 0.045 (-0.357 to 0.478)	0.09
	Low-calorie	0.160 ± 0.046 (-0.076 to 0.793)	0.250 ± 0.038 (-0.04 to 0.646)	0.085 ± 0.042 (-0.498 to 0.372)	0.03 BAND > RYGB 0.01
Amygdala	**Food**	0.086 ± 0.051 (-0.172 to 0.592)	0.121± 0.035 (-0.187 to 0.543)	-0.027 ± 0.047 (-0.694 to 0.243)	0.04 BAND > RYGB 0.02 BMI-M > RYGB 0.04
	High-calorie	0.124 ± 0.056 (-0.187 to 0.787)	0.110 ± 0.046 (-0.345 to 0.527)	-0.023 ± 0.055 (-0.690 to 0.298)	0.059
	Low-calorie	0.049 ± 0.056 (-0.263 to 0.624)	0.114 ± 0.039 (-0.087 to 0.589)	-0.011 ± 0.056 (-0.633 to 0.425)	0.24
Nucleus accumbens	**Food**	0.061 ± 0.035 (-0.21 to 0.356)	0.097 ± 0.024 (-0.058 to 0.259)	0.060 ± 0.030 (-0.182 t0 0..333)	0.67
	High-calorie	0.075 ± 0.034 (-0.295 to 0.376)	0.107 ± 0.026 (-0.063 to 0.367)	0.048 ± 0.032 (-0.281 to 0.297)	0.43

Region of interest	Contrast	BMI–M	BAND	RYGB	P value [a]
	Low-calorie	0.038 ± 0.038 (-0.28 to 0.298)	0.080 ± 0.033 (-0.209 to 0.428)	0.065 ± 0.031 (-0.217 to 0.454)	0.79
Anterior Insula	**Food**	0.0534 ± 0.025 (-0.212 to 0.256)	0.095 ± 0.034 (-0.094 to 0.496)	0.134 ± 0.037 (-0.218 to 0.532)	0.47
	High-calorie	0.062 ± 0.032 (-0.237 to 0.254)	0.102 ± 0.028 (-0.132 to 0.336)	0.127 ± 0.037 (-0.240 to 0.468)	0.64
	Low-calorie	0.038 [-0.058 to 0.107] (-0.148 to 0.310)	0.051 [-0.034 to 0.106] (-0.181 to 0.678)	0.129 [0.040 to 0.182] (-0.192 to 0.545)	0.43
Caudate	**Food**	0.031 ± 0.051 (-0.371 to 0.638)	0.141 ± 0.033 (-0.059 to 0.605)	0.087 ± 0.032 (-0.100 to 0.411)	0.23
	High-calorie	0.040 [-0.045 to 0.177] (-0.403 to 0.595)	0.013 [0.081 to 0.197] (-0.094 to 0.733)	0.038 [-0.057 to 0.150] (-0.189 to 0.415)	0.15
	Low-calorie	0.025 [-0.120 to 0.117] (-0.375 to 0.639)	0.075 [0.019 to 0.166] (-0.117 to 0.488)	0.010 [0.017 to 0.170] (-0.075 to 0.432)	0.15
CONTROL TASK					
Combined (all 3 ROIs)		0.816 ± 0.089 (0.221 - 1.815)	0.856 ± 0.077 (0.323 - 1.605)	0.798 ± 0.068 (0.415 - 1.331)	0.85
Posterior division superior temporal gyrus	**Auditory**	0.853 ± 0.134 (0.168 to 2.172)	0.942 ± 0.117 (0.065 to 2.098)	0.728 ± 0.074 (0.288 to 1.443)	0.41
Left precentral gyrus	**Motor**	0.276 ± 0.104 (-0.807 to 0.846)	0.415 ± 0.077 (-0.076 to 0.973)	0.360 ± 0.057 (-0.049 to 0.727)	0.33
Lingual gyrus	**Visual**	1.320 ± 0.169 (0.156 to 2.906)	1.212 ± 0.152 (0.152 to 2.739)	1.304 ± 0.146 (0.357 to 2.581)	0.92

Average group activation in separate and combined *a priori* regions of interest (ROI) for food category vs. objects during food evaluation task, or auditory, motor or visual cortex during control task, adjusted for age, gender and BMI. Data presented as mean ± SEM and (range).

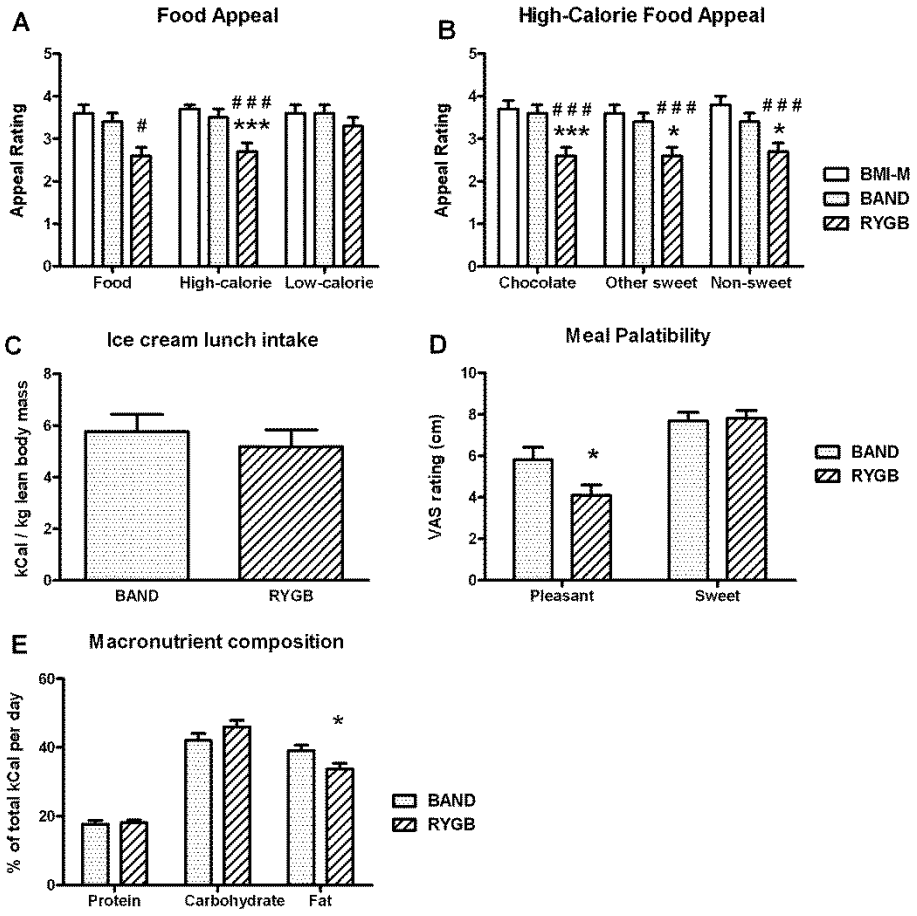
[a] P value for overall comparison of averages between groups using ANOVA, with post-hoc comparison given beneath.

Abbreviations: BAND: gastric banding, BMI-M: body mass index matched, RYGB: gastric bypass

3.4.4 Food appeal scores

During scanning, *high-calorie* food pictures, including each of the sub-categories of chocolate, non-chocolate sweet and non-sweet, were rated as significantly less appealing by RYGB than BAND and control participants (Fig. 3.3 A, B). By contrast, no differences in appeal rating for *low-calorie* food, object or blurred pictures were observed between the groups.

Figure 3.3 Food hedonics and dietary composition in obese patients after gastric bypass and gastric banding.



Comparison of (A) appeal of any food, only high-calorie or only low-calorie food pictures; (B) appeal of sub-categories of high-calorie food pictures; (C) ice-cream consumption and (D) ice-cream palatability rating at meal after fMRI scan; and (E) average percentage of total calories from fat from 3 day food diary, between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery. Data are presented as mean ± SEM. $^{\#}$P<0.05, $^{\#\#\#}$P<0.005 vs. BMI-M; *P<0.05, ***P<0.005 vs. BAND; n=20-21 per group.

3.4.5 Appetite visual analogue scales

Over the scanning period both the RYGB and BAND groups rated their 'hunger', 'pleasantness to eat' and 'volume of food they could eat' as lower than the control group, but there was no significant difference between the two surgical groups (Fig 3.4 A, E, G). RYGB patients were also less nauseated than BAND patients during scanning, but absolute nausea ratings were still low (Fig. 3.4 C).

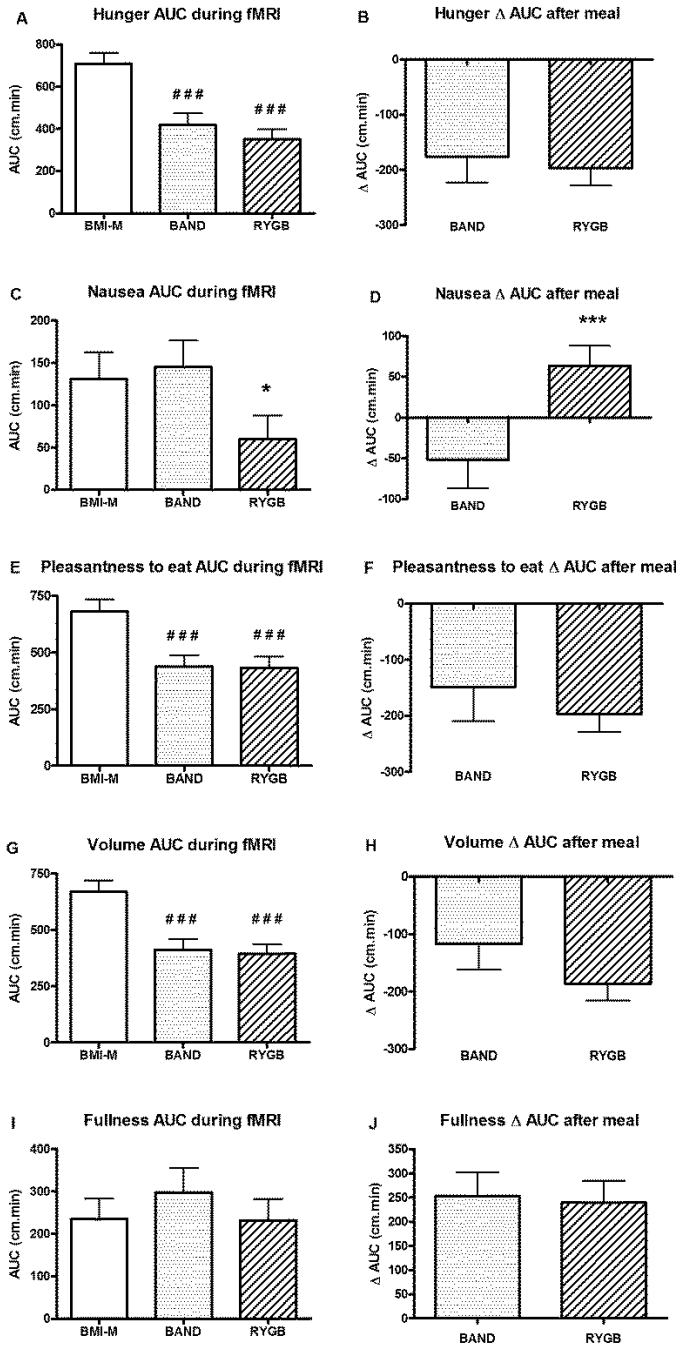
After scanning, RYGB and BAND patients consumed similar amounts of ice-cream, but RYGB patients rated it as less "pleasant to eat" than BAND patients (Fig. 3.3 C, D Table 3.5). The two surgical groups had similar decreases in hunger and increases in fullness after the meal (Fig. 3.4 B, J). RYGB patients were more nauseated after eating than BAND patients,

Table 3.5 Ice-cream meal intake and palatability in surgical groups

	BAND	**RYGB**	**P value**
n	20	21	
Lunch intake			
Total (kCal)	317.8 ± 35.8 (34.0 - 563.0)	285.5 ± 37.0 (34.0 - 604.0)	0.54
Corrected (kCal / kg LBM)	5.8 ± 0.7 (0.6 - 10.4)	5.2 ± 0.7 (0.4 - 11.8)	0.54
VAS lunch palatability (cm)			
Tastiness	6.1 ± 0.6 (0.3 - 9.5)	4.5 ± 0.6 (0.6 - 9.6)	0.07
Pleasantness to eat	5.8 ± 0.6 (0.3 - 9.6)	4.1 ± 0.5 (0.5 - 9.6)	0.047 BAND > RYGB
Sweetness	7.7 ± 0.4 (4.4 - 10.0)	7.8 ± 0.4 (3.0 - 9.7)	0.96

Data presented as mean ± SEM (range).
Abbreviations: BAND: gastric banding, BMI-M: body mass index matched, LBM: lean body mass, RYGB: gastric bypass, VAS: visual analogue scale.

Figure 3.4 Appetite visual analogue scales during fMRI and after meal



Comparison of visual analogue scale (VAS) ratings of (A, B) hunger, (C, D) nausea, (E, F) pleasantness to eat, (G, H) volume of food that could be eaten, and (I, J) fullness.

(A,C,E,G,I) levels during fMRI scanning (area under curve (AUC) +40 to +150 mins) between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery.

(B, D, F, H, J) change in levels after ice cream meal (ΔAUC +150 to +210 mins) in surgical groups.

Data are presented as mean ± SEM. $^{###}$P<0.005 vs. BMI-M; *P<0.05, ***P<0.005 vs. BAND; n=20-21 per group.

3.4.6 Dietary records

RYGB patients consumed significantly less calories than BAND patients correcting for lean body mass (Table 3.6). Percentage of energy intake derived from fat was significantly lower in the RYGB than BAND group (Fig. 3.3 E, Table 3.6).

Table 3.6 Dietary records in surgical groups.

	BAND	RYGB	P-value [a]
n	18	15	
Total energy intake			
Average daily intake (kCal/day)	1682 ± 133 (878-2620)	1191 ± 117 (587-2088)	0.01 BAND > RYGB
Average daily intake (kCal/day per kg LBM)	31.1 ± 2.9 (15.1-56.9)	21.7 ± 2.3 (8.5-38.3)	0.02 BAND > RYGB
Average daily intake (% REE)	99.6 ± 8.0 (50-160.2)	69.85 ± 7.1 (28.7-128.9)	0.01 BAND > RYGB
Macronutrient composition			
Protein (% total kCal intake)	17.7 ± 1.1 (9.0-27.0)	18.1 ± 0.8 (14.0-26.0)	0.75
Carbohydrate (% total kCal intake)	42.1 ± 2.0 (26.0-63.0)	46.0 ± 1.9 (31.0-61.0)	0.17
Fat (% total kCal intake)	39.1 ± 1.6 (28.0-54.0)	33.8 ± 1.6 (22.0-42.0)	0.03 BAND > RYGB

Data presented as mean ± SEM (range).
Abbreviations: BAND: gastric banding, BMI-M: body mass index matched, LBM: lean body mass, REE: resting energy expenditure calculated using Cunningham equation, RYGB: gastric bypass, VAS: visual analogue scale.

3.4.7 Leeds food preference questionnaire

In the operated subjects there was a similar pattern for the RYGB to have lower 'liking' and 'wanting' scores for all four food categories (high fat sweet, high fat savoury, low fat sweet and low fat savoury) (Table 3.7), although this only reached statistical significance in the high fat and low fat savoury categories. There were no significant or trends for significant differences between the surgical groups in implicit wanting (measured in reaction times to the 'wanting' rating), or in the food choice paradigm (Table 3.7).

Table 3.7 Leeds food preference scores of operated subjects

	RYGB	BAND	[a]P value
n	14	15	
Age (years)	41.9 ± 2.5 (23.0-58.0)	42.6 ± 2.7 (22.0-57.0)	0.84
Body mass index (kg/m^2)	35.7 [31.5-37.8] (27.0-49.2)	36.7 [34.1-42.2] (28.9-77.9)	0.72
WANTING High fat sweet	25.2 ± 5.1 (2.1-54.5)	39.1 ± 5.2 (11.1-73.4)	0.07
High fat savoury	26.1 [18.0-53.2] (6.4-78.5)	55.3 [26.9-60.8] (18.8-81.5)	0.04
Low fat sweet	32.1 [28.3-51.0] (8.1-67.3)	47.6 [36.0-57.8] (23.4-74.9)	0.19
Low fat savoury	38.9 ± 5.2 (12.4-82.4)	55.5 ± 3.6 (36.6-90.5)	0.01
LIKING High fat sweet	27.1 ± 5.5 (1.6-67.4)	39.5 ± 5.2 (11.4-74.3)	0.11
High fat savoury	36.1 ± 5.6 (6.6-77.3)	53.1 ± 4.5 (27.5-80.3)	0.03
Low fat sweet	41.3 ± 4.4 (10.5-67.0)	48.3 ± 4.0 (22.5-76.3)	0.25
Low fat savoury	40.5 ± 4.9 (9.8-81.6)	57.0 ± 4.0 (35.4-89.6)	0.01
IMPLICIT WANTING High fat sweet	-19.4 ± 7.6 (-53.5-31.7)	-12.1 ± 6.6 (-50.8-47.4)	0.47
High fat savoury	11.2 ± 6.9 (-44.2-46.0)	10.4 ± 6.0 (-30.4-46.3)	0.94
Low fat sweet	3.4 ± 6.8 (-31.3-52.9)	-8.2 ± 5.4 (-37.3-29.4)	0.19
Low fat savoury	4.9 ± 6.6 (-36.4-41.0)	9.8 ± 5.1 (-30.7-37.4)	0.56
CHOICE High fat sweet	17.4 ± 3.0 (1.0-38.0)	19.7 ± 2.6 (6.0-42.5)	0.56
High fat savoury	27.4 ± 2.5 (7.0-39.0)	27.8 ± 2.3 (12.5-39.5)	0.91
Low fat sweet	25.2 ± 2.6 (11.0-44.5)	21.0 ± 2.2 (10.5-37.0)	0.23
Low fat savoury	26.0 ± 2.5 (9.0-40.0)	27.5 ± 2.1 (10.5-39.0)	0.66
FAT BIAS Wanting	-9.6 ± 4.4 (-36.0-19.8)	-6.8 ± 4.2 (-26.6-26.5)	0.65
Liking	-9.3 ± 4.4 (-37.6-16.7)	-6.4 ± 4.1 (-32.8-26.4)	0.63
Implicit wanting	-8.3 ± 7.0 (-48.8-42.8)	-1.7 ± 8.0 (-44.9-53.4)	0.54
Choice	-3.2 ± 2.9 (-21.0-18.0)	-0.5 ± 3.3 (-18.5-20.5)	0.54

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).

3.4.8 Eating behaviour assessment

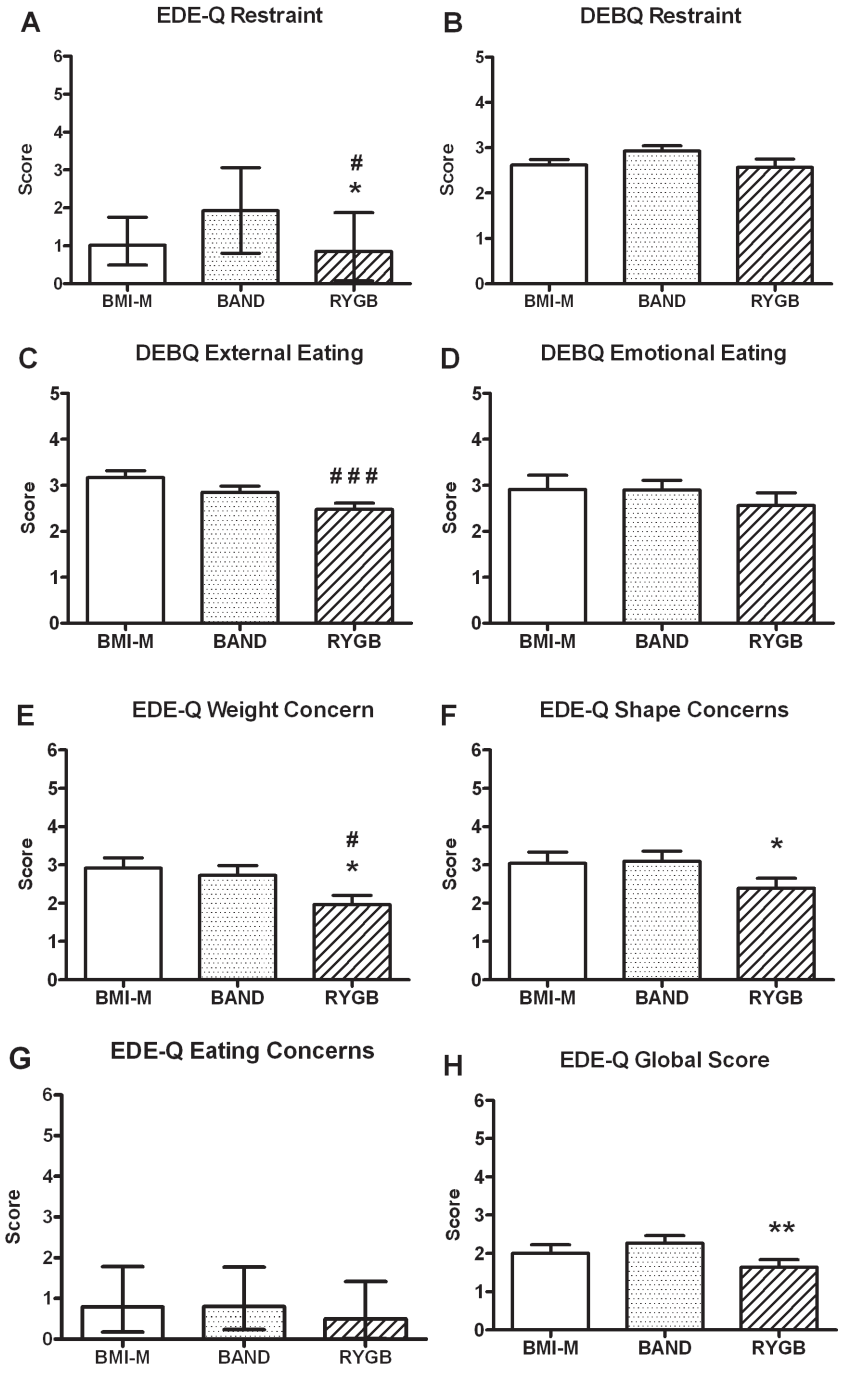
In the whole cohort, the RYGB group had healthier eating behaviour and less eating disorder psychopathology, compared with the BAND and/or control groups. The RYGB group had significantly lower scores for dietary restraint using the Eating Disorder Examination Questionnaire (EDE-Q) than both the BAND and BMI-matched groups, and a trend for lower scores using Dutch Eating Behaviour Questionnaire (DEBQ) (Restraint Scale) than the BAND group. RYGB patients scored lower on eating in response to external cues, as measured by the DEBQ (External Eating Scale) than the BMI-matched controls and a trend for lower scores than the BAND patients. RYGB patients had less symptoms of eating disorder psychopathology using the EDE-Q, with lower weight and shape concerns than the BAND and/or BMI-matched controls (Fig. 3.5, Table 3.8). Eating in response to emotional cues as measured by the DEBQ (Emotional eating Scale) did not differ between groups. In the subset of patients from the whole cohort who underwent scanning, similar results were obtained in these measures of eating behaviour (see Appendix 14)

Table 3.8 Eating behavior questionnaires from subjects in whole cohort.

	BMI-M	BAND	RYGB	P value [a]
n	25	28	30	
Dietary restraint				
EDE-Q Restraint (score /6)	0.8 [0.3 - 2.0] (0.0 - 3.2)	2.0 [0.8 - 3.1] (0.0 - 4.8)	0.5 [0.0 - 1.8] (0.0 - 4.4)	0.01 BAND > RYGB 0.01 BAND > BMI-M 0.01
DEBQ Restraint (score /5)	2.6 ± 0.1 (1.4 – 3.5)	3.0 ± 0.4 (1.4 – 4.5)	2.6 ± 0.2 (0.0 – 4.4)	0.14
Disinhibited eating (DEBQ)				
External eating (score /5)	3.1 ± 0.2 (1.5 – 5.0)	3.0 ± 0.1 (1.7 – 3.7)	2.5 ± 0.1 (0.6 – 4.2)	0.004 BMI-M > RYGB 0.001
Emotional eating (score/5)	3.1 ± 0.2 (1.0 – 5.0)	3.0 ± 0.3 (1.1 – 2.8)	2.5 ± 0.3 (0.0 – 5.0)	0.31
Disordered eating questionnaire (EDE-Q)				
Weight concerns (score /6)	3.0 ± 0.6 (0.4 - 5.6)	2.7 ± 0.3 (0.0 - 5.2)	1.9 ± 0.3 (0.0 - 5.9)	0.02 BMI-M > RYGB 0.01 BAND > RYGB 0.03
Shape concerns (score /6)	3.1 ± 0.3 (0.4 - 5.3)	3.2 ± 0.3 (0.4 - 6.0)	2.3 ± 0.3 (0.1 - 5.9)	0.05 BAND > RYGB 0.02
Eating concerns (score /6)	0.8 [0.3 - 1.8] (0.0 - 5.5)	0.8 [0.3 - 1.7] (0.0 - 4.8)	0.4 [0.0 - 1.0] (0.0 - 5.4)	0.40
Global score (score /6)	2.1 ± 0.2 (0.4 - 4.5)	2.3 ± 0.2 (0.1 - 4.5)	1.5 ± 0.2 (0.0 - 5.2)	0.02 BAND > RYGB 0.007

Data included for the whole cohort. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range), adjusted for age gender and BMI. [a] P value for overall comparison of averages between groups using ANOVA, with post-hoc comparison given beneath. Note that similar results were obtained when limiting the analysis to the scanned subjects only. Abbreviations: BAND: gastric banding, BMI-M: body mass index matched, DEBQ: Dutch Eating Behaviour Questionnaire, EDE-Q: Eating Disorder Examination Questionnaire, RYGB: gastric bypass.

Figure 3.5 Eating behavior.



(A) EDE-Q dietary restraint, (B) DEBQ dietary restraint, (C) DEBQ external eating, (D) DEBQ emotional eating, and EDE-Q (E) weight concerns, (F) shape concerns, (G) eating concerns and (H) global score of BMI-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery. Data are presented as (A,G) median and interquartile range, or (B,C,D,E,F,H) mean ± SEM. $^{\#}P<0.05$, $^{\#\#\#}P<0.005$ vs. BMI-M; *P<0.05, **P<0.01 vs. BAND; n=20-21 per group. Abbreviations**:** DEBQ: Dutch Eating Behaviour Questionnaire, EDE-Q: Eating Disorders Examination Questionnaire.

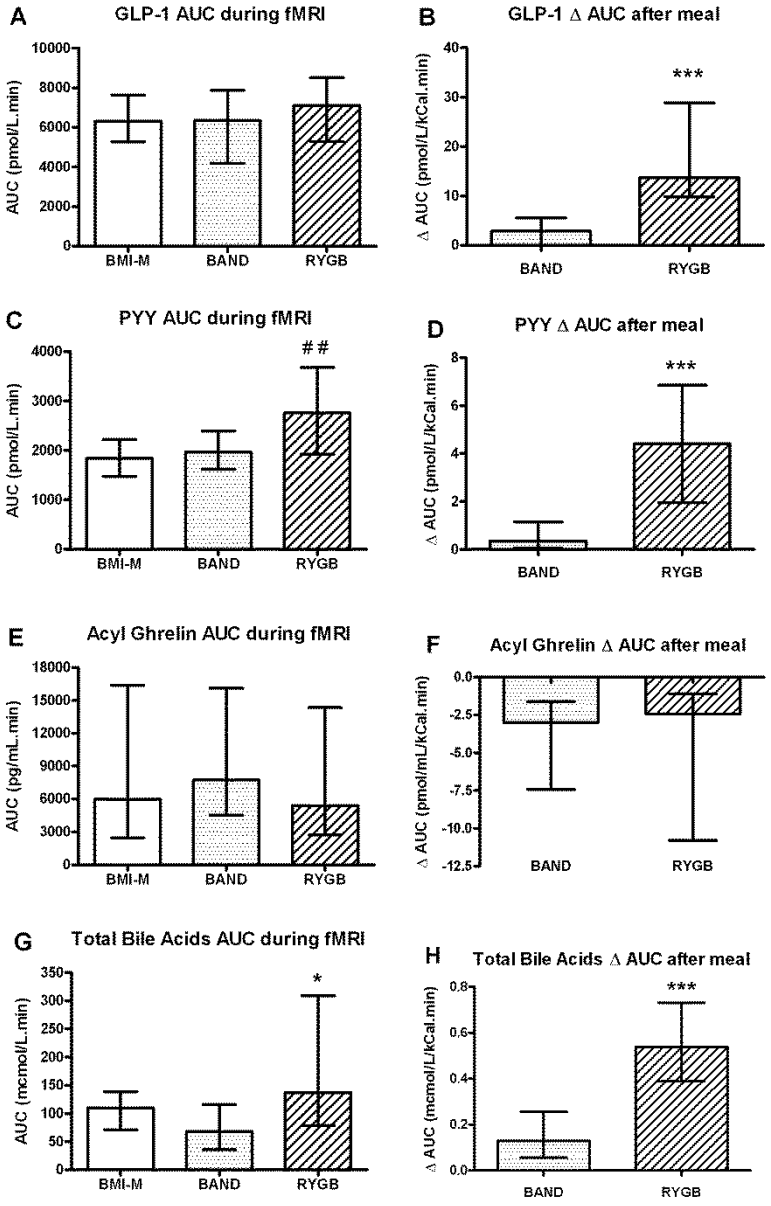
3.4.9 Metabolic and hormonal phenotyping

Plasma GLP-1 levels were similar between the three groups during scanning**,** but increased significantly more in the RYGB than BAND patients after the meal (Fig. 3.6 A, B). Plasma PYY levels during scanning were higher in the RYGB than control group, and increased more in the RYGB than BAND group after the meal (Fig. 3.6 C, D). There were no differences in plasma acyl ghrelin levels between the groups (Fig. 3.6 E, F).

Plasma levels of total and glycine-conjugated bile acids were higher in the RYGB than BAND groups both during scanning and after the meal (Fig. 3.6 G, H). The sub-fractions of primary and deoxycholic bile acids were higher in the RYGB than BAND patients only after the meal (Fig. 3.7 A-F).

Plasma glucose and insulin levels during the scanning period did not differ between the two surgical groups (Fig. 3.7 G, I). Glucose levels were lower in the BAND than the BMI-matched unoperated control group, and insulin levels were lower in the RYGB than the BMI-matched unoperated control group (Fig. 3.7 G, I). Glucose levels increased more after the meal in the RYGB compared with the BAND group (Fig. 3.7. H), and there were similar increases in insulin levels (Fig. 3.7. J).

Figure 3.6 Plasma levels of gut hormones and bile acids in obese patients after gastric bypass and gastric banding and controls.

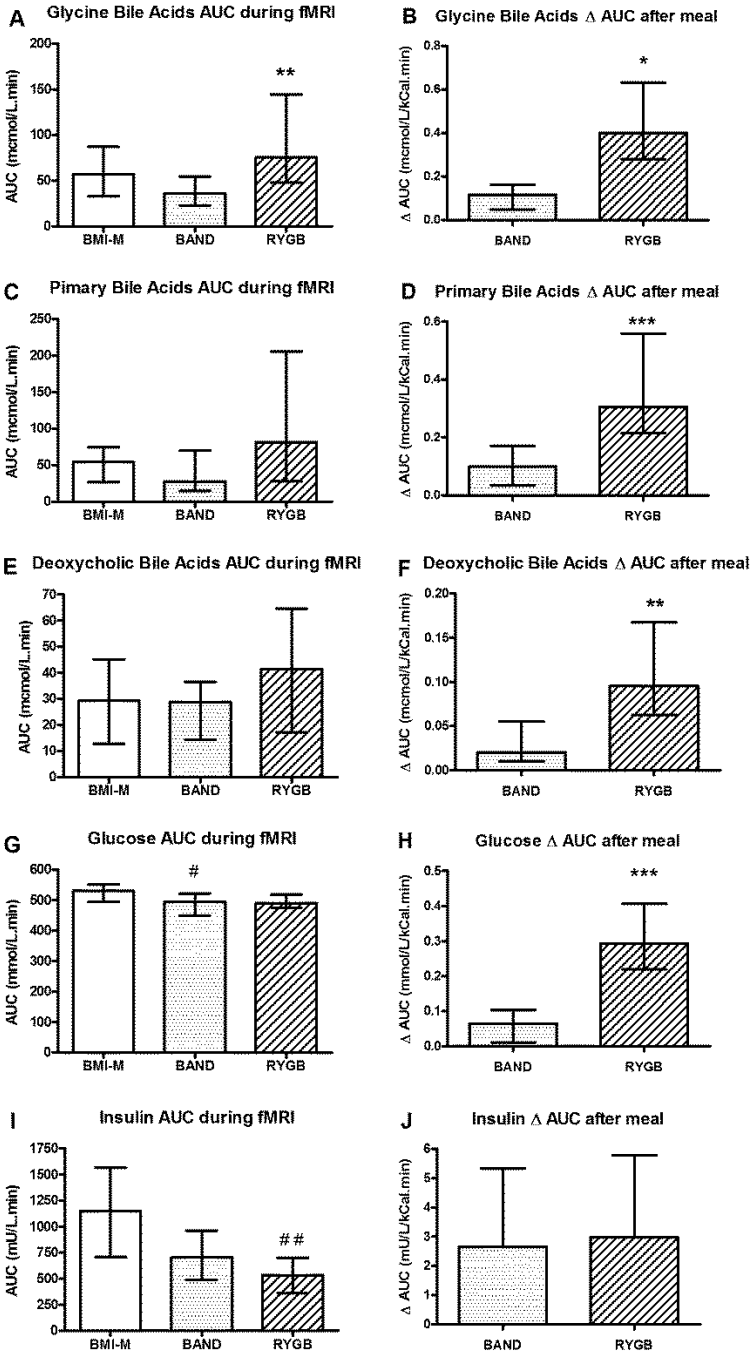


Comparison of (A,C,E) plasma hormone levels (GLP-1, PYY, acyl ghrelin, area under curve (AUC) +40 to +150 mins) and (G) total bile acid levels during fMRI scan (AUC +70 to +150 mins) between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery.

Comparison of (B,D,F) change in plasma hormone levels and (H) change in total bile acid levels after ice-cream meal (both ΔAUC +150 to +210 mins) between two surgical groups.

Data are presented as median and interquartile ranges. $^{\#\#}$P<0.01 vs. BMI-M; *P<0.05, ***P<0.005 vs. BAND; n=20-21 per group.

Figure 3.7 Plasma levels of bile acid sub-fractions, glucose and insulin

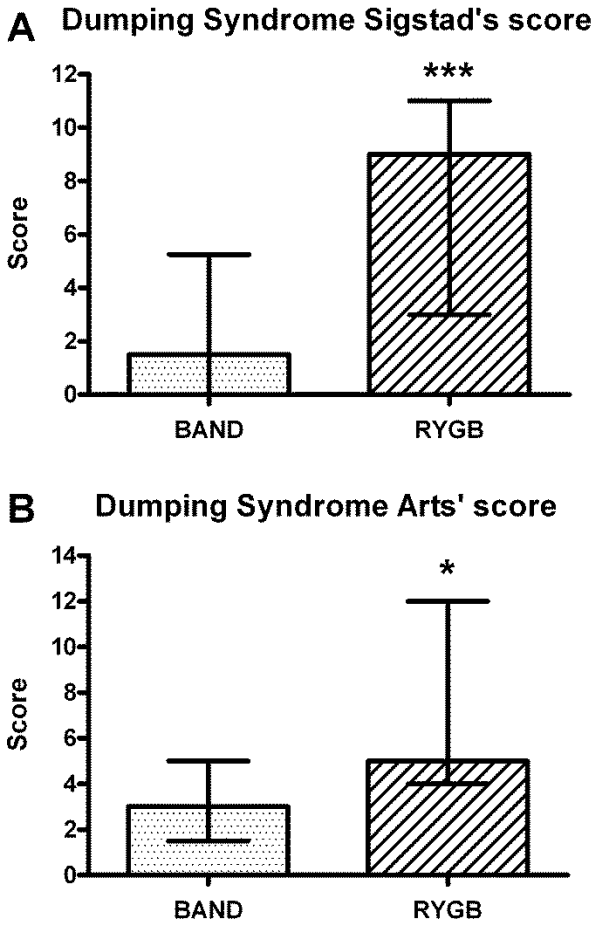


Comparison of plasma (A-F) bile acid sub-fractions (glycine, primary bile acid, deoxycholic bile acid), (G,H) glucose and (I,J) insulin levels. (A, C, E) levels during fMRI scan (area under curve (AUC) +70 to +150 mins), and (G, I) during fMRI scan (AUC +40 to +150 mins) between body mass index-matched unoperated controls (BMI-M, white), and obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery. (B, D, F, H, J) change in levels after ice-cream meal (ΔAUC +150 to +210 mins) in surgical groups. Data are presented as median and interquartile range. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. BMI-M; *P<0.05, **P<0.05, ***P<0.005 vs. BAND; n=20-21 per group.

3.4.10 Dumping symptoms and signs

Both the retrospective dumping symptom questionnaire (Arts' and Sigstad's) scores were significantly higher for the RYGB than BAND patients (Fig. 3.8 A, B, Table 3.9). The RYGB group had a greater increase in symptom of `feeling sick', but not 'sleepiness' than the BAND group after the meal (Figs. 3.4 D and Table 3.9). However there were no differences in the change in blood pressure or heart rate after the meal between the surgical groups, (Table 3.10).

Figure 3.8 Assessment of dumping syndrome in surgical groups.



Comparison of retrospective (A) Sigstad's and (B) Arts' dumping syndrome scores during first 3 months after surgery (n=18-19 per group), between obese patients after gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery. Data are presented as median and interquartile range. *P<0.05, ***P<0.005 vs. BAND.

Table 3.9 Assessment of dumping syndrome in surgical groups.

	BAND	RYGB	P value
n	20	21	
Sigstad's score	1.5 [0.0 - 5.0] (-4.0 to 11.0)	9.0 [3.0 - 11.0] (0.0 -29.0)	0.002 RYGB > BAND
Arts' score	3.0 [2.0 - 5.0] (0.0 - 8.0)	5.0 [4.0 - 12.0] (0.0 - 24.0)	0.02 RYGB > BAND
Δ Heart rate (beats per minute)	7.9 ± 1.4 (-6.0 to 20.0)	5.3 ± 1.7 (-7.0 to 21.0)	0.24
Δ Systolic BP (mm Hg)	-2.4 ± 3.8 (-23.0 to 38.0)	-10.7 ± 3.4 (-40.0 to 19.0)	0.11
Δ Diastolic BP (mm Hg)	-2.5 ± 2.9 (-28.0 to 17.0)	-3.7 ± 1.8 (-16.0 to 10.0)	0.72
VAS Sleepiness			
After meal Δ AUC (cm.min)	0.0 [-78.0 to 28.5] (-396.0 to 442.5)	-30.0 [-113.6 to 3.0] (-217.5 to 63.0)	0.34
VAS Nausea			
After meal Δ AUC (cm.min)	-19.5 [-69.8 to 0.0] (-549.0 to 186.0)	9.0 [0.0 to 79.1] (-10.5 to 408.0)	<0.001 RYGB > BAND

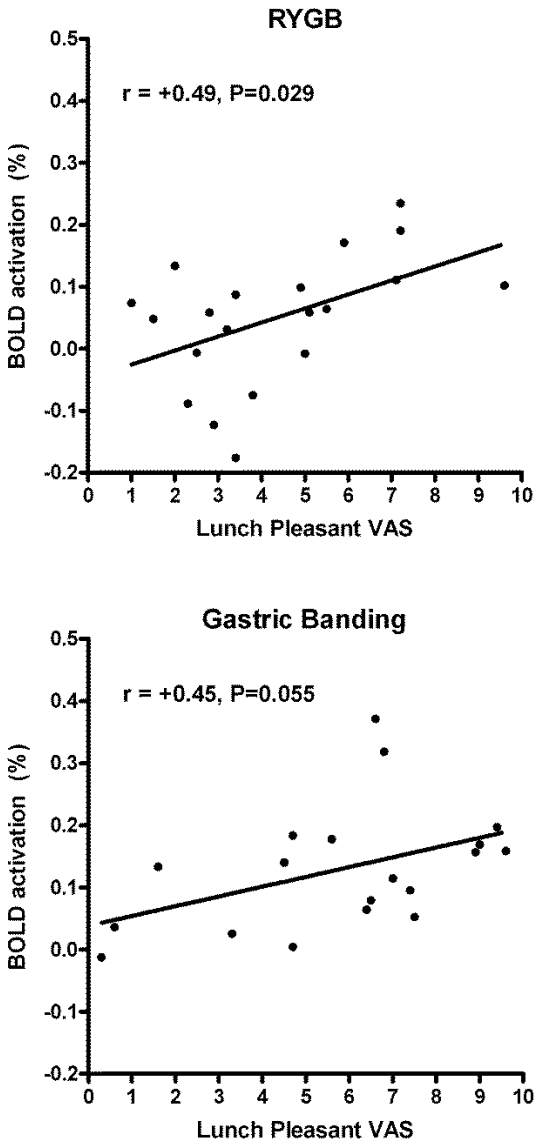
Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).
Δ heart rate and blood pressure: change between time points +150 and +210 min.
Δ AUC for VAS: change in AUC between time points +150 to +210 min.
Abbreviations: AUC: area under the curve BAND: gastric banding group, BMI-M: body mass index matched group, BP: blood pressure, mm: millimeters, RYGB: gastric bypass, VAS: visual analogue scale.

3.4.11 Correlation between outcome measures

BOLD activation to high-calorie food pictures in the whole reward system was positively correlated with VAS pleasantness ratings of the high-calorie ice-cream lunch in the RYGB group (Pearson r=+0.49, P=0.029), and a similar trend was seen in the BAND group (r=+0.45, P=0.055) (Fig. 3.9).

However within the RYGB group, there was no significant correlation between BOLD activation to any food, or high-calorie or low-calorie food pictures in the whole reward system, OFC or amygdala with any of the following secondary outcome measures: GLP-1, PYY or total bile acids area under curve (AUC) during fMRI scan (correlation coefficient -0.35 to +0.31, P=0.13-0.92); absolute GLP-1, PYY or total bile acids AUC after ice-cream meal (-0.24 to +0.29, P=0.22-1.0); or either of the dumping questionnaire scores (-0.39 to +0.27, P=0.11-1.0).

Figure 3.9 Correlation Lunch pleasantness with BOLD activation



3.4.12 Confounding variables

There were no significant differences in potential confounding variables including mood, sleep duration, time since last meal, or motion during scanning between the groups (Table 3.10).

Table 3.10 Potential confounding variables at scanning visit.

	BMI-M	BAND	RYGB	P value [a]
n	20	20	21	
PANAS positive (score /50)	32.0 ± 1.9 (16.0 - 51.0)	28.9 ± 2.0 (14.0 - 44.0)	31.0 ± 1.9 (11.0 - 44.0)	0.52
PANAS negative (score /50)	15.0 [12.0 - 20.0] (10.0 - 33.0)	13.5 [11.0 - 16.5] (9.0 - 26.0)	13.0 [11.0 - 16.5] (10.0 - 24.0)	0.33
Sleep duration previous night (hours)	6.8 [6.0 - 7.8] (4.2 - 12.0)	7.5 [7.0 - 7.5] (6.0 - 10.0)	6.5 [5.2 - 7.6] (4.3 - 9.3)	0.16
Time since supper to fMRI scan (hours)	16.4 [15.7 - 17.0] (14.8 - 19.1)	16.1 [15.6 - 16.7] (14.9 - 20.3)	16.5 [16.0 - 17.3] (15.0 - 18.6)	0.41
Absolute motion during food task (mm)	0.24 [0.19 - 0.38] (0.13 - 1.09)	0.37 [0.25 - 0.50] (0.1 - 0.9)	0.36 [0.26 - 0.52] (0.17 - 1.03)	0.13
Relative motion during food task (mm/TR)	0.10 [0.08 - 0.13] (0.05 - 0.22)	0.07 [0.15 - 0.09] (0.05 - 0.23)	0.11 [0.08 - 0.13] (0.06 - 0.36)	0.66
Absolute motion during Audio-Motor-Visual task (mm)	0.23 [0.17 - 0.43] (0.09 - 1.25)	0.28 [0.14 - 0.44] (0.09 - 0.91)	0.20 [0.19 - 0.37] (0.09 - 1.20)	0.99
Relative motion during Audio-Motor-Visual task (mm/TR)	0.09 [0.07 - 0.12] (0.05 - 0.22)	0.10 [0.07 - 0.12] (0.05 - 0.39)	0.09 [0.08 - 0.12] (0.06 - 0.35)	0.79

Data are presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range). [a] P value for overall comparison of averages between groups using ANOVA.
Abbreviations: BAND: gastric banding group, BMI-M: body mass index matched group, mm: millimeters, PANAS: positive and negative affect schedule, RYGB: gastric bypass, TR: repetition time. VAS: visual analogue scale.

3.5 Discussion

3.5.1 Summary of findings

The results of this study show that obese patients after RYGB surgery have markedly different gut-brain-hedonic responses to food compared with obese patients after BAND surgery and unoperated BMI-matched controls. In particular brain activation in certain key reward areas of the brain including the OFC, amygdala, caudate nucleus, nucleus accumbens and hippocampus was lower in obese patients after RYGB surgery than after BAND surgery, particularly to high-calorie foods. In contrast, there were no areas where lower activation to food was seen in patients who had undergone BAND compared to RYGB surgery. This observation was made both on whole brain and ROI analyses.

3.5.2 Converging evidence from longitudinal neuroimaging studies

This is the first study to investigate differences in neural reactivity to food cues between obese patients that have undergone different bariatric procedures. Though cross-sectional, these results are supported by longitudinal studies measuring changes in neural reactivity to food cues after RYGB surgery alone, using fMRI and PET neuroimaging techniques. In 2012, Ochner et al reported a study of 14 female patients one month before and one month after RYGB in which they found significant reductions in activation to food (high- and low-calorie) cues (visual and auditory) post-operatively in the dorsal striatum (lentiform nucleus and putamen), middle and superior frontal gyrus. These reductions were significantly greater for the BOLD response to high-calorie, compared to low-calorie food pictures and words. Reductions in neural reactivity for high- compared to low-calorie food cues in the lentiform nucleus, caudate, middle and superior frontal gyri, ACC, thalamus and inferior parietal lobule significantly predicted reductions in desire to eat particularly high-calorie foods (Ochner et

al. 2012). These results were similar to their previous study of a subset of 10 of the same patients, in which reductions in BOLD activation to food cues were seen in the lingual gyrus, middle and superior temporal gyri, inferior parietal lobule and precuneus, and the reduction in high- to low- food calorie cues were most pronounced in DLPFC, precuneus, dorsal cingulate, lentiform nucleus, and ventral striatum (Ochner et al. 2011).

The areas in Ochner's studies that changed after RYGB surgery differed from those areas observed to differ in activation between RYGB and BAND surgery in my study, except for the caudate. One possible explanation for this may be that activation in the areas affected in Ochner's study are similarly reduced in *both* types of surgery, and that there are other regions identified by my study which are preferentially reduced in RYGB only. In addition, Ochner's studies did not control for stage of menstrual cycle introducing a potential source of noise since this is known to affect BOLD signal to food pictures (Frank et al. 2010).

There were several other differences in study paradigm, which may account for the differences seen. For instance, although Ochner's subjects had been fasted overnight, they were also given a 250kCal meal 60 minutes before scanning, reducing the effect of fasting, but also not rendering them satiated, whereas in the current study, subjects were fasted. Ochner's paradigm made use of a combination of visual and auditory cures whereas the current study used only food pictures, which may have led to different activation patterns in itself. Whilst their subjects were asked to passively view or listen to the food cues, those in the current study were engaged in active evaluation of the food cues, thereby increasing the possibility of measuring the hedonic response to the cues and ensuring their attention to the task but also potentially increasing BOLD signal in areas of active reward evaluation, such as OFC.

Another explanation may be that the observed differences in BOLD response between the surgical groups in my study may not be as a result of reduction in activation in obese people undergoing RYGB, but in fact an *increase* in activation to food after BAND surgery in certain areas. This possibility is supported by the general *pattern* of results in the ROIs, where BOLD signal tends to be higher in the BAND than the BMI-matched controls for food compared to objects, although these differences did not reach statistical significance. The current study also improves upon Ochner's by way of a larger sample size, and by scanning patients at least 3 months after surgery to reduce the confounding effect of a liquid post-operative diet which is discontinued after 1 month, as well as by the inclusion of the BAND group to control for order effects.

There has been one longitudinal study investigating fMRI responses to food after BAND surgery. In this study, 10 obese subjects were scanned before and 3 months after BAND surgery. The authors found increased BOLD signal after surgery for food compared to non-food pictures in the fasted state in the middle frontal and superior frontal gyrus, whereas in the post-prandial state, BOLD activation in the parahippocampus, medial PFC, insula and inferior frontal gyrus was lower and in the anterior prefrontal cortex higher for food compared to non-food pictures (Bruce et al. 2011). In this study there was no statistical correction made for multiple comparisons and no control group for the effect of weight loss was used. In addition, the study suffers from the confounding order effect, in which familiarity with the cues may lead to reduced salience and therefore BOLD signal in particular areas such as the nucleus accumbens. In addition, no mention of whether the band had been inflated or not was made.

Findings from longitudinal PET studies of obese patients before and after RYGB surgery are

inconsistent and difficult to interpret given the very low numbers, lack of distinction between RYGB and sleeve gastrectomy in one study, and lack of control for the effects of weight loss (Dunn et al. 2010; Steele et al. 2010). Since D2/3 receptor availability is reduced in obesity, and assuming that this is due to down-regulation of receptors from resistance, then it is hypothesized that this should be corrected by weight loss. In a small study of 5 obese women who underwent RYGB aged in their 30's, ^{11}C-raclopride (antagonist radioligand of D2 and D3 receptors) PET studies were carried out 6 weeks pre- and post-operatively. The analysis was limited to striatum (anterior and posterior putamen, and anterior and posterior caudate), and found the predicted increases in D2/D3 receptor binding after RYGB (Steele et al. 2010). By contrast a study of 5 women in their 40's with similar mean BMI to previous study, pre- and 7 (6-11) weeks post- RYGB and VSG using PET ^{18}F-Fallypride, to measure D2 receptor availability, found decreased D2 receptor availability after surgery in the substantia nigra, caudate, putamen, ventral striatum, hypothalamus, medial thalamus and amygdala (Dunn et al. 2010).

The authors note that the discrepancies between the studies may be related to lower age and changes in pre- to post-operative depression scores, in the subjects taking part in Steele's study as well as heterogeneity in the type of operation (VSG and RYGB) in the second study. In addition, in both studies, scanning took place in the acute weight loss phase following surgery, which may have independent effects on dopamine receptor availability. In the first study, the authors begin with the assumption that pre-operatively, obese patients have low D2/D3 receptor binding, but in fact this was no different to the lean controls matched for age and sex. In addition, small subject numbers limits interpretation of both studies.

3.5.3 No differences in confounding psychological traits between groups

The observed differences in brain reward responses to food were not explicable by differences in underlying psychological traits or current mood. There were no differences in psychological measures employed, including those measuring personality traits such as impulsivity or extraversion, or current depression between groups.

Many of these traits have been known to impact on BOLD signal response to food cues. For instance negative affect (measured by PANAS) has been associated with greater BOLD activation in the OFC, ACC and insula on viewing high-calorie food pictures (Killgore et al. 2006) and impulsivity has been shown to modulate BOLD response to food cues (Scharmuller et al. 2012).

There have been a few studies that evaluate the use of psychological trait questionnaires in obese patients, reviewed in 2013 by Vainik et al. The review suggests the 'Five Factor Model' may be the most appropriate test of personality, since its sub-categories correlate well with several eating behaviours, but also notes that most personality questionnaires share the same underlying structure. A general model of high neuroticism, high assertiveness/extraversion (also interpreted in some studies as 'wanting' or reward sensitivity), low self-control and high impulsivity has emerged in studies correlating personality traits with obesity or risk of obesity (Vainik et al. 2013). Participants in this study were BMI-matched across the groups at the time of scanning, but preoperative BMI tended to be higher in the RYGB group. Therefore it is reassuring that a lack of difference in these measures suggests a lack of premorbid differences between the groups that may have led to differences in brain hedonic processing. The BDI-II (Beck et al. 1996) has been validated in

obese populations (Wadden et al. 1994; Faulconbridge et al. 2009), and negative affect using the PANAS has been shown to be associated with obesity (Pasco et al. 2013), to correlate with increased BOLD activation in the putamen, caudate and pallidum during food anticipation in bulimic women (Bohon et al. 2012), and to modulate OFC activation to low-calorie food in normal weight women (Killgore et al. 2006).

3.5.4 Lower food appeal and healthier eating behaviour in RYGB compared to BAND

Differences in brain reward responses to food were accompanied by differences in food appeal, food preference and actual eating behaviour between the groups. This is important, since neuroimaging studies are improved in validity if concurrent behavioural measures support the assumptions made on the basis of the fMRI findings (Poldrack 2006). Not only was the appeal of high-calorie foods lower in RYGB than BAND and BMI-matched controls, liking and wanting of high fat and low fat savoury foods was significantly lower and there was a trend for high and low fat sweet foods to be lower in RYGB compared to BAND patients, using the LFPQ. Furthermore, palatability of ice cream, measured by VAS ratings of pleasantness to eat, was also lower in the RYGB group compared to the BAND group. Furthermore the VAS palatability of ice cream correlated with whole reward system activation to food pictures.

It was suprising not to observe lower consumption of ice cream in the RYGB compared to BAND group. A possible explanation is that the test meal was not specifically designed to examine food preference, as subjects were not given a choice of foods of different caloric density. Analysis of macronutrient intake outside of the laboratory did reveal lower fat intake after RYGB compared to BAND.

RYGB patients had lower average calorie intake (corrected for lean body mass), and lower proportional fat intake in obese patients who had undergone RYGB compared to BAND surgery. Dietary records and questionnaires give information about food choice and actual dietary behaviour but suffer from the vagaries of being subjective in nature, and therefore subject to distortion by observation and underreporting (Barrett-Connor 1991). This appears to be particularly true of obese patients (Lissner 2002). Food diaries appear to be most accurate when recording intake over at least 3 day periods (Burrows et al. 2010).

3.5.5 Lower dietary restraint and external eating in RYGB

Dietary restraint and external eating also differed between the groups. Restraint, emotional eating and external eating have been consistently shown to be associated with obesity and overeating (Bryant et al. 2008; Herman et al. 2008) suggesting that the BAND patients may be at higher risk of disordered eating and potentially weight regain in the future than the RYGB patients. Conversely, low dietary restraint and external eating in RYGB patients compared to BMI-matched controls suggests that their eating behaviour may have been significantly changed from those patterns potentially present prior to surgery. In effect, the loss of reward attached to food may result in the possibility of liberation from constantly having to monitor and exercise restraint over food intake. Interestingly, there were no differences in emotional eating between the groups, suggesting that despite the loss of hedonic reward from food, RYGB and BAND patients may continue to use food for emotional regulation. Another study has, however found lower emotional eating after RYGB (Laurenius et al. 2012), although this study used the TFEQ and not the DEBQ, which may explain the difference in results.

The findings in the current study of higher weight and shape concerns amongst BAND patients compared to RYGB patients, despite similar BMI may also relate to unresolved emotional conflict over eating, and therefore weight, in BAND patients, epitomised by high restraint scores, not present in RYGB patients. However, another explanation is that RYGB patients had lost a higher percentage of their body weight, compared to the BAND patients in this cohort, and they may have simply experienced a greater degree of satisfaction with the degree of improvement in their body weight compared to BAND patients.

It could be argued that the Food Addiction Scale would have been a useful addition to measures of eating behaviour, given the similarities neural substrates involved in drug addiction and obesity (Volkow et al. 2008), and similarities in BOLD response to drug cues in drug addiction and food cues in obesity, although there is some controversy surrounding this (Ziauddeen et al. 2012). Also food addiction scores have been shown to correlate with BOLD activation in the ACC, OFC and amygdala in response to anticipated receipt of food (Gearhardt et al. 2011). However, this scale measures symptoms of addiction to food over the past 12 months (Gearhardt et al. 2009), and would therefore not have been suitable in this study where at the time of questionnaire completion, the time period from surgery varied from less than 3 months to more than 3 years after surgery, although it could have been shortened for the purpose of the study. A similar scale, the Power of Food Scale (PFS), also designed to examine hedonic responses to food, does not have this limitation, but at the time of study design had only just been validated in obese populations (Cappelleri et al. 2009; Lowe et al. 2009). In retrospect this would have been a useful addition to the study. However, it correlates reasonably well with the DEBQ-Emotional eating and DEBQ-External eating scales used in the study (Lowe et al. 2009). The DEBQ has been validated for use in

obese populations, and has good internal consistency and test-retest reliability (Vainik et al. 2013). The EDE-Q has been validated in the detection of disordered eating (Berg et al. 2012), and results obtained from its use in this study provide a novel addition to the literature on eating disorder psychopathology after bariatric surgery.

3.5.6 No difference in hunger and fullness between surgical groups

Interestingly, although RYGB patients reported less hedonic appeal of particularly high-calorie food, ate less proportional dietary fat, had healthier eating behaviour and had less brain reward system activation to food compared to BAND patients, there was no difference in reported hunger or fullness levels using VAS between the groups. This suggests that it may be differences in hedonic responses and reward based eating, rather than homeostatic control of hunger and satiety that leads to greater weight loss in RYGB compared to BAND surgery.

3.4.7 Fasting gut hormone and bile acid levels higher in RYGB

The gut hormone profiles are suggestive of a potential underlying mechanism for differences in brain-hedonic responses to food between the groups. This study confirmed the expected differences in post-prandial anorexigenic gut hormones and bile acid responses between groups in this study sample, in agreement with previous studies (Borg et al. 2006; Korner et al. 2006; le Roux et al. 2007; Korner et al. 2009; Nakatani et al. 2009; Patti et al. 2009; Chandarana et al. 2011; Laferrere 2011; Pournaras et al. 2012). Differences in acute post-prandial gut hormone responses are unlikely to have had an effect on fMRI findings in this part of the study, since patients were fasted at the time of scanning. However there were even in the fasted state, greater pre-meal PYY and bile acids in RYGB patients (than BAND or

BMI-M groups), introducing the possibility of longer term effects of RYGB surgery on these parameters. It is possible that repeated elevations of both GLP-1 and PYY hormones have a chronic effect on brain reward responses , since for example previous infusion studies have shown that gut hormone infusions reduce food intake even several hours after the infusion has ceased and plasma levels have returned to baseline (Batterham et al. 2007; Bryant et al. 2012).

In this study, plasma levels of bile acids were also higher in the RYGB than BAND group. RYGB modifies the anatomical location at which bile enters the upper gastrointestinal tract via the bilio-pancreatic limb of the Roux-en-Y construction. Several studies have found increased serum bile acid concentrations after RYGB surgery (Nakatani et al. 2009; Patti et al. 2009; Jansen et al. 2011; Pournaras et al. 2012). This could be an alternative modulator of central hedonic processing of food after RYGB. Indeed, bile acids cross the blood-brain-barrier (Ogundare et al. 2010), and the bile acid receptor TGR5 is present in the brain (Keitel et al. 2010).

Bile acids are known to have significant effects on glucose metabolism through a variety of pathways and may also reduce appetite through modulation of gut hormone secretion. Bile acids also stimulate small bowel production of fibroblast growth factor 19 (FGF19) and FGF19 receptors have been found in the rat hypothalamus and their expression is reduced in high fat fed animals compared to lean. Acute administration of intracranial FGF19 reduces food intake and body weight in rats, whereas an FGF19 receptor inhibitor has the opposite effect (Ryan et al. 2013). A direct role for bile acids or FGF19 after RYGB may therefore be worthy of further exploration.

3.5.8 Greater dumping symptoms in RYGB

Symptoms of dumping syndrome were higher in RYGB compared to BAND patients, suggesting a further possible mechanism underlying differences in BOLD signal to food cues, mediated by conditioned taste aversion. The mechanisms underlying dumping syndrome are poorly understood, but symptoms have been associated with elevations in PYY and GLP-1 (Gebhard et al. 2001) and may also be related to increased bile acids. Patients who had undergone RYGB reported increased symptoms of dumping in the first 3 months after surgery, such as post-prandial nausea, pain, tiredness, diarrhoea, sweating and feeling faint, compared to those that had undergone BAND surgery. Dumping symptoms are precipitated particularly by high-calorie sweet or fatty foods. Although there were no differences in physiological parameters indicative of dumping signs and symptoms during the study, such as blood pressure or pulse or post-prandial VAS scores of sleepiness, there were higher post-prandial increases in VAS scores of nausea in RYGB. On the other hand, VAS nausea scored during scanning were higher in BAND patients, although absolute ratings were low in both groups. It is possible that repeated episodes of dumping symptoms after RYGB may lead to avoidance of foods that precipitate it, and that over time, through learning, these foods therefore are associated with less hedonic appeal.

Although the stomach-derived orexigenic hormone ghrelin has stimulatory effects on food hedonics and reward system activation to food cues (Malik et al. 2008; Goldstone et al. 2010; Skibicka et al. 2011) there was no difference in plasma acyl ghrelin between surgical groups. Some studies have found reduced fasting and/or post-prandial ghrelin levels after RYGB compared to before surgery or to unoperated controls (Cummings et al. 2004; Pournaras et al. 2009; Stefater et al. 2012). This finding is however not universal, related to differences in surgical techniques, assay of total vs. active acyl ghrelin, and problems with

handling and storage of plasma samples

3.5.9 Strengths and limitations

Patients in this study were not randomized as to which surgery they receive, as it is the policy of the unit to allow patients choice of surgery. This introduced a potential source of bias since it is possible that patients choosing RYGB surgery differed in some way to those choosing BAND surgery even pre-operatively in a way that would affect their hedonic responses to food post-operatively. The groups were however similar in many respects with regard to pre-operative and post-operative demographics and obesity related co-morbidities, but did differ in the amount of weight they had lost, the prevalence of pre-operative (but not post-operative) T2DM and, probably as a result of this difference in T2DM prevalence, their pre-operative obesity related co-morbidity score. However, the RYGB group tended to have higher pre-operative BMIs than the BAND group, and therefore if anything, would have been likely to have had a *greater* hedonic response to food.

There is currently no evidence to suggest that previous T2DM should reduce brain activation and hedonic responses to food. If anything, it is likely that high-calorie sweet foods should have held more hedonic appeal to patients who had been denied them by dietary restriction in the past. On the other hand, T2DM patients may have received different or more consistent dietary advice prior to bariatric surgery compared to non-T2DM patients, and therefore may have learned to view particularly sweet foods as holding more negative consequence for them. Studies examining the effect of T2DM on food cue reactivity using fMRI are scarce, but one study has shown increased activation of the insula, OFC and basal ganglia in response to food images in T2DM compared to healthy individuals. In this study,

external eating, dietary self-efficacy and dietary self-care correlated positively with activity in the OFC and insula, whereas emotional eating positively correlated with activity in the amygdala, putamen and nucleus accumbens (Chechlacz et al. 2009). The possibility of long-standing effects of insulin resistance on brain reward area activation cannot be completely ruled, but the above study suggests that if anything this would have minimized the difference between BOLD activation in the RYGB and BAND groups. Furthermore, evidence from a recent study suggests however that RYGB reverses any insulin-induced changes in brain activation (Tuulari et al. 2013). In this study of 22 obese patients before and 6 months after bariatric surgery (type not specified) and 7 normal weight controls, a hyperinsulinemic clamp increased brain glucose metabolism in a wide-spread manner in the obese but not in controls, but postoperatively, the increase in glucose metabolism was no longer observed in the obese patients. Furthermore at, the time of scanning in my study the operated groups were similar in their prevalence of T2DM.

Percentage weight loss was greater in the RYGB group than the BAND group, although the groups were similar in BMI. Since RYGB patients tended to be heavier pre-operatively, it was not possible to match the groups for both weight loss and BMI. It is possible, but unlikely that the results of the study could be explained by this 6% difference in weight loss alone, when both groups had lost more than 20% of their body weight. If anything, greater weight loss per se is likely to lead to greater, rather than less brain reward response to food, mediated by falls in plasma leptin (Rosenbaum et al. 2008).

Although effort was made to record and where possible, reduce differences in potential confounding factors between groups, the cross-sectional nature of the study is a further limitation. The cross-sectional nature of this study also limits the ability to suggest causality

in potential mechanisms identified in the phenotyping part of the study. The number of participants in the study, although comparable with similar studies using fMRI to examine food reward (Fletcher et al. 2010; De Silva et al. 2011), and with those examining gut hormone and bile acid responses (le Roux et al. 2006; Laferrere 2011), is small. Some aspects of the study may be therefore be underpowered to detect differences between groups, resulting in Type 1 or 2 errors. However, the consistency of behavioural data with the primary endpoint of differences in BOLD signal is encouraging.

The fMRI paradigm made use of food pictures rather than food receipt itself. However, such fMRI paradigms with food pictures have been widely used to study human eating behavior (Carnell et al. 2012). Additionally fMRI responses to food pictures, anticipation of food receipt and actual food receipt all increase during food restriction (Stice et al. 2013). The use of food pictures also allows exposure, albeit visual, of the subjects to more complex, real-life food stimuli than can be achieved with the restricted nature of tastants used in fMRI experiments such as milkshakes. The use of active evaluation of the pictures during the fMRI task may have enhanced the ability to detect differences in the OFC response (Bender et al. 2009).

Interestingly however, in the food appeal ratings, lower ratings for the high- but not low-calorie food pictures was seen in the RYGB compared to BAND group, whereas in the fMRI data, differences between groups were sometimes seen for food in general (high- and low-calorie food pictures) and sometimes for low-calorie or high-calorie pictures only. It may be that there exists dissociation between perceived liking of particular foods, as measured by subjective ratings, and unconscious attraction to these, measured by BOLD signal in selected areas of the brain. However another more likely explanation is that fMRI lacks the

sensitivity to make this kind of distinction in cross-sectional studies, whereas longitudinal studies have been able to detect these distinctions (Goldstone et al. 2009; Ochner et al. 2012).

It is of note that not all reward areas activation differed between the surgical groups. In fact, different areas within the reward system act in synergy with each other, but may have distinct roles in the processing of food reward. For instance, the OFC, which did differ between groups is known to encode the reward value of food, in anticipation of food and other salient cues, whereas the striatal structure, the nucleus accumbens and caudate may be more directly involved in dopamine release in response to receipt of reward. The amygdala in turn is involved in emotional processing of reward.

It was not possible to further clarify which of the potential mediators might contribute to the reduced brain hedonic response to food after RYGB, as within the RYGB group, none were correlated with BOLD activation to food cues (in those ROIs that displayed differences between surgical groups). The ability to detect such an association may have been hindered by the sample size, cross-sectional nature of the study, and other physiological factors contributing to the variability in BOLD responses between individuals within the group (Pike 2012).

3.6 Conclusions

In conclusion, therefore, this study has demonstrated significant differences in the hedonic response to food, on a behavioural and neurological level, between patients who have undergone RYGB surgery for obesity compared to BAND surgery. RYGB patients had lower BOLD activation to high-calorie food pictures in the OFC, amygdala, caudate nucleus, nucleus accumbens and hippocampus and rated high calorie food pictures as less appealing than BAND patients did. They also rated an ice cream test meal as less palatable, showed preferential eating behaviour patterns and reported lower calorie intake, including proportionally less dietary fat intake than BAND patients. These differences were not associated with differences in hunger or psychological traits or states, such as impulsivity, mood or depression. Further hormonal and metabolic phenotyping suggested possible underlying mechanisms for the healthier eating behaviour and hedonic response to food seen in RYGB. RYGB patients had increased PYY and bile acid levels at the time of scanning, and elevated post-prandial PYY, GLP-1 and plasma bile acid levels, but no difference in acyl ghrelin levels. Finally, obese patients also reported more dumping symptoms in the first three months after RYGB compared to BAND surgery, and experienced greater post-prandial nausea on the day of scanning. These results suggest that, even in the fasted state, the gut-brain hedonic axis may be important in regulating food intake.

These results have revealed novel differences in food reward and hedonics between two successful surgical treatments of obesity. This may prompt the development of more personalized approaches to surgical choices that incorporate pre-operative assessment of food preference and craving. Other factors influencing the choice of bariatric procedure include local expertise and patient preference. There are potentially greater improvements in glycaemic control after RYGB (Dixon et al. 2012; Pournaras et al. 2012), contrasting with

shorter operation time and hospital stay, lower cost and lower mortality rates with BAND (Flum et al. 2011). However in appropriately experienced centers, absolute mortality rates are less than 0.3% for either procedure (Flum et al. 2011).

In conclusion, RYGB and BAND surgical treatments for obesity are distinct in their mechanisms of weight loss. Post-operatively patients have reduced hunger after both procedures, but there are lower brain hedonic and exaggerated gut hormone and bile acid responses to food after RYGB, that would explain its greater efficacy for weight loss. This implicates the gut-brain axis in regulating reward-driven eating behaviour, as well as homeostatic appetite, and hence body weight. Further in depth interrogation of these gut-brain mechanisms will accelerate development of efficacious, cheaper, and safer non-surgical treatments for hedonic overeating and obesity.

CHAPTER 4: EFFECT OF OCTREOTIDE ON FOOD HEDONICS IN OBESE PATIENTS AFTER GASTRIC BYPASS AND GASTRIC BANDING SURGERY

4.1 Introduction

Increasingly the distinct and specific effects of RYGB on post-prandial gut hormone secretion have been implicated in its superior weight loss. There are early and exaggerated increases in post-prandial plasma levels of anorexigenic peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and oxyntomodulin which promote satiety and which, when reversed, increase food intake (le Roux et al. 2005; Borg et al. 2006; Korner et al. 2006; le Roux et al. 2006; le Roux et al. 2007).

4.1.1 Gut hormones as mediators of weight loss in RYGB

PYY and GLP-1 are secreted mainly by L-cells in the mucosa of the ileum and colon (Adrian et al. 1985; Turton et al. 1996; Wynne et al. 2006; Janssen et al. 2013). Peripheral PYY and GLP-1 secretion reduces appetite and increases satiety via modulation of appetite centres in the brain to suppress appetite centrally, and through local effects in the gut (Punjabi et al. 2011; Campbell et al. 2013). In addition emerging evidence suggests that not only homeostatic appetite but also hedonic response to food is altered by these hormones (De Silva et al. 2012).

Evidence of the role of PYY and GLP-1 in mediating successful weight loss following RYGB has provided renewed interest in investigation of the mediation of these hormones on the gut-brain axis controlling food intake. It has long been established through animal studies that PYY and GLP-1 have direct effects on hypothalamic and brainstem appetite centres (Field et al. 2009; Parkinson et al. 2009; Field et al. 2010). PYY acts on the hypothalamic arcuate nucleus to inhibit neuropeptide Y (NPY) neurons to reduce appetite and food intake (Schwartz et al. 2000; Schwartz et al. 2002; Riediger et al. 2004; Sloth et al. 2007).

Furthermore it also decreases ghrelin levels, which may enhance anorexia (Batterham et al. 2003). Peripheral GLP-1 crosses the blood brain barrier (Kastin et al. 2002) and GLP-1 receptors are located in the paraventricular (PVN), dorsomedian (DMN), and arcuate (ARC) hypothalamic nuclei. Infusion of GLP-1 peripherally and directly into the brain ventricles reduces food intake in rats (Hayes et al. 2010; Baldassano et al. 2012).

However it has become increasingly apparent that these hormones and other may act not only on hypothalamic appetite centres, but also hedonic and reward-based eating as is evidenced by both animal and human studies (Egecioglu et al. 2011).

To date, there have been no animal studies investigating PYY action on non-homeostatic brain areas. Human subjects given a PYY infusion compared to saline, showed activation of the parabrachial nucleus, the VTA, limbic regions, ventral striatum and certain frontal cortical regions as assessed by BOLD imaging (Batterham et al. 2007). The substantia nigra, parabrachial nucleus and hypothalamic BOLD response correlated with PYY levels, while OFC activation predicted food intake and correlated negatively with hedonic ratings of food when PYY was given (Batterham et al. 2007).

GLP-1 receptors have been identified in the nucleus accumbens and VTA, and activation of these receptors with GLP-1 agonist intracerebral infusions increased c-fos expression in the nucleus accumbens, decreased intake of especially highly-palatable foods, and reduced body weight in rats (Dossat et al. 2011; Alhadeff et al. 2012). Moreover, blockade of these in the VTA and the nucleus accumbens core resulted in a significant increase in food intake. Food-reward behaviour is also reduced in rats by administration of a GLP-1 agonist, as rats no

longer prefer an environment previously paired to chocolate pellets. The peripheral administration of a GLP-1 agonist also decreased motivated behaviour for sucrose in a progressive ratio task (Dickson et al. 2012; Skibicka et al. 2012).

A combination of PYY and GLP-1 infusion reduced average BOLD activation to food pictures in combined reward regions (amygdala, caudate, insula, nucleus accumbens, OFC, and putamen) compared to saline and to GLP-1 infusion alone in healthy non-obese men (De Silva et al. 2011).

PYY also reduces gastrointestinal motility and increases water absorption in the gut, and GLP-1 inhibits gastric secretion and also reduces motility, which means that macronutrient absorption is protracted and delayed, leading to satiety and a reduction in food intake. (Wang et al. 2010). GLP-1 is also a potent anti-hyperglycaemic hormone, which stimulates insulin secretion and suppresses glucagon secretion, in a glucose dependent manner (Kreymann et al. 1987). Alterations in GLP-1 positively affect glucose metabolism after RYGB surgery (Laferrere et al. 2008; Pournaras et al. 2010; Van der Schueren et al. 2012).

GLP-1 is also expressed in taste receptors, and GLP1-receptor knock out (KO) mice exhibit reduced taste sensitivity to both nutritive and non-nutritive sweeteners (Shin et al. 2008; Martin et al. 2009), prompting the hypothesis that taste sensitivity changes observed after RYGB may be influenced by direct action of GLP-1 on taste receptors.

In obese people, post-prandial PYY secretion is attenuated compared with lean individuals (le Roux et al. 2006). PYY given intravenously to lean and obese humans reduces food intake and increases satiety (Batterham et al. 2002; Batterham et al. 2003). Peripheral infusion of GLP-1 in normal weight and obese people reduces appetite and food intake (Naslund et al. 1999; Flint et al. 2001), and Liraglutide, a GLP-1 agonist has been shown to reduce weight in obese people without T2DM (Astrup et al. 2009).

Post-prandial GLP-1 and oxyntomodulin release is exaggerated after RYGB in pre-clinical (Bueter et al. 2010) and clinical studies (le Roux et al. 2006; Morinigo et al. 2006; le Roux et al. 2007; Laferrere et al. 2008; Jacobsen et al. 2012).

Following RYGB surgery, but not BAND surgery, post-prandial secretion of PYY is also increased (Bose et al. 2010; Jacobsen et al. 2012). PYY is secreted earlier and to a greater level than that seen in lean people (Korner et al. 2006; le Roux et al. 2006), and remains higher than pre-operative levels even 2 years after surgery (Pournaras et al. 2010). Attenuated PYY secretion is associated with poor weight loss in RYGB patients (le Roux et al. 2007).

Administration of exogenous PYY to RYGB rats causes increased weight loss (Fenske et al. 2012). RYGB in PYY KO mice induces less weight loss than in wild type mice. Furthermore, gastric bypass PYY KO mice have no difference in weight loss compared to sham-operated and, suggesting that PYY is an important mediator of weight loss after RYGB (Chandarana et al. 2011). Injection of a PYY neutralizing antibody into mice who have undergone a jejeno-

ileal bypass (similar to RYGB), leads to increased food intake the following day (le Roux et al. 2006).

Reduction of PYY and GLP-1 secretion by administration of somatostatin or an analogue, increases food intake in rats (le Roux et al. 2006), and humans that have undergone RYGB surgery (le Roux et al. 2007; Fenske et al. 2012), although somatostatin also suppresses secretion of many other gut hormones including orexigenic ghrelin (see section 4.1.5). Direct blockade of PYY via receptor antagonists has not been tested in RYGB yet, but has been shown to reduce anorexic responses to gastric infusions of protein and long-chain fatty acids (Reidelberger et al. 2013).

Although PYY and GLP-1 have been shown to acutely influence food intake, the chronic effects of PYY and GLP-1 may also be important. For instance, sustained increased salivary PYY resulted in a significant long-term reduction in food intake and body weight in mice (Acosta et al. 2011), and chronic infusions of PYY reduced food intake and body weight in rats (Batterham et al. 2002; Moriya et al. 2009).

4.1.2 Glucose and insulin metabolism after RYGB

The improvements in RYGB in glycaemic control in T2DM appear to be both dependent and independent of the effect of weight loss and potentially mediated by increased incretin secretion (such as GLP-1), decreased caloric intake and improved β-cell function (Wajchenberg 2007; Laferrere et al. 2008; Weir et al. 2009; Isbell et al. 2010; Laferrere 2011; Laferrere 2011; Nannipieri et al. 2011). These improvements are sustained in the long-term

(Sjostrom et al. 2004). Reduction in peripheral insulin resistance occurs in accordance with weight loss, but hepatic insulin resistance can change earlier (Lim et al. 2011). A recent study has also shown using PET 2-deoxy-2-[18F]fluoro-D-glucose that due to early exposure of the ileum to nutrients, increased absorption of glucose in the gut occurs to support gut tissue growth after RYGB so that the intestine becomes a major tissue for glucose disposal, which may also contribute to the improvement in glycaemic control after RYGB (Saeidi et al. 2013).

The changes in insulin sensitivity and secretion may have implications for the effect of RYGB on food hedonics. Insulin reduces appetite centrally in hypothalamic centres, and affects dopamine release in the rat striatum. At low concentrations, insulin increases dopamine release but inhibits release at higher concentrations (Potter et al. 1999). As with leptin, central administration of insulin can reduce sucrose intake in rats (Figlewicz et al. 2006), and increases preference to a place associated with food reward (Figlewicz et al. 2008).

However as with leptin, insulin resistance seen peripherally in obesity may also be present in the brain, and may alter reward processing. For instance, exposure to a high-energy diet increases sucrose self-administration and prevents the ability of centrally administered insulin to reduce sucrose intake (Figlewicz et al. 2006; Cheah et al. 2012). In humans, insulin resistance is associated with attenuated striatal and prefrontal brain glucose metabolism following insulin infusion (Anthony et al. 2006). Altered resting state functional connectivity in the OFC and putamen is influenced by insulin resistance (Kullmann et al. 2012). Moreover, although intranasal insulin augments post-prandial satiety and reduces food intake in normal weight individuals, this effect is not observed in obese individuals (Tschritter et al. 2006; Hallschmid et al. 2008).

Insulin levels correlate with BOLD signal response to food imagery in the putamen and thalamus (Jastreboff et al. 2013), and with reduced activation in the OFC and thalamus to high-calorie food pictures in obese subjects (Wallner-Liebmann et al. 2010). Significant associations have also been found between postprandial changes in insulin, glucose and FFA and postprandial changes in neuronal activity in the precuneus as measured by rCBF with PET in response to a satiating liquid meal, which suggests that both insulin and these metabolites might act as modulators of postprandial neuronal events (Del Parigi et al. 2002).

Evidence from a recent study suggests that RYGB reverses insulin resistance induced changes in brain activation measured by overall brain glucose metabolism (Tuulari et al. 2013).

4.1.3 Effect of bile acid secretion on gut hormone changes after RYGB

In a rat model, delivery of bile into the ileum rather than the duodenum (similarly to RYGB), resulted in greater release of GLP-1 and PYY, reduced food intake and body weight (Pournaras et al. 2012). This study suggested that the delivery of undiluted bile (not bound up in micelles created by progressing through the stomach and proximal intestine and combining with food) to the terminal ileum stimulates bile acids to produce PYY and GLP-1 via TGR5 receptors on L-cells and may have implications for the role of bile acids in modulating PYY and GLP-1 response.

4.1.4 Suppression of gut hormones by Octreotide

Octreotide acetate (Sandostatin, Novartis Pharmaceuticals) is a somatostatin analogue and therefore mimics its action. Somatostatin suppresses the release of a number of gastrointestinal hormones including GLP-1, PYY, PP, gastrin, cholecystokinin (CCK), secretin, motilin, vasoactive intestinal peptide (VIP), gastric inhibitory polypeptide (GIP) and enteroglucagon. It also has various other effects within the gastro-intestinal system including decreasing the rate of gastric emptying, reducing smooth muscle contractions and blood flow within the intestine, suppression of the release of pancreatic hormones, including insulin and glucagon. Octreotide is safely used to treat disorders associated with high levels of gut hormones such as tumours of the pancreas and intestine (Modlin et al. 2010).

Octreotide and somatostatin also suppresses ghrelin release. When administered to children with the genetic obesity Prader-Willi syndrome over 5 days, ghrelin levels were reduced by approximately 60% (Haqq et al. 2003), similar to the reduction observed by acute administration of somatostatin infusion in another study (Tan et al. 2004).

Octreotide is a more potent inhibitor of insulin than naturally occurring somatostatin, and has a longer half-life (90 minutes). It is poorly absorbed from the gut and so is usually administered subcutaneously. It increases fasting glucose levels and lowers fasting insulin in healthy individuals (Parkinson et al. 2002), and its insulin lowering effect promotes weight loss in some but not all obese adults (Velasquez-Mieyer et al. 2003). In normal weight individuals, Octreotide reverses the satiety inducing effects of intraduodenal infusion of glucose (Lavin et al. 1996). In obese and normal-weight humans, Octreotide decreases post-

meal fullness, without altering food intake (Foxx-Orenstein et al. 2003; Cremonini et al. 2005). Peripheral somatostatin infusion also increases satiety and reduces food intake in humans over a 1 hour period, but when intraduodenal fat is introduced, food intake actually increases following somatostatin compared to saline infusion (Lieverse et al. 1995). Intracerebral Octreotide increases food intake in chicks (Tachibana et al. 2011), mice (Stengel et al. 2010) and rats (Danguir 1988) through its action on hypothalamic somatostatin receptors but potentially also through amygdala, hippocampal and striatal pathways in the brain, which also express somatostatin receptors (Viollet et al. 2008). Octreotide has been successfully used in the treatment of hyperinsulinaemic hypoglycaemia after RYGB (Myint et al. 2012). It is also used in the symptomatic treatment of dumping syndrome after RYGB, and improves symptoms but results in weight gain (Vecht et al. 1999).

Octreotide has been shown in previous studies to be an ideal compound to use to lower gut hormones after obesity surgery because its properties, dosage and side effect profile are well understood, and its effects only last a few hours. Octreotide acutely increases food intake in RYGB, but not sham-operated rats (Fenske et al. 2012), and in humans after RYGB (le Roux et al. 2007). In the latter study, 7 obese patients after RYGB were given 100mcg of subcutaneous Octreotide resulting in post-prandial suppression of both PYY and GLP-1 and an almost doubling of ice-cream test meal intake compared to 1ml saline injection. In contrast, 6 control BAND patients had no change in gut hormone secretion or food intake as a result of Octreotide administration.

Octreotide has an inhibitory effect on bile flow, but increases plasma concentrations of bile acid after glucose infusion (Sahin et al. 1999).

4.1.5 Reduced food reward in RYGB surgery

In this Chapter, possible mechanisms underlying the differences in food hedonics observed in Chapter 3 between RYGB and BAND are further explored by the reversal of post-prandial gut hormone responses using Octreotide.

In Chapter 3, reduced brain reward system activation during food evaluation were observed in the fasted state in obese patients who had undergone RYGB compared to patients of similar BMI who had undergone BAND surgery and/or BMI-matched unoperated controls. These differences were accompanied by reduced appeal and palatability of high-calorie foods, and healthier eating behaviour including less proportional fat intake in RYGB patients. Furthermore fasting PYY and post-prandial PYY and GLP-1 were higher in RYGB, but not BAND, compared to BMI-matched unoperated patients, while fasting and post-prandial bile acids were higher and dumping symptoms more frequently reported in RYGB than BAND patients, suggestive of possible underlying mechanisms.

4.2 Hypothesis

1. Lowering plasma anorexigenic gut hormones PYY and GLP-1 in fed obese patients after RYGB surgery will increase hunger, brain reward system and hedonic responses to food.

2. By contrast lowering plasma anorexigenic gut hormones PYY and GLP-1 in fed obese patients after BAND surgery will have less effect to increase hunger, brain reward system and hedonic responses to food than after RYGB surgery.

4.3 Aims

To investigate the effects of lowering the exaggerated anorexigenic gut hormone response (PYY and GLP-1) by acute administration of subcutaneous Octreotide on:

a) reward system activation during evaluation of food pictures, measured by BOLD signal in *a priori* selected regions of interest in the brain in fed obese patients after RYGB and BAND surgery,
b) hunger, food intake and hedonic responses to food in fed obese patients after RYGB and BAND surgery.

4.4 Results

4.4.1 Participant characteristics

Table 4.1 Participant numbers

	RYGB		BAND	
	Fed-Saline	Fed-Octreotide	Fed-Saline	Fed-Octreotide
Attended	9	9	9	9
Scan excluded / contra-indicated	2	2	1	1
Behavioural data included	9	9	8	8
BOLD within subject comparison included	6-7	6-7	8	8

A subset of operated subjects from Chapter 3 attended further visits for scanning and/or behavioural measures for this study. In total 9 RYGB and 9 BAND participants completed Fed-Saline and Fed-Octreotide visits and so could be included in the between group analysis of difference between Fed-Octreotide and Fed-Saline visits.

Of these, 7 RYGB and 9 BAND patients completed fMRI scans on both visits (2 RYGB subjects were not able to undergo fMRI scanning due to scanning contra-indications but completed the rest of the protocol), and 1 BAND subject's scan was excluded from all analyses due to excessive movement during scanning and poor co-operation with the paradigm. In addition for 1 RYGB subject analysis of just the OFC ROI BOLD activation was excluded due to signal drop-out in that area. Therefore 6-7 RYGB and 8 BAND subjects' scans were included in the within-subject analysis of differences between visits in brain activation to food pictures.

In this subset of patients who attended further scanning visits, as in Chapter 3, there were no significant differences between the groups in age, gender ratio, ethnic background distribution, current or pre-operative BMI, percentage body fat, current prevalence of type 2 diabetes mellitus (T2DM) or current or pre-operative binge eating disorder (BED) (Table 4.2). The RYGB group had more obesity-associated co-morbidities pre-operatively including T2DM, but not post-operatively, compared to the BAND group (Table 4.2). There were no differences between the groups in any psychological questionnaire measures of depression, mood, reward sensitivity, impulsivity or personality traits (Table 4.3).

Table 4.2 Characteristics of obese patients after gastric bypass and gastric banding at fMRI scanning

	BAND	**RYGB**	**P[a]**
n	9	9	
Age (years)	42.2 ± 3.9 (26.0 - 60.0)	47.8 ± 1.6 (42.0 - 59.0)	0.21
Gender (Male : Female)	1:8	1:8	1.00
Ethnicity: European Caucasians, n (%)	7 (78%)	9 (100%)	0.47
Pre-operative BMI (kg/m^2)	46.5 ± 2.1 (35.7 - 55.6)	51.2 ± 4.8 (35.2 - 73.7)	0.39
Current BMI (kg/m^2)	33.1 ± 2.1 (25.1 - 43.9)	36.2 ± 2.9 (23.9 - 48.3)	0.40
Current Height (m)	1.76 ± 0.03 (1.53 - 1.81)	1.96 ± 0.03 (1.60 – 1.83)	0.53
Current Weight (kg)	91.1 ± 87.0 (75.0 - 115.2)	102.8 ± 7.0 (64.4 - 129.8)	0.19
Current body fat (%)	40.5 ± 3.1 (23.6 - 53.3)	40.5 ± 4.1 (24.7 - 57.0)	0.99
Weight loss (% of pre-operative weight)	28.2 ± 4.4 (7.9 - 52.5)	28.5 ± 2.1 (20.7 - 38.2)	0.96
Time since surgery (months)	20.2 ± 4.3 (6.7 – 48.0)	15.6 ± 2.4 (5.3 – 23.5)	0.36
Pre-operative DM, n (%)	0 (0%)	5 (56%)	**0.03**
Current DM, n (%)	0 (0%)	1 (11%)	1.00
Pre-operative obesity co-morbidity score	5.1 ± 0.8 (2.0 - 9.0)	10.0 ± 1.7 (3.0 - 19.0)	**0.03**
Current obesity co-morbidity score	1.4 ± 0.5 (0.0 - 4.0)	2.9 ± 1.3 (0.0 - 10.0)	0.30
Pre-operative BED, n (%)	2 (22%)	2 (22%)	1.00
Current BED, n (%)	0 (0%)	1 (11%)	1.00

Data presented as mean ± SEM, and (range). [a] P value for comparison of averages between groups using independent t-test or Chi-squared test for frequencies. Abbreviations: BAND: gastric banding, BED: binge eating disorder; DM: type 2 diabetes mellitus, n/a not applicable; RYGB: gastric bypass.

Table 4.3 Psychological questionnaires

	BAND	RYGB	P[a]
n	9	9	
Beck Depression Inventory II (score/63)	6.7 ± 2.0 (1.0 - 18.0)	2.7 ± 0.8 (0.0 - 8.0)	0.13
Moderate-severe depression (>15), n (%)	1 (11%)	0 (0%)	0.30
On antidepressants treatment, n (%)	2 (22%)	3 (33%)	0.60
PANAS			
Negative affect (score /50)	15.8 ± 1.1 (13.0 - 21.0)	13.9 ± 0.9 (11.0 - 19.0)	0.20
Positive affect (score /50)	34.0 [29.5 - 41.5]	38.0 [28.5 - 41.0]	0.97
Behavioural activation and inhibition scale			
BAS drive (score /16)	11.2 ± 0.7 (9.0 - 15.0)	9.0 ± 1.2 (4.0 - 13.0)	0.33
BAS reward responsiveness (score /20)	17.0 ± 0.7 (14.0 - 20.0)	15.3 ± 0.9 (11.0 - 19.0)	0.35
BAS fun-seeking (score /16)	12.2 ± 0.6 (9.0 - 15.0)	10.2 ± 0.5 (8.0 - 12.0)	0.07
BIS (score /28)	19.6 ± 0.8 (16.0 - 22.0)	19.6 ± 1.0 (16.0 - 26.0)	0.86
Impulsivity			
Barratt impulsivity scale (score /120)	60.0 [50.5 - 63.5]	62.0 [52.5 - 74.0]	0.66
EPQ-R			
Extraversion (score /23)	16.6 ± 1.3 (9.0 - 21.0)	13.0 ± 1.8 (4.0 - 20.0)	0.18
Psychoticism (score /32)	6.0 ± 0.9 (2.0 - 10.0)	4.7 ± 0.8 (2.0 - 8.0)	0.13
Neuroticism (score /24)	9.8 ± 1.5 (1.0 - 17.0)	11.7 ± 1.9 (2.0 - 21.0)	0.39
Lying (score /21)	9.7 ± 1.4 (4.0 - 17.0)	10.7 ± 1.0 (6.0 - 16.0)	0.81

Data included for those participants who completed fMRI scanning. Questionnaire scores adjusted for age, gender and BMI. Data is presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).

[a] P value for overall comparison of averages between groups using independent t-test or Mann-Whitney U where not normally distributed.

Abbreviations: BAND: gastric banding, BAS/BIS: Behavioural Activation and Inhibition Scale, EPQ-R: Eysenck Personality Questionnaire, PANAS: Positive and Negative Affect Schedule, RYGB: gastric bypass.

4.4.2 Brain activation during food picture evaluation

4.4.2.1 Effect of Octreotide on brain activation to food in RYGB and BAND groups

In the RYGB group, BOLD activation in the nucleus accumbens when viewing all food and low-calorie but not high-calorie food was significantly higher at the Fed-Octreotide than Fed-Saline visit (Table 4.4, Fig. 4.1E). There were no significant or trend for significant differences in BOLD activation in the other ROIs between visits (Table 4.4, Fig. 4.1B,C,D,F). There was a trend (P=0.09) for BOLD signal in the average reward system ROIs (average of OFC, amygdala, anterior insula, nucleus accumbens and caudate) for any food and low-calorie food, but not high-calorie food, to be higher at the Fed-Octreotide than the Fed-Saline visit (Table 4.4, Fig. 4.1A).

In the BAND group, there were no significant or trend for significant differences in BOLD activation in the average reward system ROIs, or any individual ROI, to any food, high-calorie or low-calorie food between the Fed-Saline and Fed-Octreotide visits (Table 4.4, Fig. 4.2A-F).

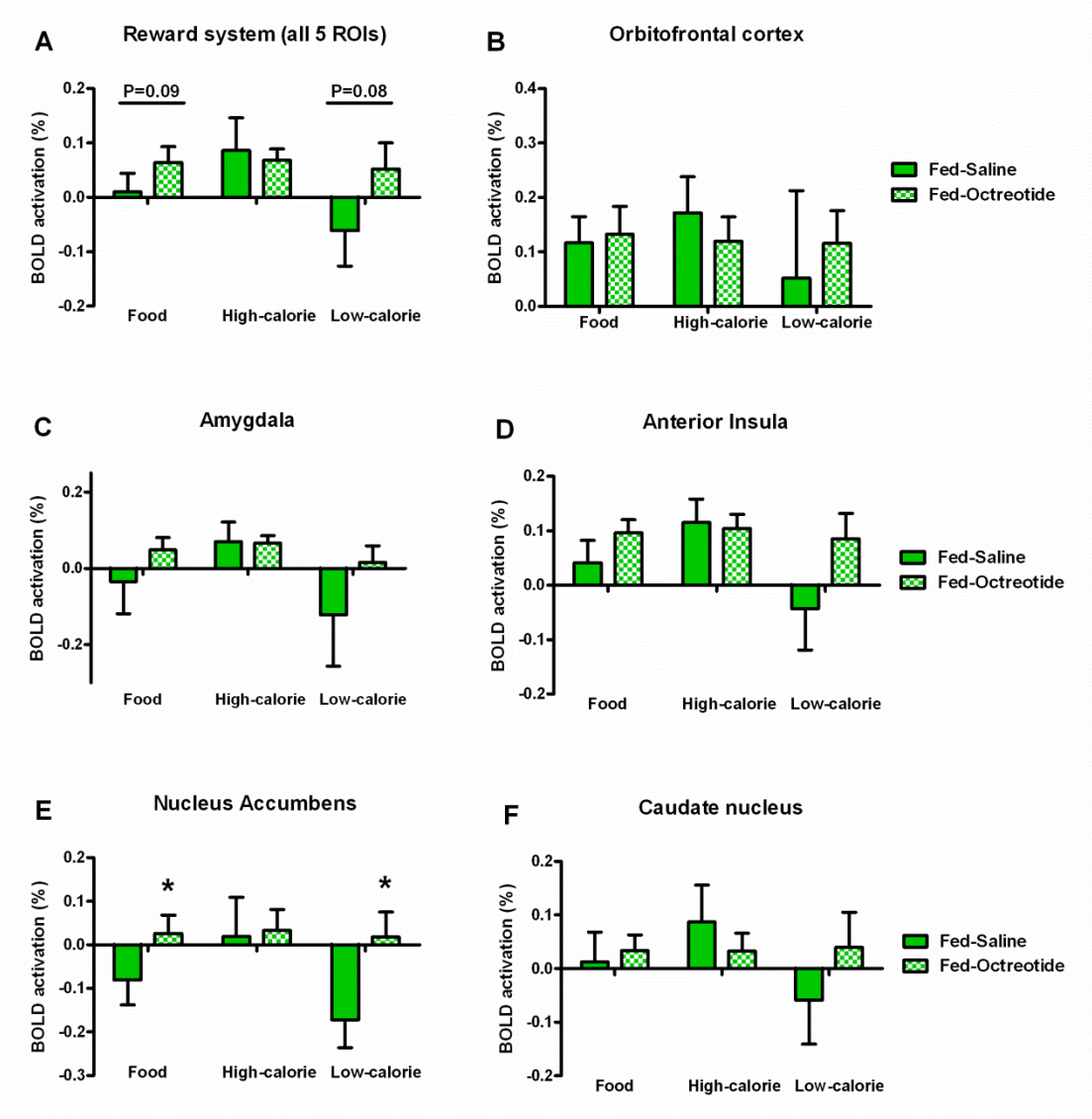
4.4.2.2 Difference in effect of Octreotide on brain activation to food between surgical groups

There were no significant or trend for significant differences between the RYGB and BAND groups in the change in BOLD activation between Fed-Saline and Fed-Octreotide visits in any ROI (Table 4.4, Fig. 4.3).

4.4.2.3 Effect of Octreotide on brain activation during auditory-motor-visual task

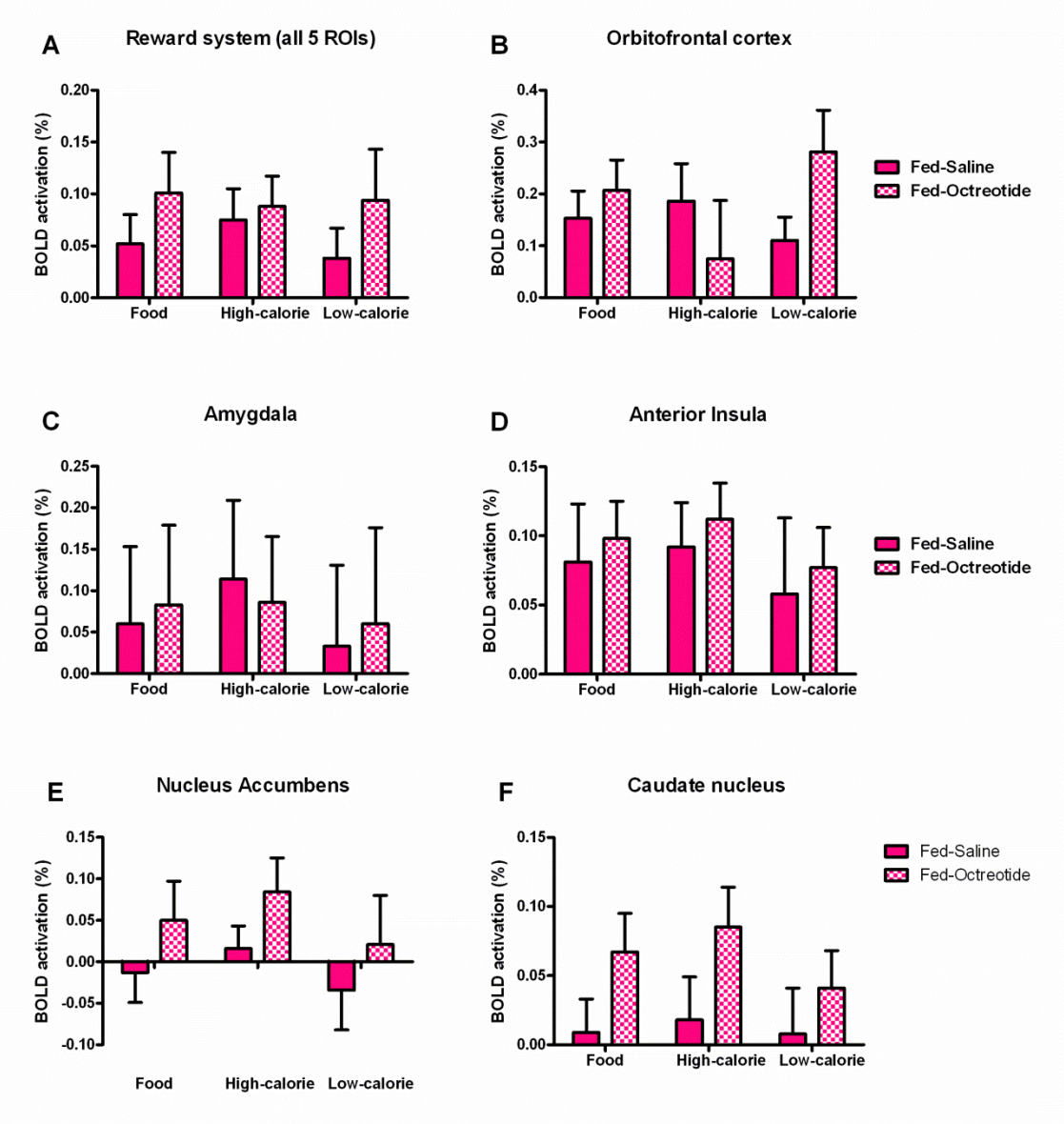
There were no significant or trend for significant effects of Octretode in the RYGB group on BOLD activation, or for difference in effects between the groups in the change in BOLD activation between Fed-Saline and Fed-Octreotide visits, in the posterior division superior temporal gyrus, left precentral gyrus or lingual gyrus respectively during the control auditory-motor-visual task (Table 4.4). The BAND group had significantly less activation in the left precentral and lingual gyrus in the Fed-Octreotide condition during the control task.

Figure 4.1 Effect of Octreotide on region of interest BOLD activation to food pictures in fed obese patients after gastric bypass surgery



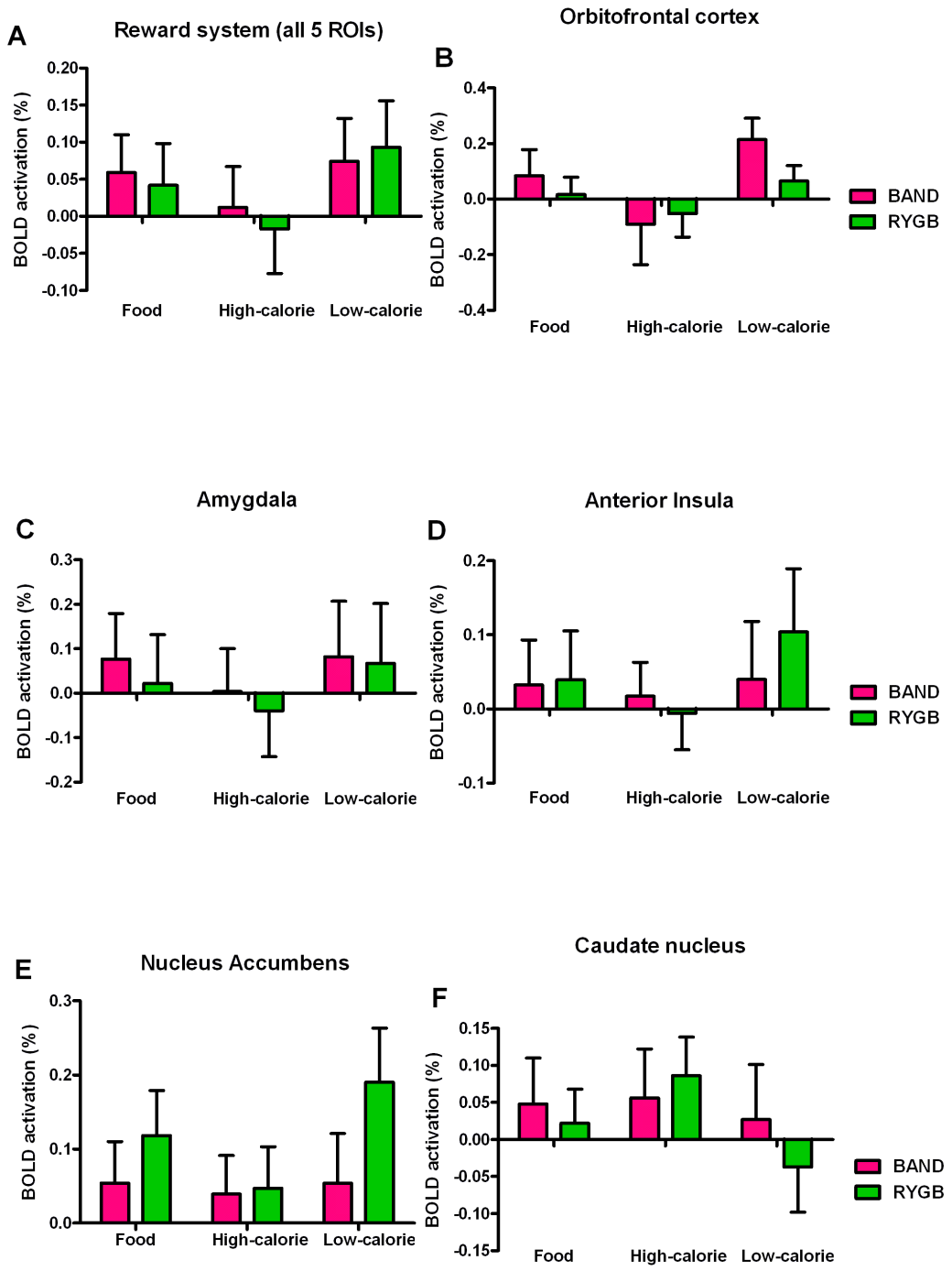
Comparison of BOLD activation to any food, only high-calorie or only low-calorie food (vs. objects), after consuming milkshake breakfast of 385kCal, in obese patients after gastric bypass (RYGB) surgery in *a priori* functional regions of interest (fROI), adjusting for age, gender and BMI, between Fed-Saline (green solid) and Fed-Octreotide (green checked) visits: (A) average in all five fROIs, (B) orbitofrontal cortex (OFC), (C) amygdala, (D) anterior insula, (E) nucleus accumbens, (F) caudate. Data are presented as mean ± SEM; n=7 per group, except OFC n=6. *P<0.05 vs. Fed-Saline

Figure 4.2 Effect of Octreotide on region of interest BOLD activation to food pictures in fed obese patients after gastric banding surgery



Comparison of BOLD activation to any food, only high-calorie or only low-calorie food (vs. objects), after consuming milkshake breakfast of 385kCal, in obese patients after gastric banding (BAND) surgery in *a priori* functional regions of interest (fROI) adjusting for age, gender and BMI, between Fed-Saline (red solid) and Fed-Octreotide (red checked) visits: (A) average in all five fROIs, (B) orbitofrontal cortex (OFC), (C) amygdala, (D) anterior insula, (E) nucleus accumbens, (F) caudate. Data are presented as mean ± SEM; n=8 per group.

Figure 4.3 Difference in effect of Octreotide on region of interest BOLD activation to food pictures in fed obese patients after gastric bypass and gastric banding



Comparison of difference in BOLD signal to any food, only high-calorie or only low-calorie food (vs. objects), after consuming milkshake breakfast of 385kCal, and administration of subcutaneous Octreotide vs. saline in *a priori* functional regions of interest (fROI) between obese patients after gastric banding (BAND, red, n=8) and gastric bypass (RYGB, green, n=7 except OFC n=6) surgery, adjusting for age, gender and BMI. (A) Average in all five fROIs, (B) orbitofrontal cortex (OFC), (C) amygdala, (D) anterior insula, (E) nucleus accumbens, (F) caudate. Data are presented as mean ± SEM**.**

Table 4.4 Region of interest activation during food evaluation and auditory-motor-visual control task

Region of interest	Contrast	RYGB			BAND			FED OCTREOTIDE – FED SALINE		
		Fed-Saline	Fed-Octreotide	P	Fed-Saline	Fed-Octreotide	P	BAND	RYGB	P [a]
n		7	7		8	8		8	7	
FOOD EVALUATION TASK										
Reward system (all 5 ROIs)	**Food**	0.010 ± 0.034 (-0.158 - 0.130)	0.064 ± 0.029 (-0.033 - 0.149)	0.09	0.052 ± 0.028 (-0.089 - 0.177)	0.101 ± 0.039 (-0.052 - 0.332)	0.37	0.049 ± 0.051 (-0.142 - 0.269)	0.054 ± 0.027 (-0.025 - 0.162)	0.84
	High-calorie	0.086 ± 0.060 (-0.101 - 0.370)	0.068 ± 0.021 (0.000 - 0.163)	0.77	0.095 [0.062 - 0.134] (-0.120 - 0.142)	0.096 [0.008 - 0.142] (-0.022 - 0.227)	0.90	0.011 [-0.079 - 0.084] (-0.164 - 0.241)	-0.019 [-0.033 - 0.127] (-0.313 - 0.153)	0.75
	Low-calorie	-0.061 ± 0.065 (-0.196 - 0.024)	0.052 ± 0.048 (-0.081 - 0.205)	0.08	0.020 [-0.026 - 0.098] (-0.048 - 0.189)	0.079 [0.038 - 0.101] (-0.076 - 0.406)	0.48	0.056 ± 0.056 (-0.126 - 0.345)	0.113 ± 0.054 (-0.045 - 0.375)	0.84
Orbitofrontal cortex *	**Food**	0.117 ± 0.048 (0.004 - 0.307)	0.133 ± 0.051 (-0.079 - 0.243)	0.81	0.153 ± 0.052 (-0.037 - 0.405)	0.207 ± 0.058 (-0.029 - 0.464)	0.59	0.054 ± 0.095 (-0.434 - 0.447)	0.016 ± 0.063 (-0.145 - 0.239)	0.77
	High-calorie	0.172 ± 0.066 (-0.043 - 0.373)	0.120 ± 0.044 (-0.059 - 0.251)	0.57	0.186 ±0.072 (-0.093 - 0.489)	0.075 ± 0.112 (-0.454 - 0.609)	0.47	-0.112 ± 0.148 (-0.673 - 0.448)	-0.052 ± 0.085 (-0.311 - 0.294)	0.76
	Low-calorie	0.052 ± 0.161 (-0.085 - 0.254)	0.116 ± 0.060 (-0.119 - 0.306)	0.31	0.110 ± 0.045 (-0.032 - 0.320)	0.281 ± 0.080 (-0.031 - 0.721)	0.09	0.171 ± 0.085 (-0.351 - 0.448)	0.064 ± 0.057 (-0.113 - 0.185)	0.35
Amygdala	**Food**	-0.035 ± 0.084 (-0.444 - 0.197)	0.048 ± 0.032 (-0.080 - 0.161)	0.23	0.061 [-0.209 - 0.236] (-0.281 - 0.497)	0.039 [-0.024 - 0.101] (-0.245 - 0.704)	0.78	0.023 ± 0.114 (-0.384 - 0.465)	0.083 ± 0.061 (-0.103 - 0.364)	0.75
	High-calorie	0.070 ± 0.051 (-0.154 - 0.217)	0.066 ± 0.019 (-0.017 - 0.112)	0.94	0.102 [-0.029 - 0.269] (-0.324 - 0.599)	0.061 [-0.037 - 0.128] (-0.192 - 0.571)	0.67	-0.028 ± 0.103 (-0.467 - 0.422)	-0.004 ± 0.047 (-0.181 - 0.137)	0.78

	Low-calorie	0.056 [-0.308 - 0.332] (0.019 - 0.090)	0.004 [-0.082 - 0.099] (-0.131 - 0.201)	0.74	0.033 0.098 (-0.397 - 0.418)	0.052 0.101 (-0.283 - 0.696)	0.88	0.007 [-0.336 - 0.384] (-0.367 - 0.519)	0.007 [-0.052 - 0.536] (-0.083 - 0.593)	0.94
Nucleus accumbens	**Food**	-0.087 [-0.426 - 0.065] (-0.172 - 0.064)	0.065 [-0.110 - 0.112] (-0.150 - 0.128)	0.04	-0.013 ± 0.036 (-0.131 - 0.163)	0.050 ± 0.047 (-0.107 - 0.274)	0.30	0.063 ± 0.056 (-0.204 - 0.308)	0.107 ± 0.034 (-0.018 - 0.215)	0.50
	High-calorie	0.041 ± 0.041 (-0.092 - 0.200)	0.033 ± 0.048 (-0.103 - 0.279)	0.82	0.016 ± 0.027 (-0.070 - 0.154)	0.084 ± 0.041 (-0.040 - 0.250)	0.18	0.068 ± 0.046 (-0.089 - 0.260)	0.014 ± 0.056 (-0.244 - 0.206)	0.93
	Low-calorie	0.115 ± 0.043 (-0.027 - 0.243)	0.018 ± 0.057 (-0.161 - 0.195)	0.03	-0.034 ± 0.048 (-0.283 - 0.152)	0.021 ± 0.059 (-0.140 - 0.270)	0.41	0.104 [-0.093 - 0.167] (-0.271 - 0.243)	0.190 [0.038 - 0.297] (0.023 - 0.487)	0.24
Anterior Insula	**Food**	-0.043 ± 0.076 (-0.403 - 0.228)	0.096 ± 0.024 (0.041 - 0.206)	0.15	0.081 ± 0.042 (-0.130 - 0.268)	0.098 ± 0.027 (-0.018 - 0.222)	0.78	0.017 ± 0.062 (-0.287 - 0.259)	0.055 ± 0.033 (-0.051 - 0.152)	0.94
	High-calorie	0.019 ± 0.090 (-0.308 - 0.332)	0.104 ± 0.026 (0.016 - 0.206)	0.80	0.092 ± 0.032 (-0.092 - 0.200)	0.112 ± 0.026 (0.003 - 0.227)	0.64	0.021 ± 0.043 (-0.118 - 0.246)	-0.011 ± 0.041 (-0.179 - 0.135)	0.76
	Low-calorie	-0.172 ± 0.064 (-0.426 - 0.065)	0.085 ± 0.047 (-0.092 - 0.248)	0.10	0.055 [-0.066 - 0.090] (-0.137 - 0.378)	0.076 [-0.010 - 0.156] (-0.033 - 0.185)	0.58	0.019 ± 0.075 (-0.411 - 0.267)	0.128 ± 0.066 (-0.093 - 0.424)	0.62
Caudate	**Food**	-0.020 [-0.063 - 0.177] (-0.202 - 0.227]	0.076 [-0.054 - 0.101] (-0.061 - 0.104)	0.74	0.009 ± 0.024 (-0.100 - 0.084)	0.067 ± 0.028 (-0.017 - 0.206)	0.29	0.058 ± 0.050 (-0.101 - 0.306)	0.021 ± 0.054 (-0.201 - 0.167)	0.89
	High-calorie	0.087 ± 0.069 (-0.139 - 0.371)	0.033 ± 0.033 (-0.091 - 0.178)	0.41	0.018 ± 0.031 (-0.130 - 0.110)	0.085 ± 0.029 (-0.022 - 0.212)	0.25	-0.009 [-0.039- 0.246] (-0.053 - 0.311)	-0.021 [-0.221 - 0.040] (-0.287 - 0.194)	0.38
	Low-calorie	-0.059 ± 0.082 (-0.393 - 0.275)	0.040 ± 0.065 (-0.172 - 0.278)	0.31	0.008 ± 0.033 (-0.120 - 0.154)	0.041 ± 0.027 (-0.073 - 0.170)	0.55	0.033 ± 0.052 (-0.151 - 0.263)	0.099 ± 0.089 (-0.269 - 0.462)	0.52

CONTROL TASK										
Post. division superior temporal gyrus	**Auditory**	0.686 [0.513 - 1.005] (0.312 - 1.765)	0.643 [0.250 - 0.944] (-0.107 - 1.084)	0.46	0.683 ± 0.152 (-0.179 - 1.239)	0.662 ± 0.174 (0.039 - 1.451)	0.90	-0.021 ± 0.164 (-0.782 - 0.597)	-0.209 ± 0.315 (-1.339 - 0.772)	0.56
Left precentral gyrus	**Motor**	0.444 [-0.211 - 0.603] (-0.604 - 0.603)	0.072 [-0.035 - 0.240] (-0.083 - 0.575)	0.60	0.646 ± 0.118 (0.290 - 1.313)	0.179 ± 0.071 (-0.078 - 0.513)	0.02	-0.467 ± 0.161 (-1.287 - 0.056)	-0.111 ± 0.172 (-0.531 - 0.585)	0.33
Lingual gyrus	**Visual**	0.562 ± 0.418 (-1.157 - 1.958)	0.474 ± 0.131 (0.093 - 0.804)	0.85	1.006 ± 0.227 (-0.025 - 1.920)	0.573 ± 0.285 (-0.404 - 2.133)	0.01	-0.433 ± 0.130 (-0.949 - 0.213)	-0.088 ± 0.455 (-1.253 - 1.883)	0.75

Average group activation in separate and combined *a priori* regions of interest (ROI) for food category vs. objects during food evaluation task, or auditory, motor or visual cortex during control task. Data adjusted for age, gender and BMI in the between group difference of effect of Octreotide. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed and (range).

[a] P value for overall comparison of averages between visits or groups using paired or independent t-test for normally distributed data or Mann-Whitney U or Wilcoxon Signed test where not normally distributed.

*OFC: 1 subject data excluded in RYGB group for both visits due to signal dropout

Abbreviations: BAND: gastric banding, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, RYGB: gastric bypass.

Abbreviations: BAND: gastric banding, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, RYGB: gastric bypass.

4.4.3 Food appeal scores

4.4.3.1 Effect of Octreotide on food appeal scores in RYGB and BAND patients

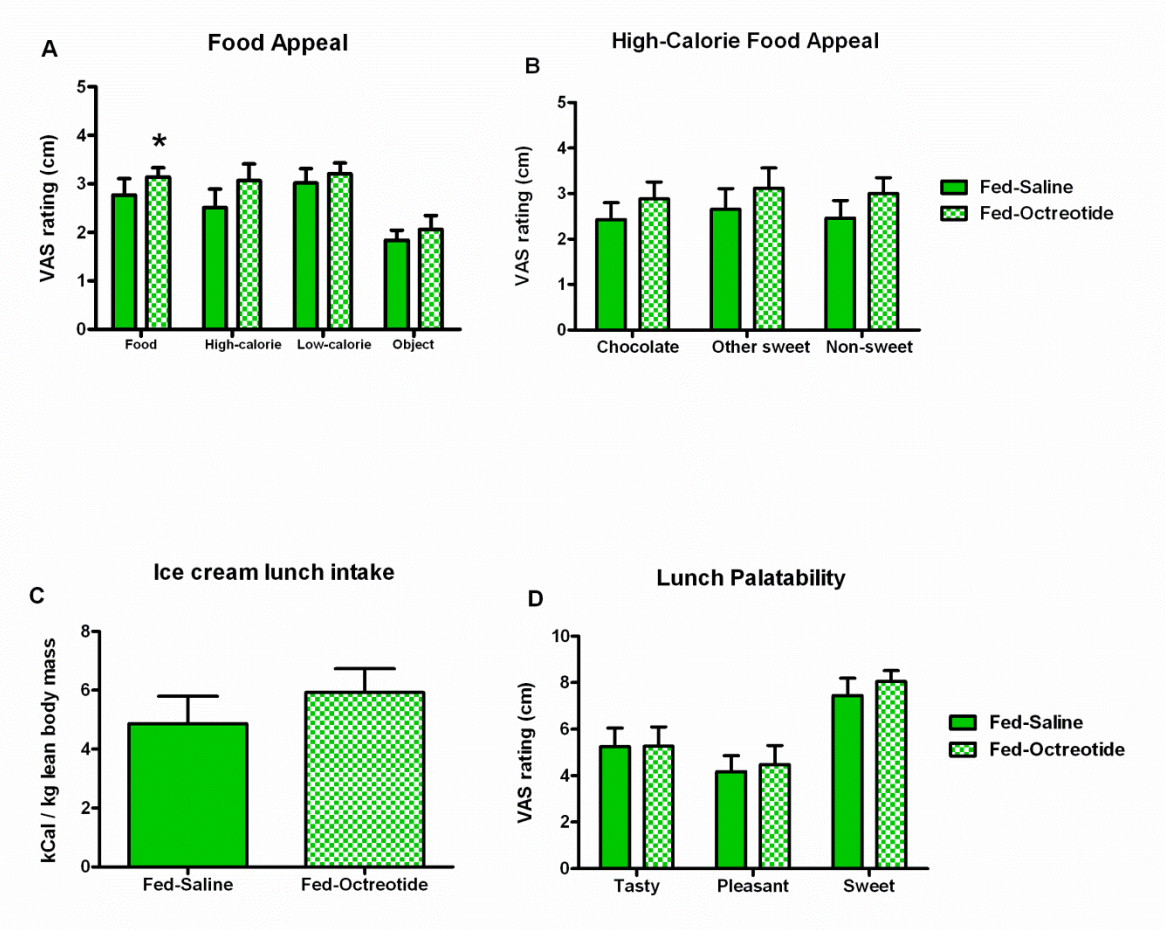
In the food evaluation task, RYGB subjects rated any food (high- and low-calorie) pictures as more appealing in the Fed-Octreotide compared with the Fed-Saline visit (Table 4.5, Fig. 4.4A). There were no significant differences in ratings of individual subcategories of high-calorie food pictures or object or blurred pictures between the Fed-Saline and Fed-Octreotide visits in RYGB subjects (Table 4.5, Fig. 4.4A,B).

There was no difference in the appeal rating of any food, high-calorie or low-calorie food, or object and blurred pictures between the Fed-Saline and Fed-Octreotide visits in the BAND group (Table 4.5, Fig. 4.5A,B).

4.4.3.2 Difference in effect of Octreotide on food appeal scores between surgical groups

There was no significant difference between the RYGB and BAND groups in the change in appeal rating for any of the picture categories between the Fed-Saline and Fed-Octreotide visits (Table 4.5, Fig. 4.6A,B)

Figure 4.4 Effect of Octreotide on food hedonics and meal palatability in fed obese patients after gastric bypass surgery

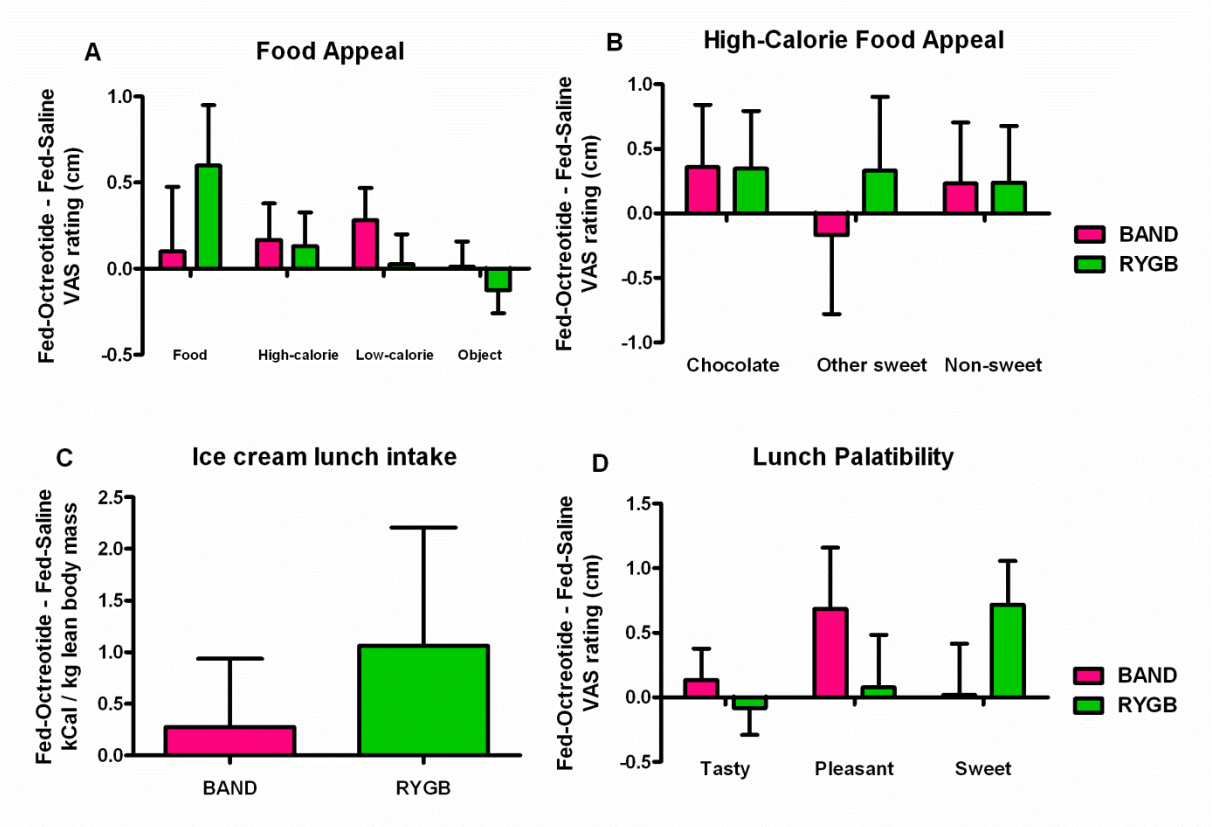


Comparison of (A) appeal of any food, only high-calorie or low-calorie food, or object pictures; (B) appeal of sub-categories of high-calorie food pictures; (C) ice cream lunch intake adjusted for lean body mass (D) VAS of ice cream lunch palatability, in obese patients after gastric bypass surgery (RYGB) between Fed-Saline (solid green) and Fed-Octreotide (green checked) visits. Data are presented as mean ± SEM; n=9 per group. *P<0.05 vs. Fed-Saline

Figure 4.5 Effect of Octreotide on food hedonics, hunger and meal palatability in fed obese patients after gastric banding surgery

Comparison of (A) appeal of any food, only high-calorie or low-calorie food, or object pictures; (B) appeal of sub-categories of high-calorie food pictures; (C) ice cream lunch intake adjusted for lean body mass, (D) VAS of ice cream lunch palatability, in obese patients after gastric bypass banding (BAND) between Fed-Saline (solid red) and Fed-Octreotide (checked red) visits. Data are presented as mean ± SEM; n=8 per group.

Figure 4.6 Difference in effect of Octreotide on food hedonics, meal palatability in fed obese patients after gastric bypass and gastric banding surgery



Comparison of difference between fed-saline and fed-Octreotide visits in (AA) appeal of any food, only high-calorie or low-calorie food, or object pictures; (B) appeal of sub-categories of high-calorie food pictures; (C) ice cream lunch intake adjusted for lean body mass, (D) VAS of ice cream lunch palatability, between obese patients after gastric banding (BAND, red) and gastric bypass (RYGB, green) surgery. Data are presented as mean ± SEM; n=8-9 per group.

Table 4.5 Appeal scores

APPEAL SCORES [b]	RYGB			BAND			FED OCTREOTIDE – FED SALINE		
	Fed-Saline	Fed-Octreotide	P[a]	Fed-Saline	Fed-Octreotide	P[a]	BAND	RYGB	P[a]
	9[c]	9[c]		8	8		8	9[c]	
Food	2.79 ± 0.28 (1.00 - 3.62)	3.14 ± 0.19 (2.22 - 3.87)	0.047	3.32 [2.88 -3.72] (1.51 - 3.82)	3.41 [2.83 - 3.83] (1.69 - 4.49)	0.46	0.03 [-0.24 - 0.50] (-0.48 - 1.14)	0.25 [0.02 - 0.63] (-0.09 - 1.25)	0.28
High-calorie	2.48 ± 0.40 (1.00 - 4.23)	3.07 ± 0.34 (1.00 - 4.28)	0.13	3.28 ± 0.36 (1.28 - 4.57)	3.40 ±0.38 (1.28 - 4.38)	0.68	0.02 [-0.53 - 0.57] (-0.82 - 1.50)	0.20 [0.04 - 0.79] (-0.15 - 3.18)	1.00
Chocolate	2.38 ± 0.37 (1.00 - 4.24)	2.88 ± 0.37 (1.00 - 4.20)	0.18	3.09 ±0.46 (1.33 - 4.68)	3.64 ± 0.40 (1.25 - 4.65)	0.17	0.09 [-0.09 - 1.55] (-0.29 - 2.37)	-0.01 [-0.17 -0.83] (-3.05 - 3.05)	0.32
Sweet non-chocolate	2.60 ± 0.46 (1.00 - 4.20)	3.12 ± 0.44 (1.00 - 4.55)	0.14	3.20 ± 0.47 (1.15 - 5.00)	3.22 ±0.46 (1.28 - 5.00)	0.97	-0.08 [-0.82 - 0.41] (-1.13 - 2.04)	0.22 [-0.08 - 0.81] (-4.15 - 3.55)	0.74
Savoury	2.48 ± 0.39 (1.00 -4.26)	3.00 ± 0.35 (1.00 - 4.21)	0.15	3.06 ± 0.39 (1.37 - 4.43)	3.35 ±0.38 (1.32 - 4.47)	0.52	0.03 [-0.75 - 1.11] (-1.01 - 2.73)	0.08 [-0.08 - 0.86] (-3.21 – 2.95)	1.00
Low-calorie	3.43 [2.91 - 3.63] (1.00 - 3.75)	3.43 [2.61 - 3.79] (2.20 - 3.98)	0.59	3.00 ±0.30 (1.74 - 3.93)	3.17 ± 0.29 (2.10 - 4.70)	0.22	0.18 ± 0.13 (-0.28 - 0.77)	0.12 ± 0.22 (-1.23 - 1.24)	0.84
Object	2.02 [1.26 - 2.73] (1.04 - 3.12)	2.34 [1.13 - 2.86] (1.02 - 3.08)	0.53	2.27 ± 0.29 (1.07 - 3.36)	2.49 ± 0.20 (1.62 - 3.30)	0.30	0.22 ± 0.20 (-0.37 - 1.24)	0.08 ± 0.12 (-0.31 - 0.84)	0.56
Blurred	1.14 [1.04 -2.26] (1.01 - 2.90)	1.14 [1.03 - 1.86] (1.00 - 3.05)	0.53	1.55 [1.06 - 2.56] (1.01 - 3.29)	1.32 [1.03 -2.48] (1.02 -3.84)	0.78	-0.03 ± 0.10 (-0.43 - 0.55)	-0.09 ± 0.13 (-0.84 - 0.53)	0.72

Data included for those participants who attended study visits, and includes 2 RYGB subjects who completed rest of the paradigm, including food evaluation task, but were not eligible for fMRI scanning. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).

[a] P value for overall comparison of averages between visits or groups using paired or independent t-test for data that is normally distributed or Mann-Whitney U or Wilcoxon signed test where not normally distributed.

[b] 1 = Not at all, 5 = A lot

[c] Includes scores of 2 subjects who did not complete fMRI scanning but did complete rest of the paradigm

Abbreviations: BAND: gastric banding, Fed Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide injection prior to scanning, RYGB: gastric bypass

Table 4.6 Food palatability and ice-cream intake

	RYGB			BAND			FED OCTREOTIDE - FED SALINE		
	Fed-Saline	**Fed-Octreotide**	**P[a]**	**Fed-Saline**	**Fed-Octreotide**	**P[a]**	**BAND**	**RYGB**	**P[a]**
n	9[c]	9[c]		8	8		8	9[c]	
Lunch intake									
Total (kCal)	301.7 ± 59.5 (37.0 - 563.0)	349.3 ± 48.3 (103.0 - 563.0)	0.41	280.1 ± 47.9 (113.0 - 451.0)	293.3 ± 55.8 (113.0 - 563.0)	0.70	13.3 ± 32.9 (-113.0 - 113.0)	47.8 ± 54.8 (-225.0 - 301.0)	0.61
Corrected (kCal/kg LBM)	4.9 ± 0.9 (1.0 -0.1)	5.9 ± 0.8 (1.8 - 9.9)	0.38	5.2 ± 0.9 (1.7 - 8.9)	5.5 ± 1.1 (1.7 - 10.8)	0.70	0.3 ± 0.7 (-2.2 - 2.8)	1.1 ± 1.1 (-4.7 - 7.4)	0.57
VAS lunch palatability (cm)									
Tastiness	5.2 ± 0.8 (1.9 - 8.6)	5.3 ± 0.8 (2.5 - 8.7)	0.91	7.4 [5.3 - 7.9] (3.3 - 8.3)	6.8 [5.1 - 7.7] (1.9 - 7.9)	0.36	-0.4 [-0.7 - 0.4] (-5.1 - 1.7)	0.1 [-0.4 - 0.3] (-0.9 - 1.2)	0.37
Pleasantness to eat	4.2 ± 0.7 (1.2 - 7.4)	4.5 ± 0.8 (1.3 - 8.7)	0.51	6.8 ± 0.5 (3.8 - 8.5)	6.3 ± 0.7 (1.8 - 8.3)	0.61	-0.5 ± 0.9 (-6.7 - 1.5)	0.3 ± 0.4 (-1.1 - 3.2)	0.43
Sweetness	8.6 [5.2 - 9.2] (3.7 -9.6)	8.5 [6.9 - 9.2] (5.4 - 9.8)	0.10	7.3 ± 0.4 (5.3 - 8.5)	7.3 ± 0.5 (5.1 - 9.1)	0.45	0.2 ± 0.2 (-0.6 - 1.0)	0.6 ± 0.4 (-0.7 - 3.0)	0.36
VAS breakfast palatability (cm)									
Tastiness	4.2 ± 1.0 (0.0 - 8.8)	3.8 ± 1.0 (0.0 - 9.1)	0.58	4.5 ± 0.7 (1.3 - 8.0)	4.8 ± 0.6 (1.7 - 7.0)	0.57	0.3 ± 0.4 (-1.9 - 1.7)	0.4 ± 0.7 (-5.3 - 2.0)	0.46
Pleasantness to eat	4.1 ± 1.0 (0.0 - 9.1)	4.1 ± 1.1 (0.0 - 9.3)	0.92	4.1 ± 0.9 (0.3 - 8.1)	4.8 ± 0.5 (2.0 - 6.4)	0.41	0.7 ± 0.7 (-3.1 - 4.1)	0.0 ± 0.4 (-2.0 - 2.2)	0.47
Sweetness	6.2 ± 0.6 (3.7 - 8.5)	6.0 ± 0.8 (2.4 - 9.2)	0.66	6.5 ± 0.5 (4.5 - 8.1)	7.0 ± 0.7 (3.9 - 9.8)	0.41	0.6 ± 0.6 (-2.9 - 2.1)	-0.2 ± 0.5 (-2.3 - 2.2)	0.33

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed and (range).

[a] P value for overall comparison of averages between visits or groups using paired or independent t-test for data that is normally distributed or Mann-Whitney U or Wilcoxon signed test where not normally distributed. [c] Includes scores of 2 subjects who did not complete fMRI scanning but did complete rest of the paradigm.

Abbreviations: BAND: gastric banding, BMI-M: body mass index matched, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, LBM: lean body mass, RYGB: gastric bypass, VAS: visual analogue.

4.4.4 Test meal intake and palatability

4.4.4.1 Effect of Octreotide on food intake and palatability in RYGB and BAND

There was no significant difference in ice-cream test meal intake between the Fed-Saline and Fed-Octreotide visits in the RYGB or the BAND group (Table 4.6, Fig. 4.4 C, D and Fig. 4.5 C, D). There were also no significant differences in palatability ratings of the milkshake breakfast between the two visits in either RYGB or BAND groups (Table 4.6).

4.4.4.2 Difference in effect of Octreotide on food intake and palatability between surgical groups

There were no significant differences between the RYGB and BAND groups in the change in ice-cream intake or palatability ratings between the Fed-Saline and Fed-Octreotide visits (Table 4.6, Fig. 4.6 C, D).

4.4.5 Appetite visual analogue scales

4.4.5.1 Effect of Octreotide on visual analogue scales in RYGB and BAND

During scanning, there were no significant differences in VAS ratings of 'hunger', 'fullness', 'pleasantness to eat' and 'volume of food they could eat' between the Fed-Saline or the Fed-Octreotide visit in either the RYGB or BAND group (Table 4.7, Fig. 4.7 and 4.8).

The RYGB group reported significantly less reduction in the volume of food they felt able to eat after the ice cream lunch meal on the Fed-Octreotide visit than on the Fed-Saline visit, whereas there was no significant difference between visits in the BAND group. There were

no differences in the change in VAS ratings of 'hunger', 'fullness', 'pleasantness to eat' after the ice cream lunch meal between the Fed-Saline and Fed-Octreotide visits in either the RYGB or BAND group (Table 4.7, Fig. 4.7 and 4.8).

4.4.5.2 Difference in effect of Octreotide on visual analogue scales between surgical groups

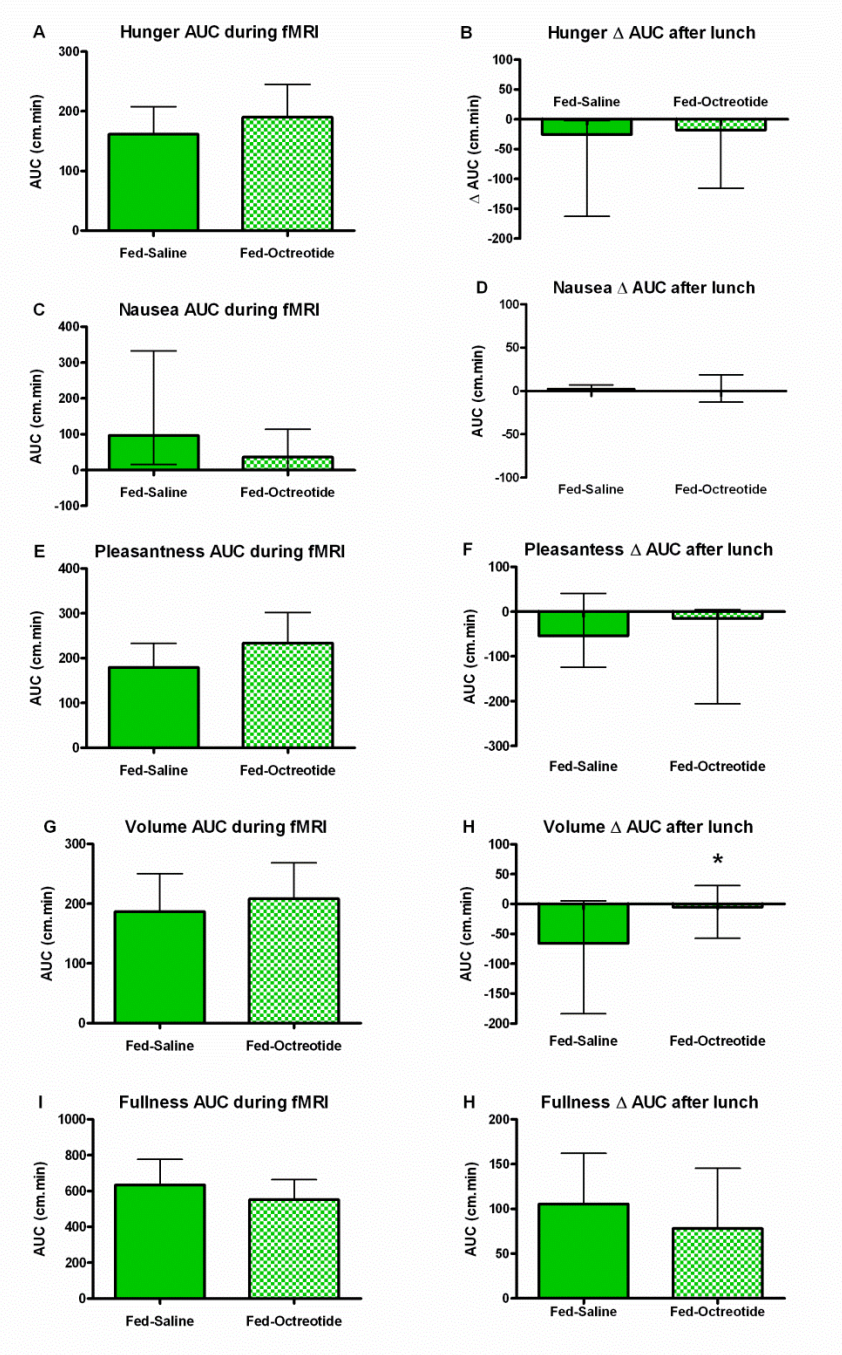
There were no significant differences between the RYGB and BAND groups in the change in VAS ratings of 'hunger', 'fullness', 'pleasantness to eat' or 'volume of food they could eat' between Fed-Saline and Fed-Octreotide visits (Fig. 4.9).

Table 4.7 Visual analogue scales of appetite

	RYGB			BAND			FED OCTREOTIDE – FED SALINE		
	Fed-Saline	**Fed-Octreotide**	**P[a]**	**Fed-Saline**	**Fed-Octreotide**	**P[a]**	**BAND**	**RYGB**	**P[a]**
n	9[c]	9[c]		8	8		8	9[c]	
VAS Hunger									
Pre-lunch AUC (cm.min)	161.3 ± 46.5 (0.0 - 390.0)	190.2 ± 54.9 (0.0 - 484.3)	0.41	190.1 ± 60.8 (1.0 - 470.0)	212.5 ± 64.2 (12.8 - 528.5)	0.68	22.5 ± 51.2 (-235.8 - 184.8)	29.0 ± 33.5 (-170.3 - 178.0)	0.91
After meal Δ AUC (cm.min)	-25.5 [-162.8 – -2.3] (-225.0 - 264.0)	-18.0 [-115.5 - 0.0] (-313.5 - 16.5)	0.95	-50.3 [-182.3 - -1.1] (-400.5 - 7.5)	-39.8 [-232.9 - -5.3] (-313.5 - 51.0)	0.78	-11.3 [-90.0 - 59.30 (-142.5 - 361.5)	9.0 [-75.0 - 22.5] (-264.0 - 138.0)	1.00
VAS Pleasantness to eat									
Pre-lunch AUC (cm.min)	179.2 ± 53.2 (1.0 - 493.0)	233.8 ± 68.0 (0.0 - 552.5)	0.24	231.5 [15.5 - 386.0] (0.0 - 625.0)	229.5 [65.6 - 444.6] (20.8 - 582.5)	0.90	4.0 [-83.4 - 223.2] (-349.3 - 287.8)	15.0 [-20.6 -172.1] (-134.5 - 276.3)	0.89
After meal Δ AUC (cm.min)	-54.0 [-123.8 - 40.5] (-336.0 - 91.5)	-15.0 [-205.5 - 4.5] (-472.5 - 36.0)	0.44	-96.6 ± 49.2 (-313.5 - 109.5)	-131.6 ± 71.0 (-490.5 - 45.0)	0.63	-35.1 ± 70.6 (-267.0 - 282.0)	-45.2 ± 54.6 (-351.0 - 211.5)	0.91
VAS Volume									
Pre-lunch AUC (cm.min)	186.9 ± 63.2 (9.0 - 504.0)	208.1 ± 60.3 (0.0 - 488.0)	0.39	249.8 ± 73.6 (4.0 - 551.0)	225.0 ± 67.0 (13.5 - 559.3)	0.70	-24.8 ± 62.6 (-377.3 - 172.3)	22.4 ± 24.1 (-62.3 - 139.3)	0.49
After meal Δ AUC (cm.min)	-66.0 [-183.8 - 5.3] (-310.5 - 36.0)	-5.3 [-57.4 - 30.8] (-280.5 - 37.5)	0.02	-87.0 [-254.3 - 20.6] (-307.5 - 145.5)	-51.0 [-256.9 - -4.9] (-291.0 - 4.5)	0.61	-3.0 [-103.5 - 83.3] (-322.5 - 280.5)	6.8 [2.6 - 96.4] (0.0 - 120.0)	0.11
VAS Fullness									
Pre-lunch AUC (cm.min)	633.9 ± 142.6 (157.0 - 1067.0)	550.9 ± 111.7 (109.3 - 972.0)	0.99	603.0 ± 119.5 (226.0 – 1051.0)	664.1 ± 112.3 (264.8 - 1042.0)	0.60	61.0 ± 110.7 (-355.3 - 671.0)	-97.5 ± 51.3 (-336.5 - 190.5)	0.22
After meal Δ AUC (cm.min)	105.3 ± 56.7 (-13.5 - 541.5)	77.8 ± 67.4 (-216.0 - 408.0)	0.80	152.1 ± 69.1 (-75.0 - 463.5)	95.8 ± 52.4 (-75.0 - 288.0)	0.49	-56.3 ± 77.7 (-370.5 - 304.5)	-27.5 ± 104.6 (-757.5 - 301.5)	0.83

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range). Pre-lunch AUC: area under the curve between time point +40 and +150 min. After lunch meal Δ AUC: change in area under the curve between time point +150 and +240 min. [a] P value for overall comparison of averages between visits or groups using paired or independent t-test for data that is normally distributed or Mann-Whitney U or Wilcoxon signed test where not normally distributed. [c] Includes scores of 2 subjects who did not complete fMRI scanning but did complete rest of the paradigm.

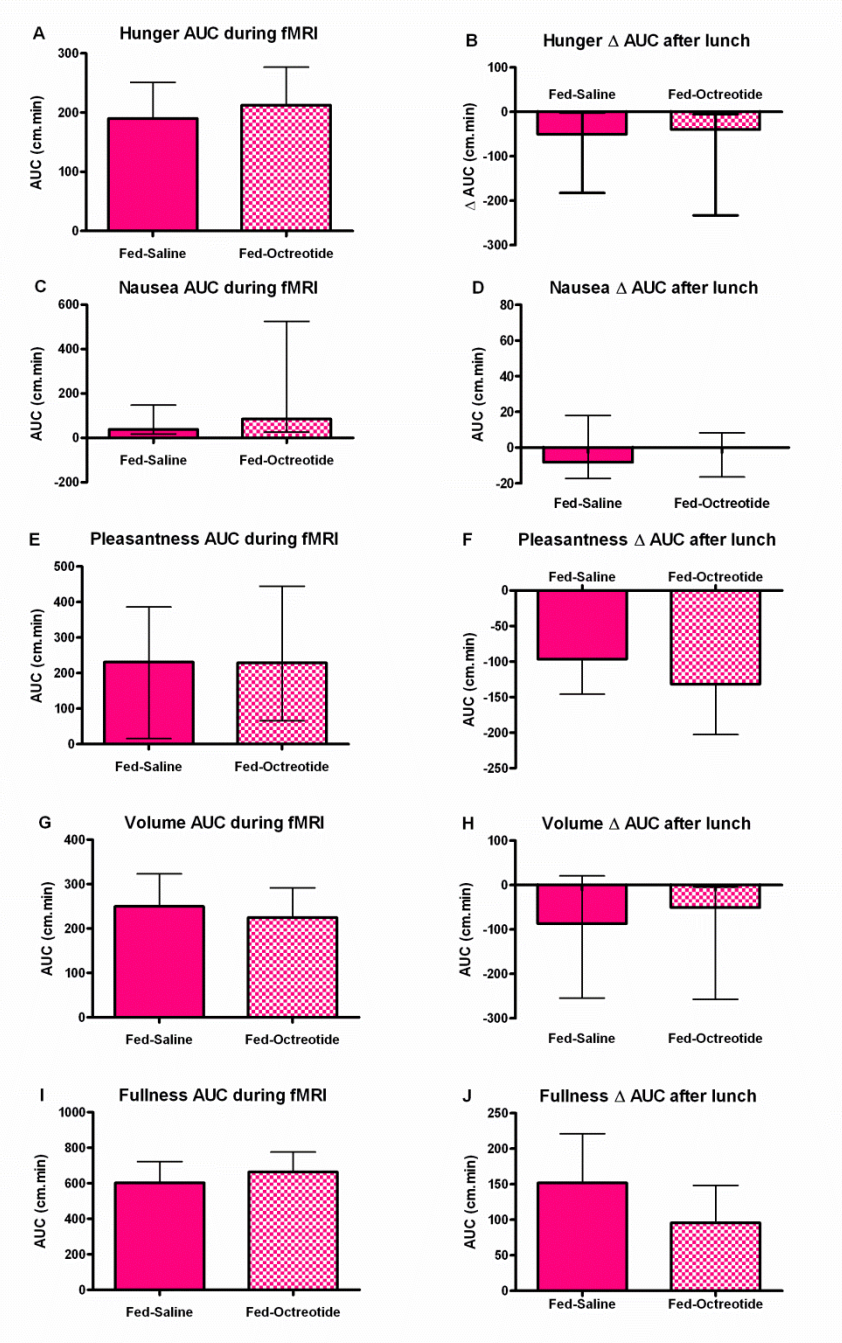
Figure 4.7 Effect of Octreotide on appetite visual analogue scales in fed obese patients after gastric bypass surgery



Comparison of visual analogue scale (VAS) ratings of (A, B) hunger, (C, D) nausea, (E, F) pleasantness to eat, (G, H) volume of food that could be eaten, and (I, J) fullness.

(A,C,E,G,I) levels during fMRI scanning (area under curve (AUC) +40 to +150 mins) and (B, D, F, H, J) change in levels after ice cream lunch meal (ΔAUC +150 to +210 mins) , in obese patients after gastric bypass surgery (RYGB) between Fed-Saline (solid green) and Fed-Octreotide (green checked) visits. Data are presented as mean ± SEM (A,E,G,I,J) or median and interquartile ranges (B,C,D,F,H), where not normally distributed; n=9 per group. *P<0.05 Fed-Octreotide vs. Fed-Saline

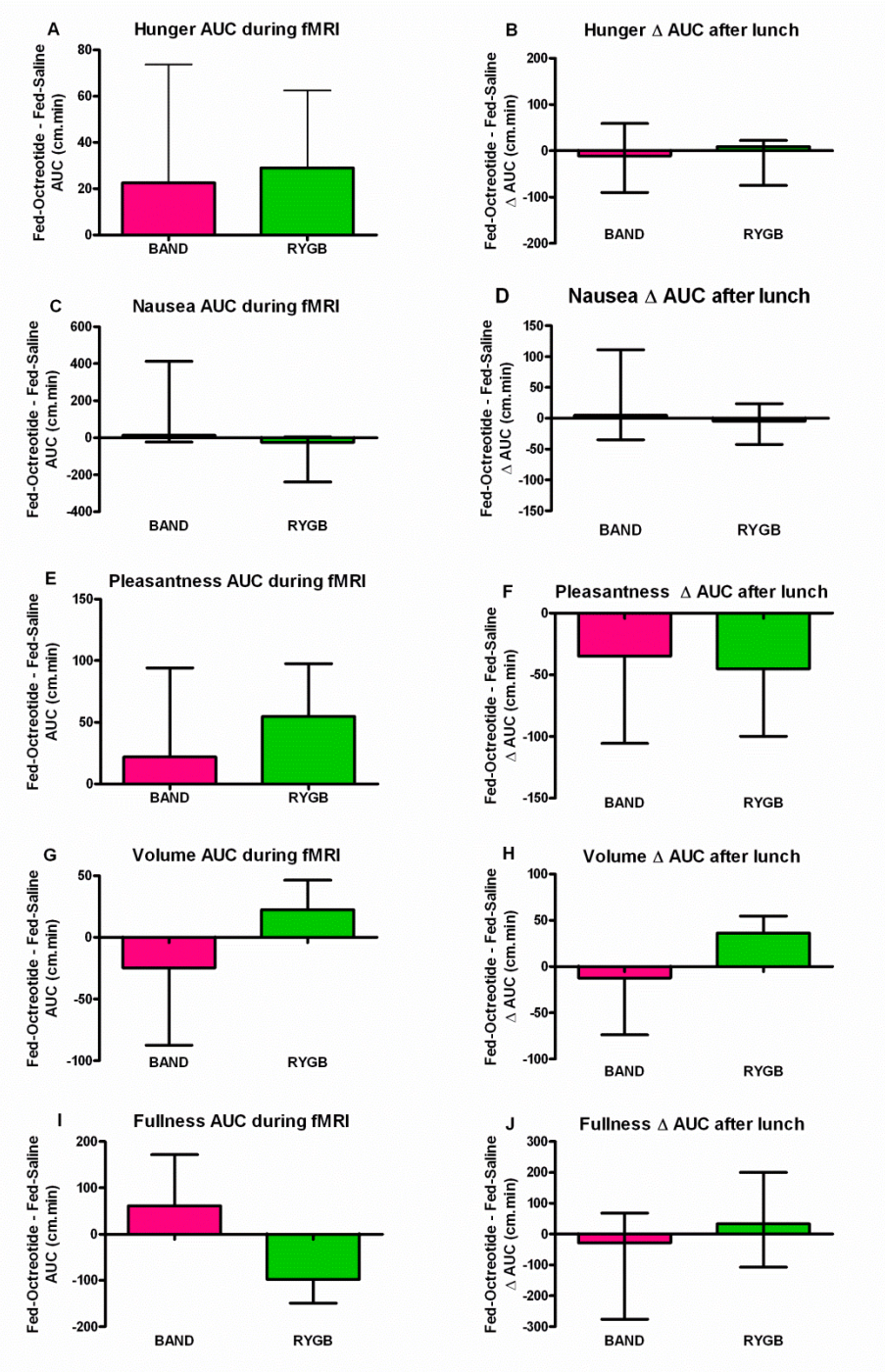
Figure 4.8 Effect of Octreotide on appetite visual analogue scales in fed obese patients after gastric banding surgery



Comparison of visual analogue scale (VAS) ratings of (A, B) hunger, (C, D) nausea, (E, F) pleasantness to eat, (G, H) volume of food that could be eaten, and (I, J) fullness.

(A,C,E,G,I) levels during fMRI scanning (area under curve (AUC) +40 to +150 mins) and (B, D, F, H, J) change in levels after ice cream lunch meal (ΔAUC +150 to +210 mins) in obese patients after gastric bypass banding (BAND) between Fed-Saline (solid red) and Fed-Octreotide (checked red) visits. Data are presented as mean ± SEM (A,G,I,J) or median and interquartile ranges (B,C,D,E,H), where not normally distributed; n=8 per group.

Figure 4.9 Difference in effect of Octreotide on appetite visual analogue scales in fed obese patients after gastric banding and gastric bypass surgery



Comparison of difference in visual analogue scale (VAS) ratings of (A, B) hunger, (C, D) nausea, (E, F) pleasantness to eat, (G, H) volume of food that could be eaten, and (I, J) fullness, (A,C,E,G,I) levels during fMRI scanning (area under curve (AUC) +40 to +150 mins) and (B, D, F, H, J) change in levels after ice cream lunch meal (ΔAUC +150 to +210 mins) between obese patients after gastric banding (BAND, red) and gastric bypass (RYGB, green) surgery between Fed-Octreotide and Fed-Saline condition. Data are presented as mean ± SEM (A,E,F,G,H,I) or median and interquartile ranges (B,C,D,J), where not normally distributed. n= 8-9 per group.

4.4.6 Metabolic and hormonal phenotyping

4.4.6.1 Effect of Octreotide on plasma insulin and glucose in RYGB and BAND

In RYGB patients, plasma glucose levels increased more after the ice-cream lunch meal at the Fed-Saline visit than the Fed-Octreotide condition (Table 4.8, Fig. 4.10B). There were no significant differences between visits in pre-lunch plasma glucose or insulin levels or post-prandial changes in plasma insulin levels (Table 4.8, Fig. 4.10A, C, D) in the RYGB group. Due to technical problems with analysing samples as a result of haemolysis, insulin levels were only available for n=5-6 of the RYGB subjects. There were also no differences in the plasma triglyceride levels between visits in RYGB group (Table 4.8, Fig. 4.10 E, F).

In BAND patients, pre-lunch insulin levels were higher at the Fed-Saline visit than the Fed-Octreotide visit (Table 4.8, Fig. 4.11C). Pre-lunch triglyceride levels were also higher at the Fed-Saline than the Fed-Octreotide visit (Table 4.8, Fig. 4.11E). There were no significant differences in BAND patients between visits in the pre-meal plasma glucose levels or post-prandial change in plasma glucose, insulin or triglycerides (Table 4.8, Fig. 4.11 A, B, D, F) (although there was a trend toward significance in the post-lunch triglyceride change).

4.4.6.2 Difference in effect of Octreotide on insulin and glucose between surgical groups

The increase in glucose levels pre-lunch between Fed-Saline and Fed-Octreotide visits, and decrease in post-lunch glucose levels between Fed-Saline and Fed-Octreotide visits, were both greater for the RYGB compared with the BAND group (Table 4.8, Fig. 4.12A,B). There were no significant differences between the RYGB and BAND groups in the change in insulin levels between the Fed-Saline and Fed-Octreotide visits (Table 4.8, Fig. 4.12C,D). Again this comparison of insulin levels only included 5-6 subjects in the RYGB group.

Table 4.8 Plasma glucose, insulin and triglyceride results

	RYGB			BAND			FED OCTREOTIDE – FED SALINE		
	Fed-Saline	**Fed-Octreotide**	**P[a]**	**Fed-Saline**	**Fed-Octreotide**	**P[a]**	**BAND**	**RYGB**	**P[a]**
n	9[c]	9[c]		8	8		8	9[c]	
Glucose									
Pre-lunch AUC (mmol/L.min)	589.4 ± 42.8 (461.0 - 832.5)	777.1 ± 58.2 (442.5 - 1018.0)	0.06	564.6 ± 43.2 (418.0 - 784.5)	530.3 ± 66.8 (349.0 - 967.5)	0.54	-34.3 ± 53.7 (-261.0 - 183.0)	187.7 ± 83.4 (-390.0 - 505.5)	0.05
After meal Δ AUC (mmol/L/kCal.min)	0.2 ± 0.0 (0.0 - 0.5)	0.0 ± 0.0 (-0.1 - 0.1)	0.02	0.0 ± 0.0 (-0.2 - 0.1)	0.1 ± 0.1 (-0.1 - 0.3)	1.00	0.13 ± 0.06 (-0.20 - 0.30)	-0.20 ± 0.04 (-0.50 to -0.10)	0.001
Insulin									
Pre-lunch AUC (mmol/L.min)	1484.9 ± 538.7* (0.0 - 4009.0)	1172.1 ± 219.2* (0.0 - 2254.5)	0.11	2574.8 ± 825.5 (0.0 - 6087.5)	1662.6 ± 323.5 (0.0 - 2623.0)	0.04	-1743.4 ± 997.3 (-4269.5 - 341.5)	-1519.3 ± 631.1* (-3082.5 to -288.5)	0.85
After meal Δ AUC (mmol/L/kCal.min)	2.3 ± 1.2* (-0.4 - 5.3)	0.3 ± 0.1* (-0.1 - 0.7)	0.14	1.3 ± 1.1 (-2.4 - 6.4)	-1.0 ± 1.0 (-7.1 - 0.6)	0.34	-1.33 ± 1.25 (-6.40 - 2.80)	-2.10 ± 1.05* (-4.60 - 0.40)	0.68
HOMA -IR	0.9 ± 0.0* (0.7 - 1.0)	0.9 ± 0.1* (0.5 - 1.2)	0.16	0.9 ± 0.1 (0.6 - 1.3)	1.6 ± 0.3 (0.7 - 3.7)	0.04	0.39 ± 0.14 (0.00 - 1.10)	0.13 ± 0.05* (0.00 - 0.20)	0.22
Triglycerides									
Pre-lunch AUC (mmol/L.min)	138.9 ± 10.3 (94.7 - 170.7)	130.2 ± 5.7 (95.2 - 154.5)	0.36	122.2 ± 18.9 (63.1 - 191.5)	105.6 ± 13.0 (50.8 - 159.3)	0.03	-16.7 ± 5.7 (-32.7 - 9.3)	-8.8 ± 9.0 (-43.9 - 39.4)	0.50
After meal Δ AUC (mmol/L/kCal.min)	0.0 ± 0.0 (-0.1 - 0.0)	0.0 ± 0.0 (0.0 - 0.0)	0.56	0.0 ± 0.0 (0.0 - 0.1)	0.0 ± 0.0 (-0.1 - 0.0)	0.05	0.00 [-0.08 - 0.00] (-0.10 - 0.00)	0.00 [0.00 - 0.00] (0.00 - 0.10)	0.28

Data presented as mean ± SEM and (range). Pre-lunch AUC: area under the curve between time point +40 and +15 0min. After meal Δ AUC: change in area under the curve between time point +150 and +210 min per kCal of lunch eaten.

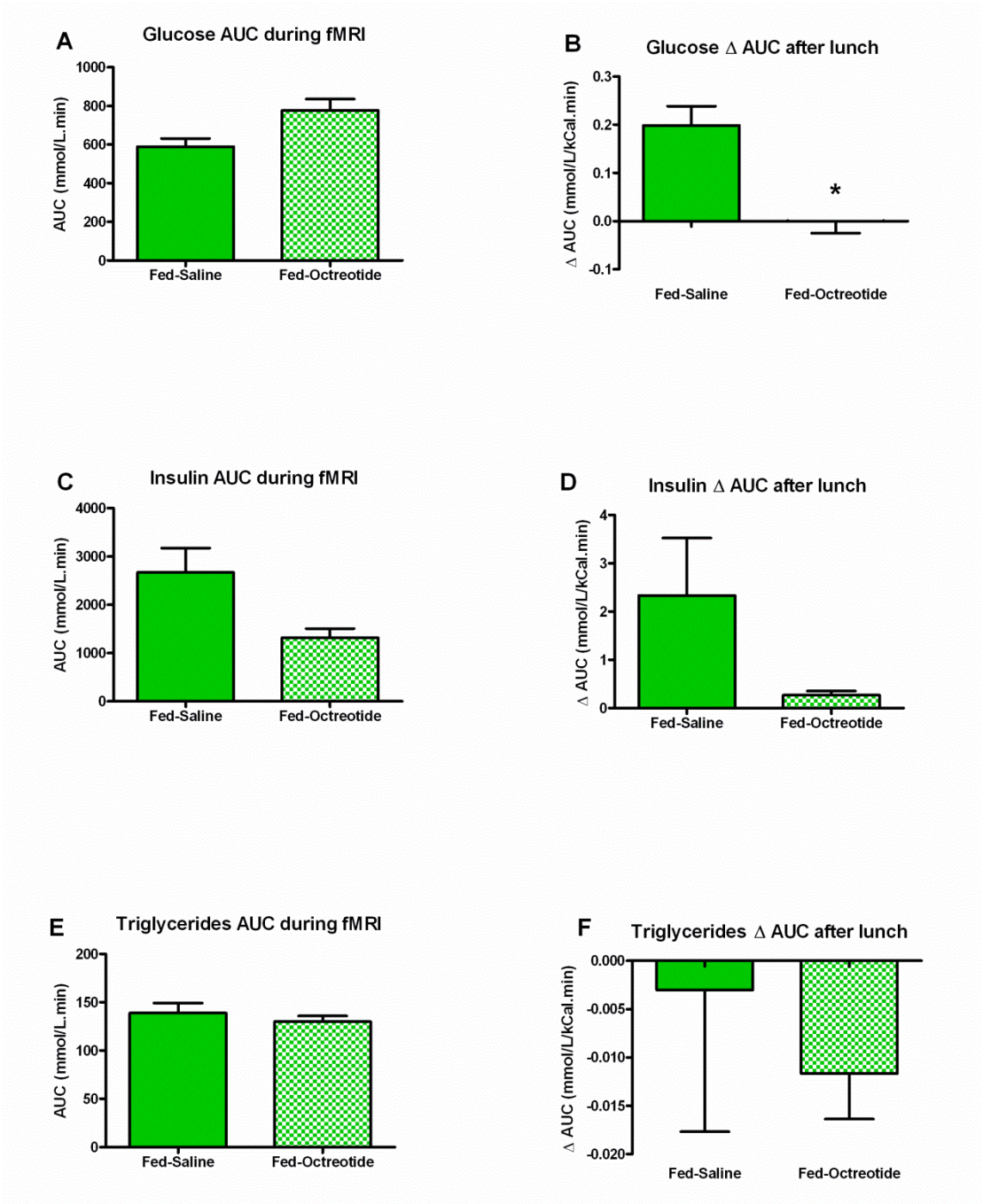
[a] P value for overall comparison of averages between visits or groups using paired or independent t-test.

[c] Includes scores of 2 subjects who did not complete fMRI scanning but did complete rest of the paradigm.

[*] n=5-6 for insulin and HOMA-IR levels in RYGB group

Abbreviations: AUC: area under the curve, BAND: gastric banding group, BMI-M: body mass index matched group, BP: blood pressure, mm: millimeters, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning, Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, RYGB; gastric bypass

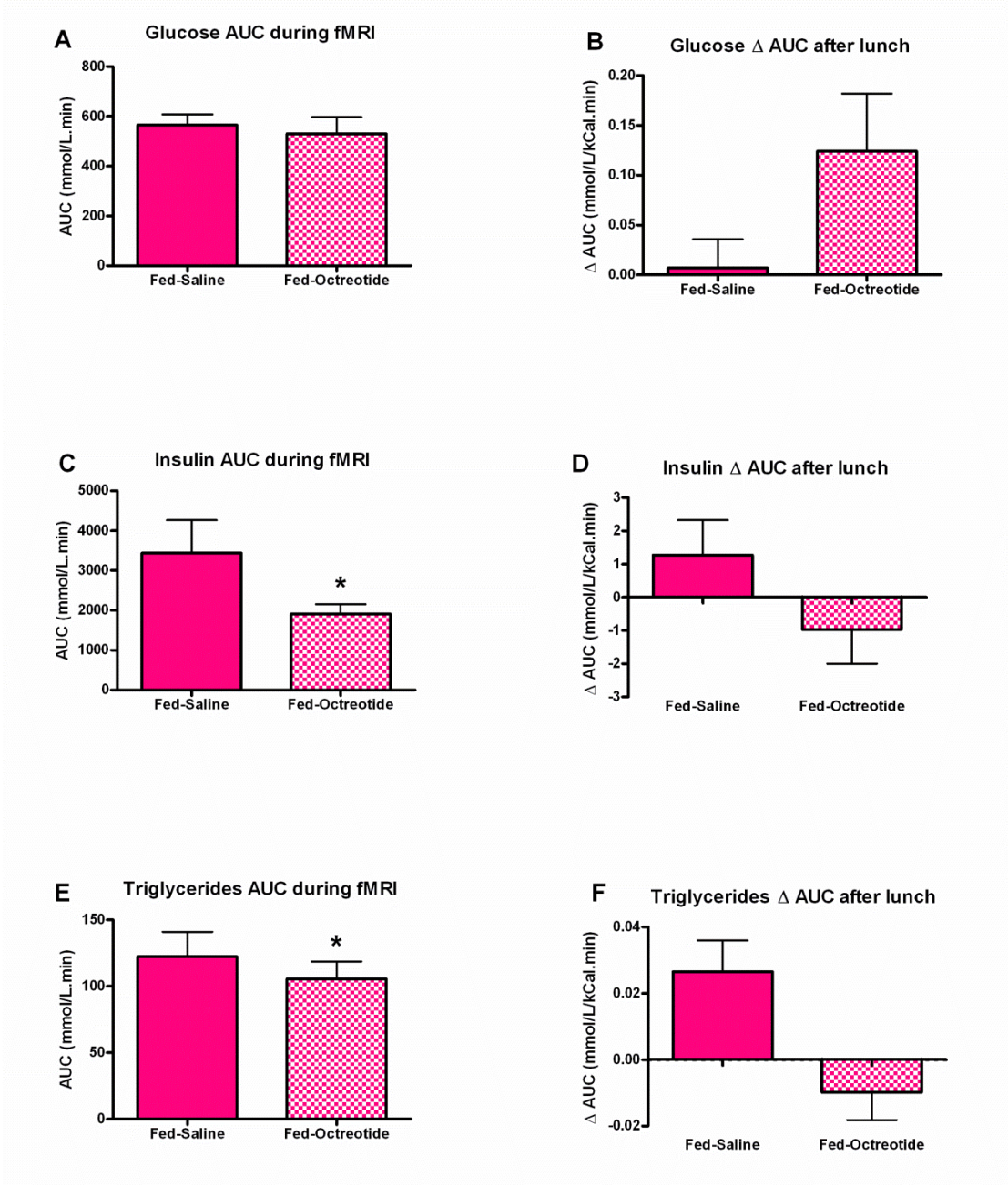
Figure 4.10 Effect of Octreotide on plasma glucose, insulin and triglycerides in fed obese patients after gastric bypass surgery.



Comparison of (A,C,E) plasma levels of glucose, insulin and triglycerides, area under curve (AUC) +40 to +150 mins) and (B,D,F) change in glucose, insulin and triglycerides after ice-cream meal (ΔAUC +150 to +210 mins) in obese patients after gastric bypass (RYGB) surgery in the Fed-Saline (solid green) and Fed-Octreotide (hatched green) condition.

Data are presented as mean ± SEM. *P<0.05 vs. Fed-Saline; n=9 per group except C and D where n=6.

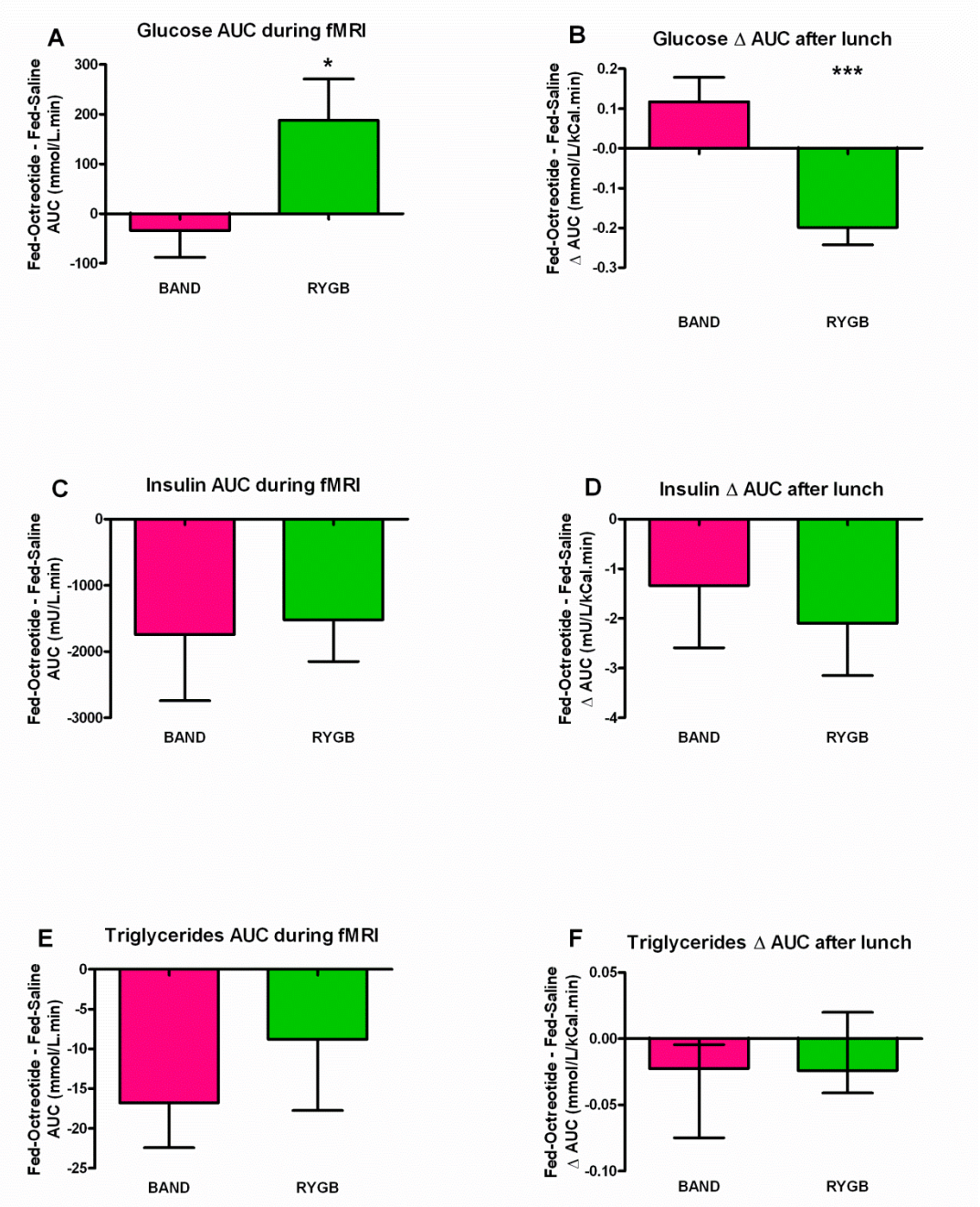
Figure 4.11 Effect of Octreotide on plasma glucose, insulin and triglycerides in fed obese patients after gastric banding surgery.



Comparison of (A,C,E) plasma levels of glucose, insulin and triglycerides, area under curve (AUC) +40 to +150 mins) and (B,D,F) change in glucose, insulin and triglycerides after ice-cream meal (ΔAUC +150 to +210 mins) in obese patients after gastric banding (BAND) surgery in the Fed-Saline (solid red) and Fed-Octreotide (hatched red) condition.

Data are presented as mean ± SEM. *P<0.05 vs. Fed-Saline; n=8 per group.

Figure 4.12 Difference in effect of Octreotide on plasma glucose, insulin and triglycerides in obese patients after gastric bypass and gastric banding surgery



Comparison of difference between Fed-Saline and Fed-Octeotide visits in (A,C,E) plasma levels of glucose, insulin and triglycerides, area under curve (AUC) +40 to +150 mins) and (B,D,F) change in glucose, insulin and triglycerides after ice-cream meal (ΔAUC +150 to +210 mins) in obese patients after gastric banding (BAND, red) and gastric bypass (RYGB, green) surgery.

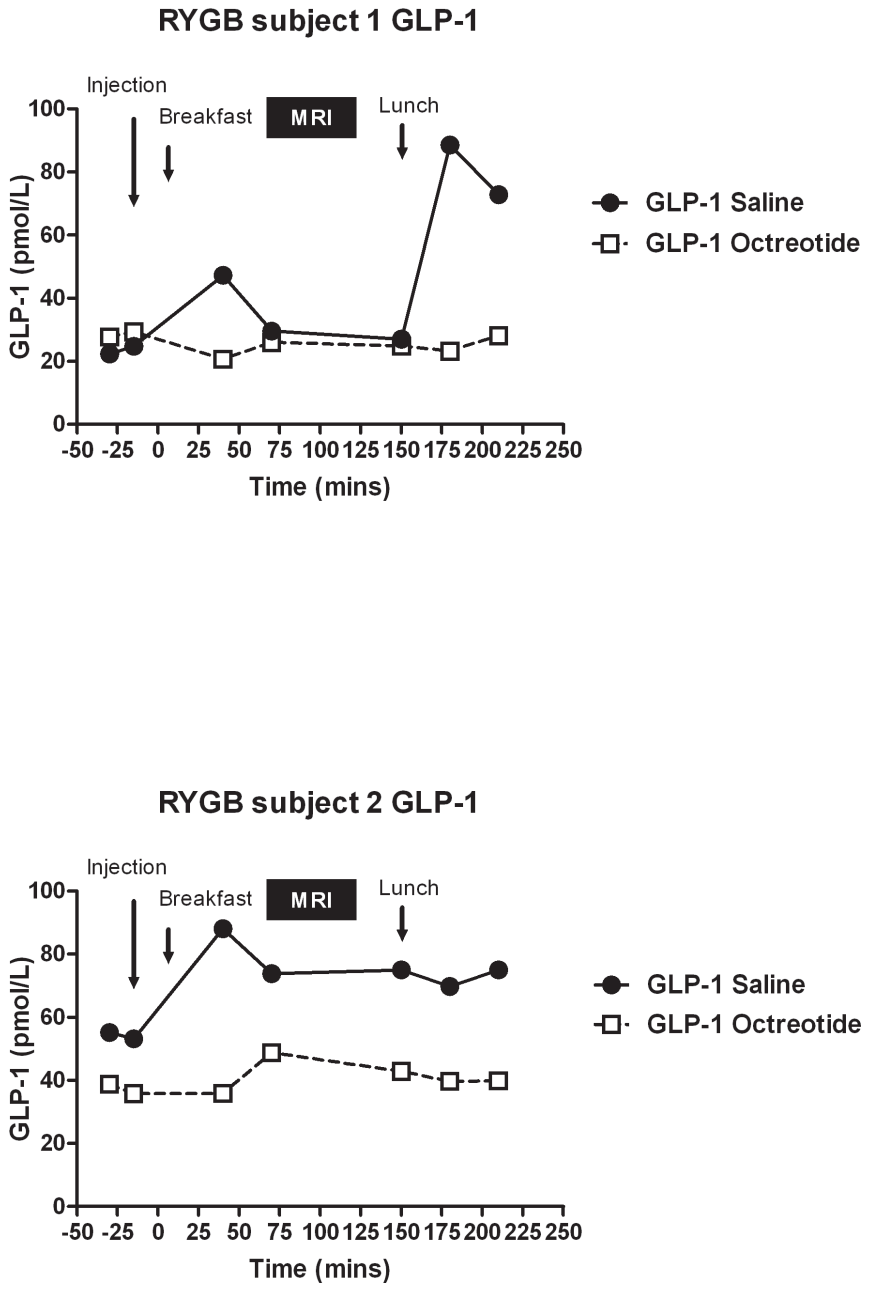
Data are presented as mean ± SEM (A-E) or median and interquartile ranges (F), where not normally distributed. *P<0.05 vs. BAND, ***P<0.0005; n=8-9 per group, except C,D where n=6-9.

4.4.7 Effect of Octreotide on plasma PYY and GLP-1 levels in RYGB

Plasma GLP-1 and PYY levels were not measured for all the participants as due to inter-assay variation the samples are being kept to be assayed in a single run when more participants have been scanned (see Chapter 6). A preliminary assay was performed from 2 participants after RYGB (Subject 1: male, White British, age 42 years, BMI 34kg/m^2 and Subject 2: female, Pakistani, age 59 years, BMI 32.6kg/m^2), in order to confirm the expected suppression of PYY and GLP-1 by Octreotide over the duration of the study paradigm. Subject 1 only was given a smaller breakfast than the standard (251 kCal compared to 385 kCal) due to adjustments being made in the protocol at that time, but otherwise both subjects followed the standard protocol in every other way, except that they did not undergo fMRI scanning. The participants ate 563 kCal (9.9 kCal/kg lean body mass [LBM]) and 338 kCal (8.3 kCal/kg LBM) of the ice cream test meal, respectively. Both PYY and GLP-1 levels were markedly lower after both breakfast and lunch on the Fed-Octreotide visit compared to the Fed-Saline visit in both subjects, confirming the long duration of action of this somatostatin analogue (Fig. 4.13 and Fig. 4.14).

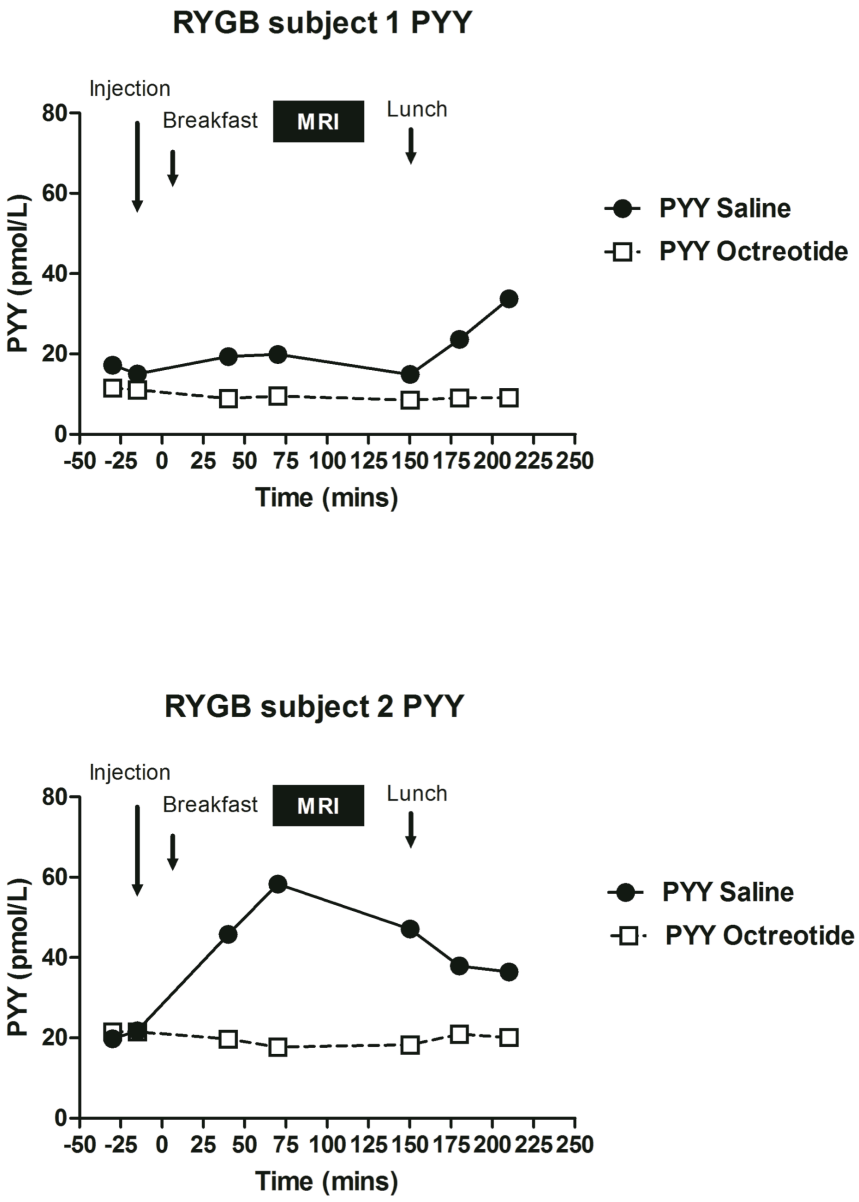
Plasma levels of total and glycine-conjugated bile acids and ghrelin were not measured in these pilot samples, or the whole study participants for the same reason as above.

Figure 4.13 Effect of Octreotide on plasma GLP-1 levels in fed obese patients after gastric bypass surgery



Comparison of plasma GLP-1 levels over the course of the visits between Fed-Saline (● and solid line) and Fed-Octreotide (□ and dashed line) visits in two patients after gastric bypass surgery.

Figure 4.14 Effect of Octreotide on plasma PYY levels in fed obese patients after gastric bypass surgery



Comparison of plasma PYY-1 levels over the course of the visits between Fed-Saline (● and solid line) and Fed-Octreotide (□ and dashed line) visits in two patients after gastric bypass surgery.

4.4.8 Dumping symptoms

There was no difference in physiological markers of dumping syndrome (heart rate, blood pressure) or VAS ratings of 'sleepiness' or 'nausea' between visits in either the RYGB or BAND group (Table 4.9). There was also no difference between the RYGB and BAND groups in the difference between dumping signs or symptoms between Fed-Saline and Fed-Octreotide visits (Table 4.9).

4.4.9 Confounding variables

By chance, the BAND group had slept on average approximately 1.5 hours more the night before the Fed-Octreotide than the Fed-Saline visit. There were no other significant differences in potential confounding variables including anxiety, stress, mood, time since last meal, or motion during scanning between the groups at either visit (Table 4.10).

Table 4.9 Assessment of dumping syndrome symptoms

	RYGB			BAND			FED OCTREOTIDE – FED SALINE		
	Fed-Saline	**Fed-Octreotide**	**P[a]**	**Fed-Saline**	**Fed-Octreotide**	**P[a]**	**BAND**	**RYGB**	**P[a]**
n	9[c]	9[c]		8	8		8	9[c]	
Δ Heart rate (beats per minute)	-1.9 ± 1.8 (-8 – 10)	-1.8 ± 3.0 (-18 – 13)	0.81	-2.9 ± 2.5 (-16 – 4)	3.8 ± 2.1 (-3 – 18)	0.06	-6.9 ± 3.1 (-24 – 12)	0.2 ± 3.4 (-15 – 18)	0.16
Δ Systolic BP (mm Hg)	-1.9 ± 3.8 (-19 – 21)	-4.3 ± 7.3 (-45 – 24)	0.76	-2.6 ± 6.8 (-30 – 27)	5.1 ± 6.3 (-29 – 26)	0.63	5.5 ± 10.8 (-56 – 35)	1.0 ± 6.5 (-33 – 28)	0.71
Δ Diastolic BP (mm Hg)	0.2 ± 2/4 (-11 – 12)	2.0 ± 3.9 (-12 – 31)	0.61	-1.8 ± 3.0 (-21 – 5)	-3.3 ± 4.1 (-24 – 12)	0.86	-0.75 ± 4.1 (-17 – 19)	-2.3 ± 5.1 (-37 – 23)	0.82
VAS Sleepiness									
After meal Δ AUC (cm.min)	-41.3 [-170.3 - -3.0] (-202.5 - 12.0)	-109.5 [-234.8 - 43.5] (-304.5 - 95.5)	0.76	0.0 [-163.5 - 103.5] (-406.5 - 328.5)	2.3 [-78.0 -24.8] (-147.0 - 61.5)	0.69	10.5 [-261.8 - 146.3] (-418.5 - 211.5)	7.5 [-46.5 - 7.9] (-297.0 - 342.0)	0.96
VAS Nausea									
After meal Δ AUC (cm.min)	2.3 [0.0 – 7.1] (-15.0 - 375.0)	0.0 [-12.8 – 18.8] (-46.5 - 160.5)	0.50	-8.3 [-17.3 – 78.0] (-166.5 – 28.5)	0.0 [-16.5 - 8.3] (-237.0 - 127.5)	0.89	4.5 [-34.9 - 111.0] (-250.5 - 168.0)	-4.5 [-42.4 - 23.6] (-375.0 - 153.0)	0.57

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range).
Δ heart rate and blood pressure: change between time points +150 and +210 min. Δ AUC for VAS: change in AUC between time points +150 to +210 min.
[a] P value for overall comparison of averages between visits or groups using paired or independent t-test for data that is normally distributed or Mann-Whitney U or Wilcoxon signed test where not normally distributed. [c] Includes scores of 2 subjects who did not complete fMRI scanning but did complete rest of the paradigm

Abbreviations: AUC: area under the curve BAND: gastric banding group, BMI-M: body mass index matched group, BP: blood pressure, mm: millimeters, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, RYGB; gastric bypass

Table 4.10 Potential confounding variables at scanning visit

	RYGB			BAND		
	Fed-Saline	**Fed-Octreotide**	**P[a]**	**Fed-Saline**	**Fed-Octreotide**	**P[a]**
n	7	7		8	8	
PANAS positive (score /50)	33.1 ± 3.1 (14.0 - 48.0)	29.8 ± 3.1 (16.0 - 47.0)	0.16	32.6 ± 2.8 (24.0 - 47.0)	33.9 ± 2.3 (26.0 - 43.0)	0.80
PANAS negative (score /50)	12.5 ± 11.5 (10.0 - 19.0)	11.0 [10.5 -14.5] (10.0 - 16.0)	0.20	32.6 ± 2.8 (10.0 - 19.0)	11.0 [10.0 - 13.5] (10.0 - 23.0)	0.57
Sleep duration previous night (hours)	6.7 ± 0.4 (4.8 - 8.5)	6.3 ± 0.3 (4.5 - 7.5)	0.24	6.5 ± 0.2 (5.5 - 7.5)	8.0 ± 0.5 (6.3 - 10.8)	0.04
Time since supper to fMRI scan (hours)	14.8 ± 0.3 (13.3 - 16.5)	15.5 ± 0.3 (14.6 - 17.5)	0.11	14.8 ± 0.5 (13.4 - 17.3)	15.0 ± 0.5 (13.7 - 18.1)	0.98
Absolute motion during food task (mm)	0.54 ± 0.10 (0.30 - 1.04)	0.41 ± 0.06 (0.20 - 0.69)	0.08	0.48 ± 0.13 (0.15 - 1.47)	0.50 ± 0.08 (0.18 - 0.85)	0.09
Relative motion during food task (mm/TR)	0.13 [0.08 - 0.22] (0.08 - 0.48)	0.15 [0.07 - 0.25] (0.04 - 0.25)	0.33	0.14 [0.08 - 0.18] (0.06 - 0.32)	0.11 [0.09 - 0.13] (0.06 - 0.22)	0.67
Absolute motion during Audio-Motor-Visual task (mm)	0.23 [0.20 - 0.87] (0.11 - 1.08)	0.23 [0.18 - 0.57] (0.16 - 1.32)	0.27	0.28 [0.15 - 0.62] (0.10 - 2.98)	0.23 [0.14 - 0.35] (0.13 - 2.18)	0.83
Relative motion during Audio-Motor-Visual task (mm/TR)	0.11 [0.07 - 0.19] (0.06 - 0.41)	0.09 [0.08 - 0.26] (0.07 - 0.29)	0.73	0.10 [0.07 - 0.32] (0.07 - 0.56)	0.09 [0.06 - 0.15] (0.06 - 0.31)	0.73
Stress pre-lunch (cm.min)	15.8 [2.4 - 49.3] (0.0 - 94.0)	31.8 [7.7 - 158.4] (0.0 - 294.5)	0.11	49.5 [18.9 - 255.0] (10.3 - 895.0)	38.3 [16.0 - 212.3] (0.0 - 402.3)	0.76
Anxiety pre-lunch (cm.min)	21.6 [0.6 - 86.1] (0.0 - 151.3)	38.2 [3.6 - 144.9] (0.0 - 305.0)	0.18	48.8 [21.6 - 239.5] (1.5 - 338.8)	53.5 [7.1 - 214.6] (4.0 - 419.0)	0.76

Data included for subjects who attended fMRI scanning. Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (range). [a] P value for overall comparison of averages between visits or groups using paired or independent t-test for data that is normally distributed or Mann-Whitney U or Wilcoxon signed test where not normally distributed. Abbreviations: BAND: gastric banding group, mm: millimeters, PANAS: positive and negative affect schedule, Fed-Saline: standardized milkshake breakfast (385kCal) and subcutaneous saline injection prior to scanning; Fed-Octreotide: standardized milkshake breakfast (385kCal) and subcutaneous Octreotide and Insulin injection prior to scanning, RYGB; gastric bypass, TR: repetition time. VAS: visual analogue scale.

4.5 Discussion

4.5.1 Summary of results

In this pilot study, exploration of the role of exaggerated post-prandial secretion of PYY and GLP-1 in RYGB on food hedonics was made by administration of Octreotide to suppress plasma GLP-1 and PYY levels. Acute Octreotide administration increased BOLD activation in the nucleus accumbens to any food or just low-calorie food in the picture evaluation task in fed obese patients after RYGB surgery. Octreotide also increased the subjective appeal of any food pictures in the RYGB group, but did not significantly alter ice cream palatability. Although Octreotide did not change any of the measures of food hedonics in the BAND group, there were no significant differences between the RYGB and BAND groups. However these interpretations will be limited as final subject numbers in the RYGB and BAND groups were low with n=6-9 per group depending on the outcome measure.

Octreotide lowered satiety (measured by 'volume able to eat' VAS) following an ice cream test meal in obese patients after RYGB, but had no effect on reported hunger or the amount of ice cream consumed in either RYGB or BAND patients. Octreotide had no effect on satiety measures in the BAND group, but again there were no significant differences between groups. Octreotide had no effect in either group on dumping symptoms. Preliminary analysis confirmed the ability of acute Octreotide administration to completely prevent the exaggerated post-prandial increases in plasma PYY and GLP-1 after RYGB.

4.5.2 Fasted compared to fed brain activation to food cues in RYGB

One previous study has investigated the BOLD response to food stimuli in RYGB patients in the fed state, thereby attempting to capture the effect of acute post-prandial gut hormone response on BOLD signal to food cues. In a longitudinal study, 5 RYGB patients were fed a small liquid meal (250 ml, 250 kCal) or 250 ml water, 45 min prior to scanning, after an overnight fast. No difference in BOLD activation to food cues (pictures and spoken words) was seen in the fed state one month after surgery compared to one month before surgery, even though the same patients had a reduction in BOLD activation to food cues after surgery in the insula, medial frontal gyrus, DLPFC, pre-central gyrus and middle and superior temporal gyri in the fasted state (Ochner et al. 2012). Although limited in number, this result runs counter to the hypothesis that changes in neural responsiveness to food after RYGB are due to acute post-prandial release of gut hormone acting on reward centres (Ochner et al. 2012). Due to the small number of subjects in the current study and the difficulties in distinguishing nutritional from order effects, the results between the Fasted-Saline and Fed-Saline visits were not directly compared in this study in either the RYGB or BAND groups.

4.5.3 Effect of Octreotide on brain activation to food pictures

Although cross-sectional in nature, this study revealed increased activation in the nucleus accumbens to food cues (hypothesised to be associated with dopaminergic release in response to reward anticipation) in the fed state in RYGB patients when gut hormone release was suppressed by Octreotide. One explanation for this discrepancy between this and the earlier longitudinal study may be that patients were scanned on average 16 months after RYGB surgery in the current study as opposed to 1 month in Ochner's study. There may be acute effects immediately after surgery that blunt the hedonic and satiating effects of the post-prandial state. Another possibility is that the relatively small size of meal in Ochner's

study did not induce a sufficiently exaggerated gut hormone response to alter BOLD response in the Fed visit. However that does not explain why a difference was seen in BOLD response in the fasted condition in the same patients.

Interestingly these effects of Octreotide were not seen in the BAND group, but further comment is difficult because of the small numbers of subjects, such that direct comparison showed no difference between surgical groups. It is also of note that while the stimulatory effect of Octreotide was seen in the nucleus accumbens fROI in RYGB, and this ROI did show lower activation in the RYGB than BAND groups in the response to food pictures when fasted, the latter study did not show any change in the nucleus accumbens in fROI analysis (Chapter 3). Meanwhile the lower OFC and amygdala activation to food pictures in RYGB than BAND seen from the fROI analysis when fasted (Chapter 3) was not reflected by an increase in OFC or amygdala activation with Octreotide in RYGB.

4.5.4 Effect of Octreotide on food intake

In the current study, Octreotide did not induce the expected increase in food intake that le Roux et al. have previously shown after RYGB (2007). In their study, which measured appetitive but not hedonic response to food, the same dose of Octreotide (100mcg) was administered to 6 RYGB and 7 BAND patients after a 12 hour fast, and a subsequent increase of nearly 50% was seen in ice cream intake in the RYGB but not BAND patients. This increase in food intake was associated with a reduction in fullness on VAS in the RYGB group only, and reduction in post-prandial PYY and GLP-1 secretion in both groups. The post-prandial PYY levels were lower at 30 minutes, were most reduced at 60min, and remained reduced at 90min after Octreotide administration compared to saline, whereas for GLP-1 the trough

was at 30min with sustained reduction at 90min.

The two paradigms made use of the same dosage of Octreotide to reverse gut hormone responses, and identical test meals were given. The most notable difference in the two paradigms was the fact the patients were fasted for 12 hours in le Roux's study and that the test meal was given 60 minutes after the injection in the fasted state, whereas in my paradigm, patients were fed a 385kCal milkshake breakfast after an overnight fast, and the test meal was given 135 minutes after the injection in the fed state. The reason breakfast was given was to be able to measure BOLD responses to food pictures in a fed state, when the anorexigenic gut hormone response would have been at its maximum.

One possible result is that by the time the test meal was employed, the effects of Octreotide on the gut hormone response had largely worn off by the time of lunch. Since scanning took place at only 90 minutes after the injection, Octreotide effects on brain activation may still have been evident at this time, which may explain why a difference in BOLD signal was detected in the RYGB patients, albeit only in one ROI. However preliminary examination of the sustained duration of action of Octreotide on plasma PYY and GLP-1 levels after RYGB using my paradigm suggests that this is unlikely to be an explanation, although the hormone levels have yet to be measured in the subjects having test lunch meals.

Another possible explanation for the lack of difference in food intake after Octreotide may be that the milkshake breakfast was too similar to the ice cream lunch and therefore may have induced sensory specific satiety to the ice cream (an observed reduction in hedonic appeal of a substance associated with repeated ingestion thereof).

4.5.5 Effect of Octreotide on glucose and insulin

In addition the effects of Octreotide on anorexigenic gut hormone suppression may be less pronounced in a fed state, due to concomitant suppression of insulin secretion, and hence increase in glucose levels (Cheah et al. 2012), which may counter the effect of lowering PYY and GLP-1 on food hedonic responses, appetite measures and food intake. To counter the suppression of insulin levels by Octreotide, Actrapid was co-administered with Octreotide to all patients (see Section 2.7). In RYGB patients, administration of Actrapid appears to have attenuated the expected post-prandial increase in glucose, but only after the ice cream lunch meal, and not after breakfast (Fig. 4.10A,B). Although Actrapid is a short-acting insulin, its onset of action is 30 minutes after administration, which may have been sufficient to reduce only the post-lunch and not post-breakfast glucose rise.

Unfortunately it is difficult to interpret the co-incident changes in plasma insulin levels, since there were many missing insulin samples for the RYGB group. In addition it is difficult to compare between the absolute AUC plasma levels in the post-breakfast state (Fig. 4.10, 4.11, 4.12 A,C,E) and the delta AUC plasma levels in the post-lunch state (Fig. 4.10, 4.11, 4.12 B,D,E) given that the delta may be dependent upon the starting value.

In addition, high pre-operative (but not post-operative) rates of T2DM in the RYGB group may mean that this group has persistently impaired β-cell function which is influencing the results, particularly in the comparison between the effects of Octreotide on glucose and insulin levels between the RYGB and BAND groups, since pre-operative T2DM rates were much lower in the BAND group.

4.5.6 Other possible mediators affected by Octreotide

Plasma levels of orexigenic ghrelin are also suppressed by Octreotide (Haqq et al. 2003) and somatostatin (Tan et al. 2004). Since ghrelin is known to increase appetite, food intake (Druce et al. 2005; Neary et al. 2006), appeal of food and OFC, amygdala, hippocampal and striatal BOLD activation to food pictures (Malik et al. 2008; Goldstone et al. 2010), suppression of ghrelin by Octreotide may have resulted not only in attenuation of the expected increase in appetite and food intake, but also attenuation of the increase in BOLD and hedonic responses to food pictures at the Octreotide visit in RYGB.

Previous studies show contradictory results in the effect of RYGB and BAND surgery on ghrelin levels, probably due to inconsistencies in measurement techniques (Cummings et al. 2002; Faraj et al. 2003; Geloneze et al. 2003; Leonetti et al. 2003; Lin et al. 2004; Morinigo et al. 2004; Chan et al. 2006; Sundbom et al. 2007), particularly the lack of measurement of the active acyl form of ghrelin, or perhaps differences between fasting and post-prandial levels. In those studies that did measure acyl ghrelin, two showed reduced fasting acyl ghrelin 2 weeks and 6 months post-RYGB (Fruhbeck et al. 2004; Jacobsen et al. 2012), and one showed increased fasting acyl ghrelin at 6 and 12 months post-RYGB (Holdstock et al. 2003). It is difficult to say how this may have affected my results, but in Chapter 3, there were no differences in fasting ghrelin levels between the surgical groups. Ghrelin levels in this part of the study have not been yet been measured, but it is possible that differences between the visits and groups were diluted by the suppression of acyl ghrelin by Octreotide.

The effect of Octreotide on bile acid secretion is to reduce bile flow but increase plasma bile acid levels (Sahin et al. 1999). Since bile acids may be a potential mediator for the increase in

reward system BOLD activation to food stimuli either directly or indirectly in RYGB (see Section 3.4.8), this effect may also have counteracted any increase in BOLD response in the Fed-Octreotide condition and reduced the difference between visits in RYGB.

Octreotide suppresses release of not only PYY and GLP-1 but many other anorexigenic gut hormones including CCK and oxyntomodulin. It is therefore possible that the observed effect on BOLD response in the nucleus accumbens in RYGB patients may be due to suppression of the effects of another gut hormone on food hedonics. Although increasing evidence of the action of PYY and GLP-1 on reward and hedonic based eating, outlined in Section 4.1.1 does suggest a particular role for these hormones, it is not possible to determine specificity of the hormonal effect in this paradigm.

The lack of any effect of Octreotide on auditory, motor or visual cortex activation during the control fMRI task in either surgical group suggests the absence of any non-specific effect of Octreotide on BOLD signal e.g. on neurovascular coupling. However Octreotide itself may have effects on appetite and food intake and potentially BOLD signal to food cues. Animal studies have shown increased food intake after Octreotide in mice, rats and chicks (Danguir 1988; Stengel et al. 2010; Tachibana et al. 2011). By contrast human studies have shown increased satiety but no change in food intake following Octreotide administration in normal weight and obese subjects (Foxx-Orenstein et al. 2003; Cremonini et al. 2005). Another study found somatostatin infusion increased satiety and reduced food intake in healthy volunteers, but this was reversed after infusion of fat into the duodenum, resulting in increased food intake after somatostatin. Somatostatin receptors are located widely in the brain, not only in the hypothalamus but also the amygdala, hippocampal and striatal pathways in the brain (Viollet et al. 2008). Octreotide is believed to cross the blood-brain

barrier (Fricker et al. 2002) supported by its use in treating neuroendocrine tumours. Therefore it is conceivable that Octreotide may have intra-cerebral effects of its own, which may have affected appetite, food hedonics or brain activation to food cues in my experiment. However there is no reason to believe that there would be difference between the two surgical groups in its action.

4.5.7 Alternative methods of assessing influence of GLP-1 and PYY on brain and hedonic responses to food in RYGB

GLP-1 and PYY antagonists and genetically manipulated animal models are more clearly able to delineate a specific role for these hormones in altering hedonic responses to food after bariatric surgery. Indeed counter to the expected hypothesis, KO mice lacking the GLP-1 receptor did not differ in weight loss or food choices after VSG compared to wild-type mice (Wilson-Perez et al. 2013). The GLP-1 antagonist, Exendin(9-39), has been shown to reverse the glycaemic improvements seen in RYGB and VSG surgery in animal and human studies, although no change in food intake or weight was noted, and food hedonics were not measured in these studies (Kindel et al. 2009; Chambers et al. 2011; Salehi et al. 2011). GLP-1 agonists have also been shown to further reduce food intake in RYGB mice (Fenske et al. 2012). PYY KO mice have less weight loss than normal mice after RYGB surgery (Chandarana et al. 2011) and injection of a PYY neutralizing antibody into mice who had undergone a jejeno-ileal bypass (a similar procedure to RYGB), led to increased food intake the following day (le Roux et al. 2006). Direct blockade of PYY via receptor antagonists has not been tested in RYGB yet, but has been shown to reduce anorexic responses to gastric infusions of protein and long-chain fatty acids (Reidelberger et al. 2013).

These methods carry their own problems however. For example, the degree of receptor blockade by GLP-1 antagonist Exendin(9-39) is uncertain, and it may have partial agonist effects which lead to a reduction in the overall antagonist effect. Genetic approaches have the advantage of unequivocal long-term disruption of the GLP-1 pathway, but could result in developmental compensation within the animal thereby leading to an underestimation of the role of GLP-1. Furthermore the finding of synergistic or additive actions of PYY and GLP-1 on appetite and brain reward responses to food means that blockade of both PYY and GLP-1 may be need to see attenuation of the effects of RYGB (De Silva et al. 2012).

4.5.8 Other limitations

The number of subjects in Ochner's fasted/fed study in RYGB were low but were of sufficient power to identify a change in BOLD signal between visits in the fasted state. My study is very likely not to have yielded the expected results due to insufficient power to detect differences between visits and groups. These pilot results provide a basis for planning extension of this study with larger numbers (see Chapter 6).

As in Chapter 3, the groups were not different in psychological traits that could have affected BOLD activation to food pictures. BAND subjects did differ between visits in the number of hours of sleep the night before the visit. They had approximately 1.5 hours less sleep on the Fed-Saline than the Fed-Octreotide visit. Since sleep deprivation can increase the neural responsivity to food (St-Onge et al. 2012), it is possible that this chance occurrence has partially masked some of the potential difference between groups in activation to food that may have been present. No other confounding variables measured were different between the groups.

Unfortunately the gut hormone analyses are not available for this part of the study at this time, as further study visits are planned and the hormone assays are best carried out in a single run to improve accuracy. However, the test sample results suggest that Octreotide is having the desired effect of reducing the post-prandial elevations in particularly PYY and GLP-1 seen after RYGB surgery.

4.6 Conclusions

In summary, this cross-sectional study of RYGB and BAND patients has yielded preliminary evidence of an increase in BOLD activation of the nucleus accumbens to food pictures and their associated appeal in RYGB but not BAND patients when given the somatostatin analogue, Octreotide, which is expected to reverse the post-prandial exaggerated gut hormone responses in RYGB. Octreotide also reduced post-meal satiety in the RYGB group but did not change the palatability or intake of an ice cream test meal.

The preliminary nature of this small study precludes extensive interpretation especially of the difference between surgical groups. In addition the lack of specificity of Octreotide action, and various factors additional to changes in plasma PYY and GLP-1 may have influenced the results. These include effect on insulin and plasma glucose levels, suppression of ghrelin and other gut hormones such as CCK and oxyntomodulin, and potential increases in plasma bile acid levels. Furthermore Octreotide itself may affect satiety and brain-hedonic responses examined in this study.

Nonetheless, the results would be in agreement with the hypothesis that acute post-prandial anorexigenic gut hormone responses may indeed influence hedonic responses to food in patients after RYGB surgery, although perhaps in different areas of the reward system to those where chronic effects of repeated elevations may exercise an effect. Future measurement of acyl ghrelin, PYY, GLP-1 and bile acids and correlation of BOLD activation in a larger group of RYGB and BAND patients will extend to and add to this work.

CHAPTER 5: STRUCTURAL BRAIN DIFFERENCES

AFTER BARIATRIC SURGERY FOR OBESITY

5.1 Introduction

Brain structural changes or abnormalities within normal or disease populations can be visualized in a number of ways, but voxel-based morphology (VBM, for grey matter) and diffusion tensor imaging (DTI, for white matter) have emerged as effective ways of measuring subtle effects of disease and age-related neurodegeneration on specific structures and tissue types at a group level.

VBM is a neuroimaging analysis technique that measures voxel-wise grey matter (grey matter) volume and topographical differences in brain structures across populations or across time, using high-resolution structural MRI T1 scans (Ashburner et al. 2003). Brain tissue is extracted from scans to exclude superfluous tissue, such as skull tissue, and the images undergo tissue-type segmentation to separate out grey matter from white matter and cerebrospinal fluid. Each individual's structural scan is then registered to a template constructed from a standardized brain (the average of a large number of control brains) by spatial warping. The grey matter density in each voxel across the brain is then calculated. Voxel-wise comparisons across individuals are then made correcting for multiple comparisons. A region of interest approach can also be used, in which pre-selected clinically or behaviourally relevant anatomical regions of interests are selected and the average grey matter density within these voxels compared between groups (see Sections 1.5.4 and 2.14 for more detail).

VBM has been a particularly useful tool in detecting early signs of neurodegeneration in conditions such as Alzheimer's disease and cerebrovascular disease (Ferreira et al. 2011; Pan et al. 2012) and as a measure of response to treatment (Bottini et al. 2012). Its use has widened in the last decade to include exploration of neuroanatomical abnormalities in psychiatric illnesses including affective and psychotic illnesses (Fusar-Poli et al. 2011; Ivleva

et al. 2012; Haller et al. 2013; Lai 2013; Piras et al. 2013) and anorexia nervosa (Titova et al. 2013). It has also been used to examine brain morphological associations with less disease-orientated cognitive constructs such as personality traits, chronic pain and addiction (Pan et al. 2012; Ivo et al. 2013; Liu et al. 2013; Obermann et al. 2013).

FIRST (FMRIB's Integrated Registration and Segmentation Tool) is a newer, model-based segmentation tool, which, using Bayesian principles and a library of training data, to model the most probable average shape and likely variations of a given brain structure, across a population. An average volume measurement of the particular anatomical structure in question across the population can then be calculated. This technique aims to replace previous volumetric analyses that made use of manual tracing around structures, which were open to inconsistency in agreement of structure borders (Konrad et al. 2009; Patenaude et al. 2011). Volumetric differences between groups in areas of interest are also calculated using T1 images but are processed in subject as opposed to standard brain space.

A number of VBM cohort studies of adults younger than 70 years have found that grey matter (and white matter) volume may be *increased* in the OFC, dorsal striatum, peri-hippocampal areas and amygdala in obesity or *positively* associated with increased BMI (Pannacciulli et al. 2006; Haltia et al. 2007; Horstmann et al. 2011; Orsi et al. 2011; Taki et al. 2012). However, one study of adults younger than 70 years, found BMI to be *negatively* associated with grey matter volume in frontal, hippocampal, caudate, striatal and gustatory cortex areas (Kurth et al. 2012). There appears to be a distinction in results of grey matter volume associations with BMI based on age. For instance, in studies of adults older than 70 years, *lower* grey matter volume in frontal, striatal (putamen), peri-hippocampal areas, gustatory cortex and amygdala was associated with increasing BMI (Ho et al. 2010; Raji et al. 2010; Walther et al. 2010). In adolescents, OFC grey matter volume was *reduced* in obesity

(Maayan et al. 2011), and reduced frontal grey matter volumes and increased striatal and hippocampal white matter volumes predicted future weight gain (Yokum et al. 2012).

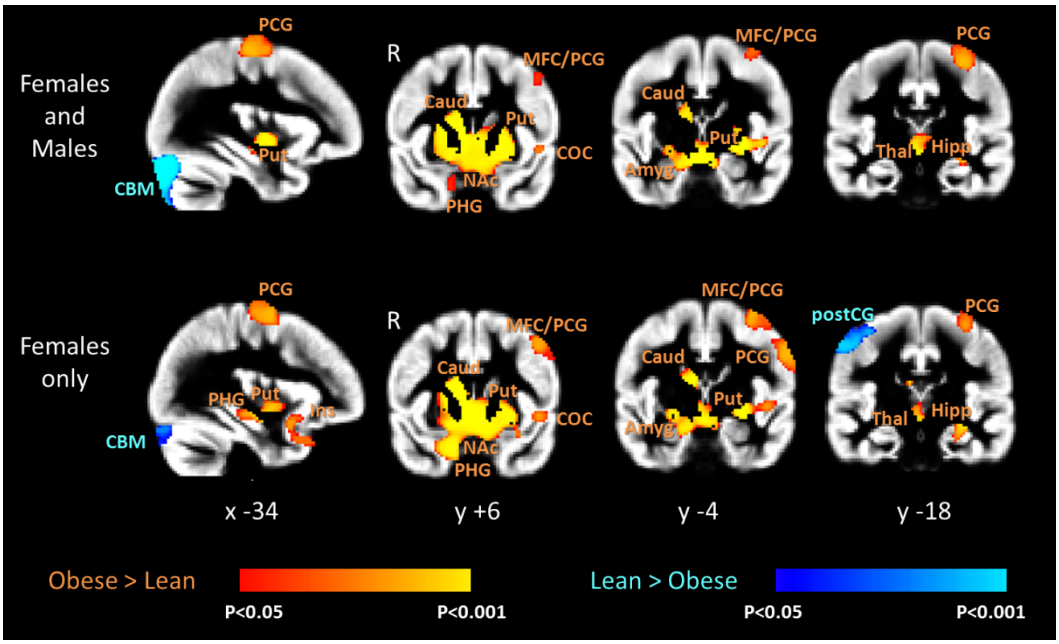
Disordered eating such as binge eating (Schafer et al. 2010) and increased disinhibition on TFEQ (Maayan et al. 2011) and food preference (Cohen et al. 2011) have also been associated with increased OFC grey matter volume (see Section 1.8.1, Table 1.3 for more detail)

In an unpublished VBM study examining a large cohort of adults under 70yr across a range of BMIs, our Group (Natalie White, MSc Clinical Neuroscience project) has recently found obese subjects to have increased grey matter volume in the caudate, putamen, nucleus accumbens, amygdala, precentral gyrus, middle temporal gyrus, parahippocampal gyrus, and the left middle frontal gyrus on whole brain analysis (adjusting for age and gender, TFCE, corrected $P<0.05$) (Figure 5.1, see Appendix 14 for detailed results and MNI coordinates). This analysis compared a group of obese (age mean ± SD 37.0 ± 10.6 y, BMI median [interquartile range 25%-75%] 35.1 [32.6- 42.5] kg/m^2) with lean (age 33.5 ± 12.0 y, BMI 22.8 [21.0- 24.3] kg/m^2) subjects.

Similarly, in a region of interest analysis, obese subjects had significantly higher grey matter volume compared to the obese subjects in the bilateral nucleus accumbens ($P<0.001$), amygdala ($P< 0.03$), caudate nucleus ($P<0.001$), pallidum, ($P< 0.001$), and putamen ($P< 0.001$) (adjusted for age, gender and ICV) (see Appendix 15 for statistics and post-hoc results).

Subcortical volumetric analysis using FIRST of the same patients found reduced nucleus accumbens volume in the obese compared to normal weight subjects, adjusted for age and gender (see Appendix 16). The results of this study are summarized in Table 5.1.

Figure 5.1 Comparison of GM volume between obese and normal weight subjects



Whole brain VBM results for male and female subjects (top, n=50) and female subjects only (bottom, n=34), displaying difference in grey matter density between obese and lean subjects. Orange shows brain regions with greater GM volume in obese than lean, and blue lower in obese than lean. Results overlaid onto GM template for lean and obese groups. Co-ordinates given in MNI space. Abbreviations: PCG, Precentral gyrus; Put, Putamen; CBM, Cerebellum; PHG, Parahippocampal gyrus; Ins, Insula; Caud, Caudate; MFC, Middle frontal gyrus; COC, Central Opercular cortex; NAc, Nucleus accumbens; Amyg, Amygdala; Thal, Thalamus; Hipp, Hippocampus; postCG, Postcentral gyrus; R, Right.

DTI is a technique that uses MRI to measure quantitatively the coherence and direction of white matter based on the pattern of diffusion of water molecules within these tracts. Diffusion occurs predominantly along one axis in white matter and occurs preferentially along intact white matter parallel to the direction of the tract. Diffusion perpendicular to the tract is limited by the presence of cell membranes and myelin. Both the rate and direction of diffusion of water molecules carries information and this information is used as a measure of

the structural integrity of the white matter tracts. Fractional anisotropy refers to how parallel the flow of water is compared to the main direction of the tract, and mean diffusivity, the average rate of diffusion of water in all directions within a voxel.

DTI has been widely used to investigate white matter tract integrity in psychiatric illness, including schizophrenia, depression, obsessive compulsive disorder and autism (White et al. 2008), as well as anorexia nervosa (Frieling et al. 2012). Brain insult resulting from trauma results in reduced white matter tract integrity, as do most psychiatric illnesses. This is generally evidenced by reduced fractional anisotropy (FA), and sometimes increased mean diffusivity (MD) (Assaf et al. 2008).

Several DTI studies have demonstrated an association of *reduced* FA with increased BMI, indicative of reduced white matter tract integrity, in the middle and superior cerebellar peduncles, parts of the midbrain, the internal capsule, cingulum and peri-hippocampal tracts (Verstynen et al. 2012), as well as the corpus callosum and fornix (Mueller et al. 2011; Stanek et al. 2011).

There are a number of reasons why obese people may have altered grey matter and white matter structure in different parts of the brain compared to normal weight individuals. Obesity itself may lead to atrophy or damage in brain structure, through cerebrovascular disease, inflammation or lack of micronutrients due to poor diet (e.g. diet low in fish oils or high in saturated fat). For instance, high fat and sugar diets can directly damage the brain in animal studies by reducing hippocampal brain-derived neurotrophic factor (Molteni et al. 2002), whereas omega-3 fish oils may have a protective role in brain function and structure (Luchtman et al. 2013). Obesity, and particularly associated metabolic syndrome, is increasingly seen as a chronic inflammatory condition, leading to activation of astroglia and microglia and high levels of pro-inflammatory cytokines in the hippocampus in animal

studies (Thirumangalakudi et al. 2008). This inflammation leads to cognitive deficits and associated structural brain damage (Fung et al. 2012; Yates et al. 2012). One VBM study found the inflammatory marker, fibrinogen, to be associated with increased grey matter volume in the OFC (Cazettes et al. 2011). Obesity related atrophy might therefore result in *reduced* grey matter volume, especially in older adults, whereas obesity-related inflammation may result in *increased* grey matter volume, as seen in younger adults. These inflammatory processes in the brain are potentially reversable by bariatric surgery. RYGB has been shown in animals to reduce hippocampal microglial infiltration, and improve memory in a task designed to test hippocampal function (Grayson et al. 2013).

Brain structural changes associated with behaviour related to obesity may also be important. For instance, impulsivity was negatively correlated with ACC (Lee et al. 2013), OFC (Hesslinger et al. 2002) and VMPFC volumes (Matsuo et al. 2009), and with reduced FA in the frontostriatal white matter tracts (Gruber et al. 2011; Peper et al. 2013). Increased reward sensitivity positively correlated with grey matter volume in the somatosensory cortex (Moreno-Lopez et al. 2012; Weng et al. 2013) and OFC (Tanabe et al. 2009; Weng et al. 2013), and reduced FA in frontal, corpus callosum tracts (Weng et al. 2013) and para-hippocampal gyrus (Yuan et al. 2011). On the other hand addiction (non-alcohol) is associated with reduced ACC, DLPFC, amygdala and hippocampus volumes (Cousijn et al. 2012). Therefore there may be structural alterations within the brains of obese people which are unrelated to obesity itself, but are related to behavioural correlates of obesity, either predisposing to, or caused by obesity.

As evidenced from Chapter 3, there were significant differences in hedonic responses to food, including fMRI activation to food pictures and the appeal and palatability of high-calorie foods between obese patients who had undergone RYGB compared to BAND surgery.

This preferential effects on food hedonics in RYGB may potentially lead to differences in grey and white matter structure in areas involved in encoding reward value and sensitivity (nucleus accumbens, caudate, putamen), emotional processing (amygdala) and behavioural conditioning to food (hippocampus, OFC, DLPFC) between these groups if making the assumption that neural pathways that are used more frequently increase in volume or density over time. RYGB surgery also results in eating behaviour that differs significantly from that seen in BAND surgery. Proportionally less calories from fat were consumed by RYGB patients, compared to BAND patients, and this in itself may differentially affect brain structure in these groups (Molteni et al. 2002).

Possible mechanisms underlying the lower hedonic response in obese people after RYGB compared to BAND, were further explored in Chapter 4. Administration of Octreotide, a somatostatin analogue, to both RYGB and BAND subjects, reversed exaggerated anorexigenic gut hormone responses in RYGB patients. This resulted in increased NAcc activation to, and increased appeal rating of food pictures in the RYGB group only, albeit only small numbers (n=7) were included in the analysis. These results, although preliminary, support the potential mechanism of PYY and GLP1 in reducing the hedonic response to food in RYGB. One previous study has shown that postprandial PYY levels are positively associated with caudate grey matter volume in normal weight adults (Weise et al. 2012), providing another possible link between changes after RYGB and brain structure.

Weight loss itself, through either RYGB or BAND surgery may also result in changes in grey and white matter in obesity. Longitudinal studies have shown that weight loss through dieting in obesity results in increased white matter volume in the parahippocampal gyrus, fusiform gyrus and temporal gyrus, and in reduced FA in the cingulate and temporal areas (Haltia et al. 2007).

In this third part of the cross-sectional study I therefore explored, using VBM, sub-cortical volume analysis (FIRST) and DTI, whether grey or white matter structural differences, particularly in brain reward systems, exist between patients who have undergone RYGB or BAND surgery for obesity. Furthermore, comparison with BMI-matched unoperated controls gives an indication as to whether weight loss itself, independent of BMI, may result in structural changes to the brain, for example, repair of potential obesity-related microstructural damage to white and grey matter or reversal of structural differences related to obesity, over-eating or associated psychological traits.

5.2 Hypothesis

1. Reduced activation in OFC and amygdala to food pictures was seen in obese patients who had undergone RYGB, compared to BAND surgery, associated with healthier eating behaviour in RYGB patients, whereas unhealthy eating behaviour such as binge eating, is associated with increased OFC grey matter volume. It is therefore hypothesized that grey matter volume and density would be lower in the RYGB compared to the BAND group in the OFC and amygdala, after adjusting for age, gender, BMI and ICV.

2. Increased grey matter volume in the OFC, striatum (nucleus accumbens, caudate and putamen), peri-hippocampal areas and amygdala has been reported in obese adults under the age of 70 years. Therefore, based on the assumption that obesity-related changes in brain structure are reversible with weight loss it is hypothesized that grey matter volume in these regions would be lower in operated patients (RYGB and/or BAND), compared to BMI-matched unoperated controls.

3. Increased BMI and behavioural traits linked to obesity such as reward sensitivity, have been associated with reduced white matter integrity in frontostriatal, corpus callosum and

peri-hippocampal tracts. It is therefore hypothesized that white matter integrity in these tracts would be greater in operated patients (RYGB and/or BAND), compared with BMI-matched unoperated controls, and perhaps also greater in the RYGB compared to the BAND group, since RYGB subjects had healthier food hedonic responses.

5.3 Aims

The aims of this cross-sectional study were to determine using VBM, volumetric and DTI analysis whether grey matter volume, and white matter integrity in areas of the brain associated with reward, emotional and cognitive processing:

1. differed between obese subjects who underwent RYGB compared to BAND surgery,
2. differed between obese subjects who underwent bariatric surgery (RYGB and BAND) and unoperated BMI-matched controls,
3. correlated with BMI independently of group

5.4 Results

5.4.1 VBM results

5.4.1.1 Participant characteristics

There were no significant differences between the three groups in age, gender ratio, ethnic background distribution, current BMI, percentage body fat or prevalence of binge eating disorder (BED) at the time of scanning. The two surgical groups had similar pre-operative BMI and pre-operative prevalence of BED. The RYGB group had more obesity-associated co-morbidities pre-operatively, but not post-operatively, compared to the BAND group (Table 5.1).

Table 5.1 Participant demographics at time of structural brain scans used for VBM analysis

	BMI-M	BAND	RYGB	P [a]
n	20	19	19	
Age (years)	39.1 ± 2.3 (20.0 - 55.0)	39.8 ± 2.5 (22.0 - 59.0)	42.9 ± 1.9 (23.0 - 59.0)	0.46
Gender (Male : Female)	3:17	1:17	2:18	0.86
Ethnicity: European Caucasians, n (%)	10 (50%)	14 (74%)	15 (79%)	0.14
Pre-operative BMI (kg/m^2)	n/a	45.5 ± 1.3 (36.5 – 57.0)	51.1 ± 2.5 (34.7 – 74.6)	0.07
Current BMI (kg/m^2)	35.9 ± 1.9 (24.7 - 55.6)	35.5 ± 1.4 (24.8 - 50.0)	36.3 ± 2.0 (23.4 - 54.2)	0.96
Current Height (m)	1.66 [1.59 - 1.69] (1.49 - 1.78)	1.66 [1.61 - 1.74] (1.56 - 1.79)	1.65 [1.60 - 1.68] (1.52 - 1.85)	0.59
Current Weight (kg)	87.0 [77.9 -118.8] (65.5 - 162.5)	96.5 [88.8 - 108.7] (75.2 - 121.7)	98.7 [84.6 - 118.2] (63.6 - 144.0)	0.57
Current body fat (%)	44.3 [35.2 - 50.7] (26.0 - 54.0)	43.3 [38.6 - 50.0] (21.7 - 54.1)	43.1 [34.4 - 50.0] (16.8 - 68.2)	0.97
Weight loss (% of pre-operative weight)	n/a	23.2 ± 2.5 (22.6 - 52.0)	29.1 ± 1.4 (17.7 - 40.0)	0.05
Pre-operative obesity co-morbidity score	n/a	5.5 ± 0.5 (1.0 - 10.0)	9.7 ± 1.0 (3.0 - 19.0)	0.001
Current obesity co-morbidity score	0.0 [0.0 - 5.5] (0.0 - 18.0)	1.0 [0.0 - 2.0] (0.0 - 9.0)	1.0 [0.0 - 3.0] (0.0 - 10.0)	0.86
Pre-operative BED	2 (10%)	4 (22%)	4 (22%)	0.57
Post-operative BED		2 (11%)	1 (5%)	0.55

Data are presented as mean ± SEM or median [IQR], and range in brackets. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests or, if not normally disturbed, Mann Whitney U test and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test or, if not normally distributed, Friedman ANOVA on Ranks with *post hoc* Dunn's test. Below statistically significant P values (<0.05) for the overall ANOVA, the statistically significant pairwise comparisons and the direction of the result are shown using > or <. BMI-M: body mass index matched, BAND: gastric banding, RYGB: gastric bypass.

Abbreviations: BAND: gastric banding, BED: binge eating disorder; DM: type 2 diabetes mellitus, DTI: Diffusion Tensor Imaging; n/a not applicable; RYGB: gastric bypass; VBM: voxel-based morphometry

5.4.1.2 Region of interest analysis

Total grey matter, white matter (WM) and cerebrospinal fluid (CSF) volume was calculated using the program, SIENAX, on an individual and group level for the three groups. There was no significant difference between the groups for any of these measures (Table 5.2). Intracranial volume (ICV) was calculated by adding total brain (GM + WM) volume with CSF volume. Individual ICV measurements were also used as a covariate on an individual level in the VBM analysis between the three groups.

Table 5.2 Whole brain volume measurements using SIENAX

	BMI-M	BAND	RYGB	BMI-M vs. BAND+RYGB P-value
n	**20**	**19**	**19**	
CSF	29.5 ± 2.1 (12.0 – 46.7)	28.0 ± 2.6 (13.6 – 45.1)	34.4 ± 2.7 (16.8 – 70.0)	0.18
Peripheral GM	666.6 ± 7.8 (610.7 - 738.4)	663.1 ± 9.8 (580.9 – 727.6)	767.2 ± 9.7 (710.7 – 845.9)	0.22
Total GM	792.5 ± 8.9 (729.9 – 860.2)	791.7 ± 10.6 (702.1 – 864.9)	646.4 ± 8.4 (574.1 – 732.3)	0.12
Total WM	779.1 ± 7.3 (730.2 – 846.9)	772.0 ± 9.0 (718.6 – 871.8)	775.4 ± 8.6 (707.2 – 842.9)	0.83
Total brain (GM + WM)	1,571.6 ± 14.3 (1,480.9 – 1,676.1)	1,563.8 ± 16.1 (1,420.6 – 1,652.4)	1,542.6 ± 15. (1,425.4 – 1,676.1)	0.39
Intracranial volume (ICV)	1,158.4 ± 31.0 (992.6 – 1,560.7)	1,542.6 ± 15.2 (1,425.4 – 1,676.1)	1,185.6 ± 20.9 (984,6 – 1,319.0)	0.77

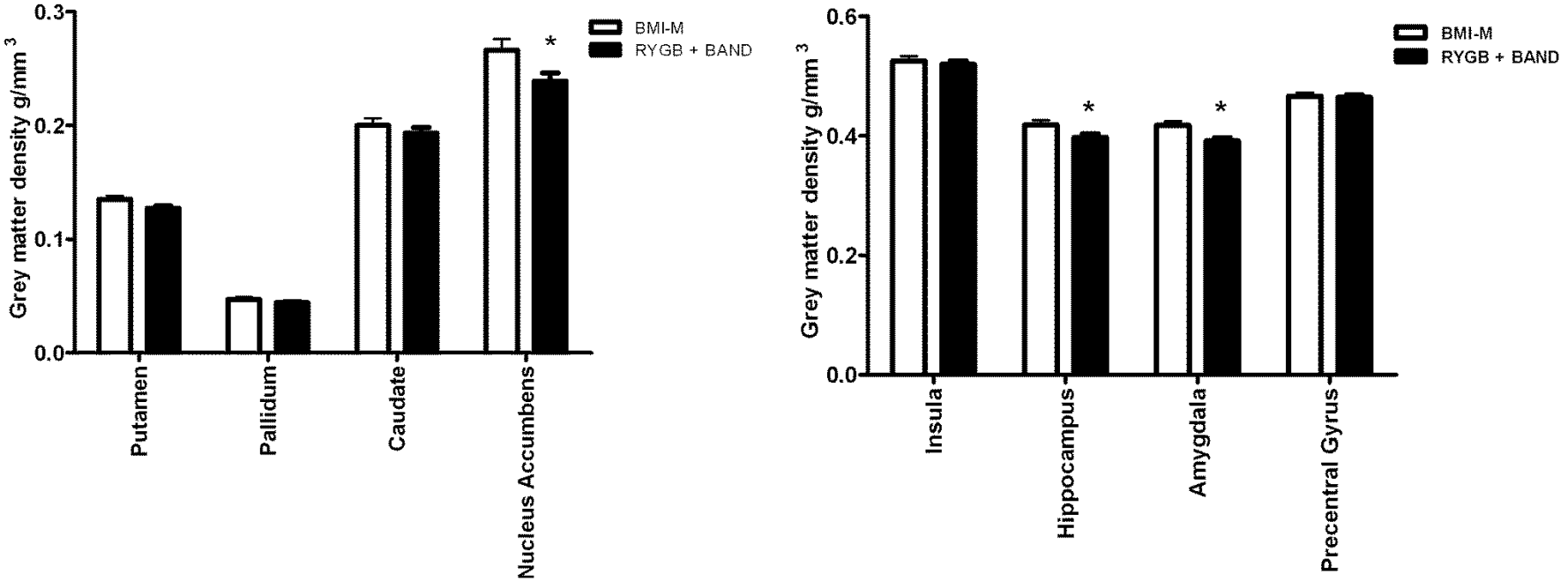
Data are presented as mean ± SEM cm^3, and range in brackets. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Data are corrected for age and gender, and normalized for skull size, except for ICV. Comparisons used one-way ANOVA with *post hoc* Fisher's LSD test.

Abbreviations: BMI-M: body mass index matched, BAND: gastric banding; CSF: Cerebrospinal Fluid; GM: GM; RYGB: gastric bypass; WM: White Matter.

ROIs were obtained using Harvard anatomical masks for 8 *a priori* defined ROIs: bilateral nucleus accumbens, amygdala, caudate, hippocampus, insula, pallidum, putamen and precentral gyrus (as a control area). After adjusting for age, gender and BMI, grey matter volume was significantly lower in obese patients who had undergone bariatric surgery (i.e. RYGB and BAND patients combined) compared to BMI-matched unoperated controls in the amygdala, nucleus accumbens and hippocampus (Table, 5.3, Fig 5.2). In comparison between all 3 groups, amygdala grey matter volume was also significantly lower in both RYGB and BAND groups than BMI-matched unoperated controls. There was however no significant difference in grey matter volume between RYGB and BAND patients in any of the ROIs (Table 5.3, Fig 5.3).

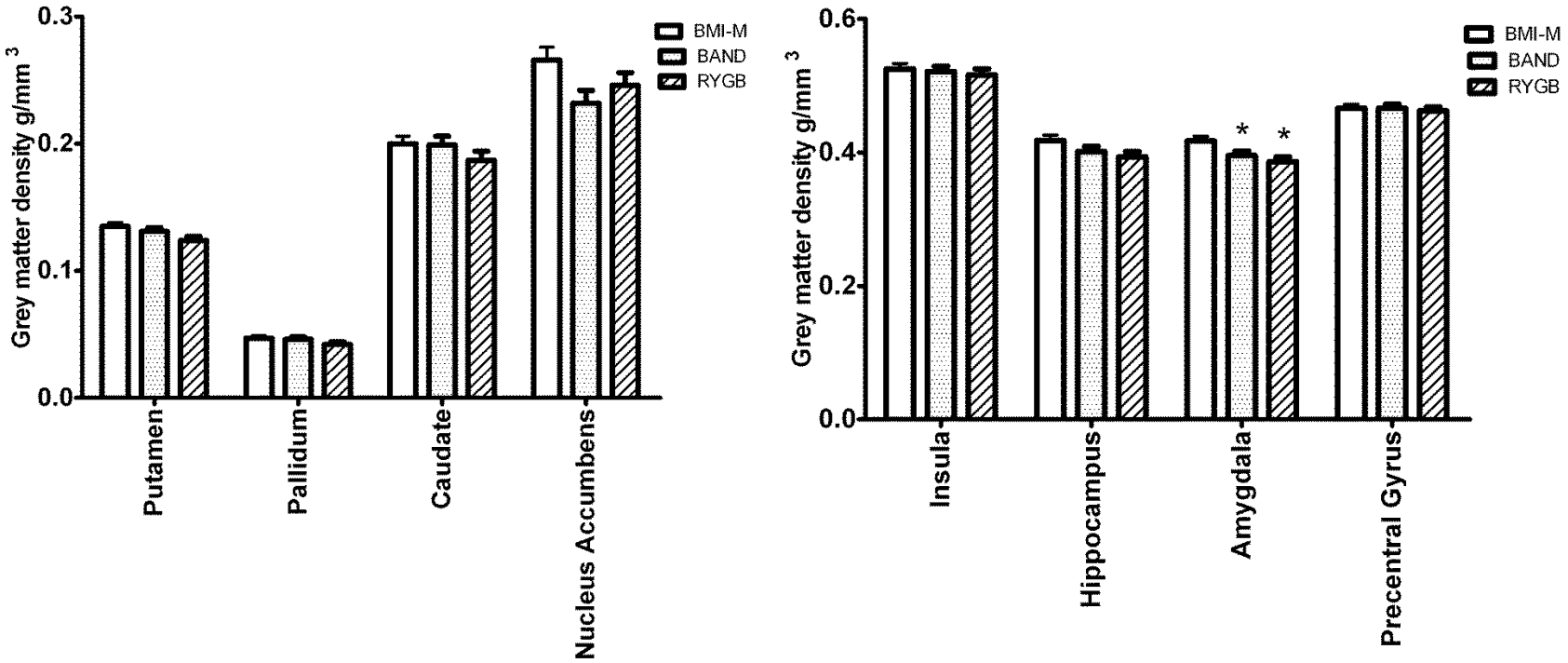
After adjusting for age, gender, BMI *and* ICV, grey matter volume in the amygdala remained significantly lower in the operated group (RYGB and BAND combined) and RYGB group alone compared to the BMI-matched unoperated controls (Table 5.4, Fig 5.4), but the findings in the nucleus accumbens and hippocampus did not reach significance. No new ROIs showed any significant difference in grey matter volume between groups when also adjusting for ICV (Table 5.4, Fig 5.5).

Figure 5.2 Comparison of GM density (adjusted for age, gender and BMI) between obese patients after bariatric surgery and controls



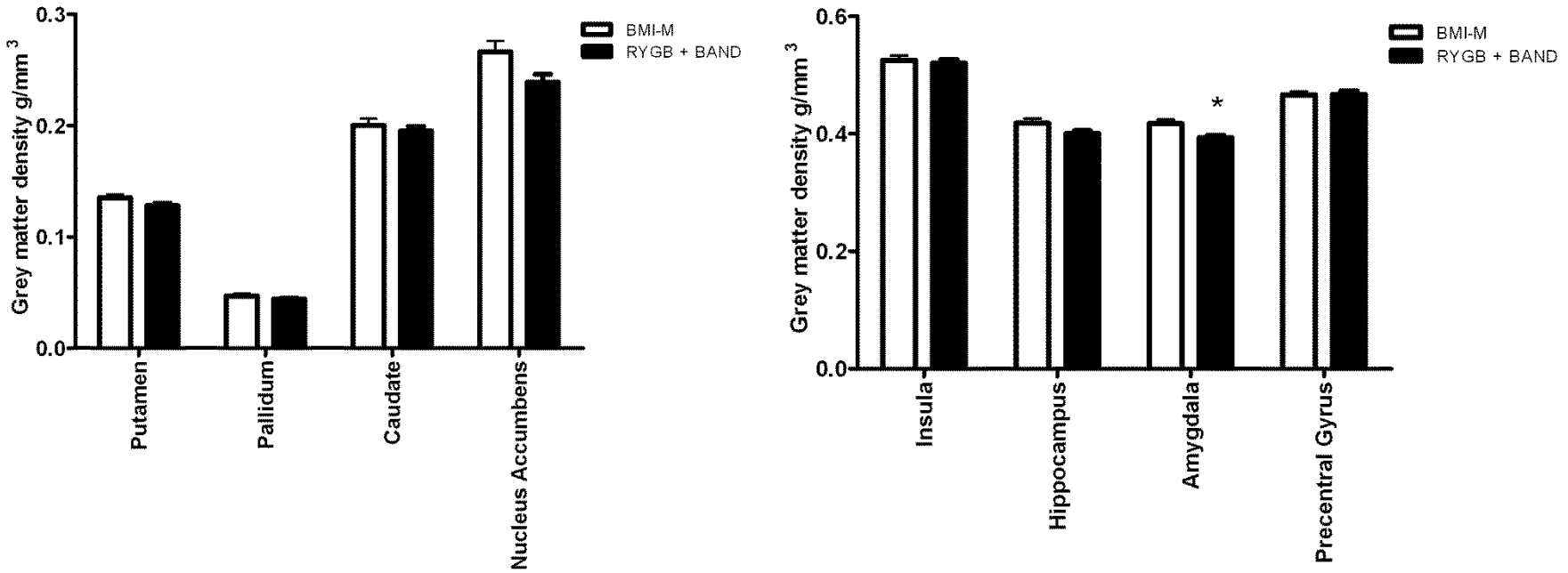
Comparison of GM density using voxel-based morphometry, in bilateral *a priori* regions of interest, between BMI-matched controls (BMI-M) (n=20, white) and obese patients who have undergone gastric bypass (RYGB) or gastric banding (BAND) surgery (n=38, black), adjusting for age, gender and BMI. Data are presented as mean ± SEM. *P<0.05 vs. BMI-M

Figure 5.3 Comparison of GM density (adjusted for age, gender and BMI) between obese patients after gastric bypass, gastric banding surgery and controls



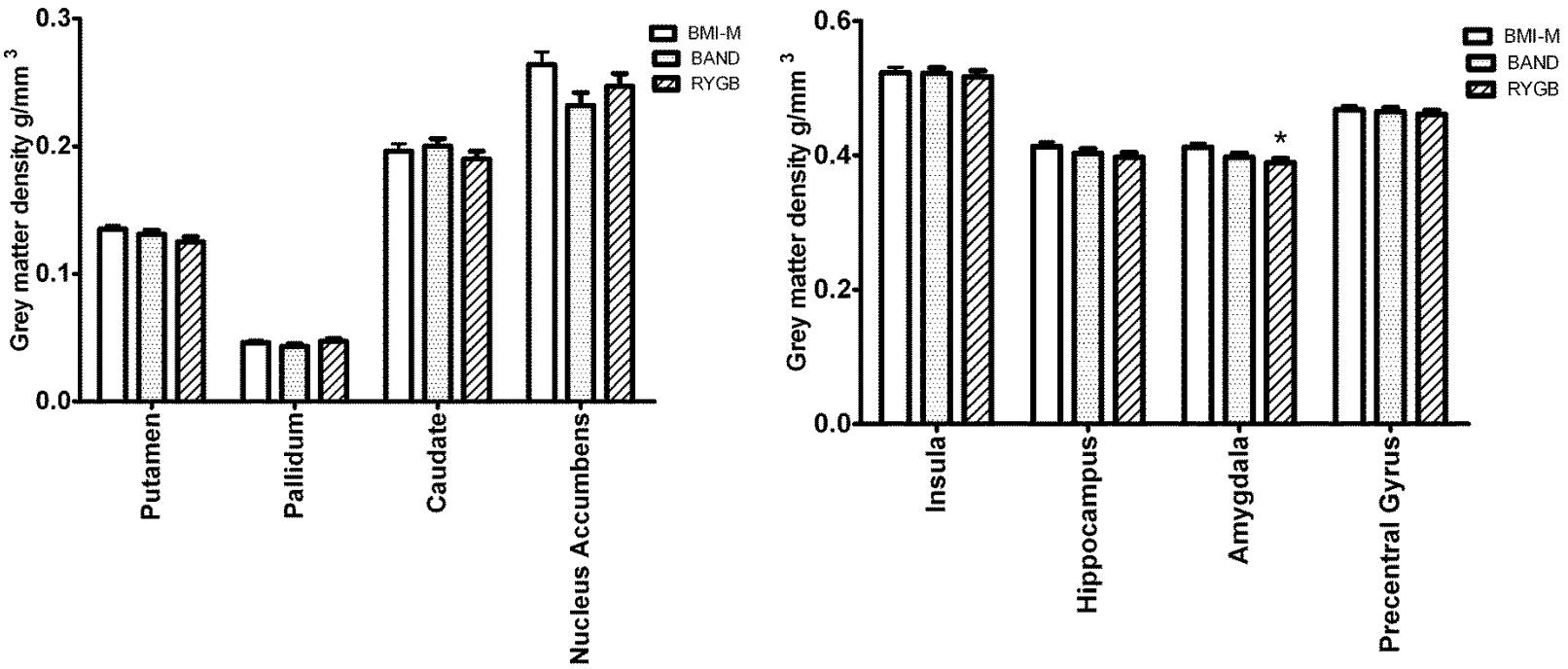
Comparison of GM density using voxel-based morphometry, in bilateral *a priori* selected regions of interest, between BMI-matched controls (BMI-M) (white), obese patients who have undergone banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery adjusting for age, gender and BMI. Data are presented as mean ± SEM. n=19-20 per group. *P<0.05 vs. BMI-M

Figure 5.4 Comparison of GM density (adjusted for age, gender, BMI and ICV) between obese patients after bariatric surgery and controls



Comparison of GM density using voxel-based morphometry, in bilateral *a priori* regions of interest, between BMI-matched controls (BMI-M) (n=20, white) and obese patients who have undergone gastric bypass (RYGB) or gastric banding (BAND) surgery (n=38, black), adjusting for age, gender, BMI and ICV. Data are presented as mean ± SEM. *P<0.05 vs. BMI-M

Figure 5.5 Comparison of GM density (adjusted for age, gender, BMI and ICV) between obese patients after gastric bypass, gastric banding surgery and controls



Comparison of GM density using voxel-based morphometry, in bilateral *a priori* selected regions of interest, between BMI-matched controls (BMI-M) (white), obese patients who have undergone banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery adjusting for age, gender, BMI and ICV. Data are presented as mean ± SEM. n=19-20 per group. *P<0.05 vs. BMI-

Table 5.3 Voxel-based morphometry region of interest analysis adjusted age, gender, BMI (and ICV)*

	BMI-M	BAND	RYGB	BMI-M vs. BAND vs. RYGB Adjusted P	BAND vs. RYGB Adjusted P	BMI-M vs. BAND+RYGB Adjusted P
n	**20**	**19**	**19**			
Bilateral Accumbens	0.266 ± 0.012 (0.156 - 0.382)	0.230 ± 0.091 (0.168 - 0.306)	0.246 ± 0.092 (0.189 - 0.309)	0.07 (0.10)*	0.32 (0.36)*	0.03 (0.06)*
Bilateral Amygdala	0.411 [0.386-0.443] (0.362 - 0.474)	0.397 [0.367-0.418] (0.355 - 0.459)	0.383 [0.363-0.417] (0.346 - 0.437)	0.006 (0.03)* BAND<BMI-M 0.029 RYGB<BMI-M 0.002 (0.009)*	0.30 (0.40)*	0.002 (0.01)*
Bilateral Caudate	0.202 ± 0.009 (0.129 - 0.301)	0.199 ± 0.006 (0.166 - 0.266)	0.185 ± 0.007 (0.130 - 0.228)	0.36 (0.55)*	0.17 (0.21)*	0.40 (0.89)*
Bilateral Hippocampus	0.416 ± 0.010 (0.342 - 0.502)	0.402 ± 0.006 (0.344 - 0.450)	0.395 ± 0.008 (0.320 - 0.398)	0.09 (0.32)*	0.49 (0.67)*	0.04 (0.16)*
Bilateral Insula	0.524 ± 0.011 (0.435 - 0.617)	0.513 ± 0.009 (0.439 - 0.582)	0.526 ± 0.006 (0.483 - 0.595)	0.76 (0.89)*	0.79 (0.96)*	0.62 (0.75)*
Bilateral Pallidum	0.046 [0.042–0.050] (0.034 - 0.058)	0.045 [0.039-0.051] (0.030 - 0.069)	0.042 [0.040-0.046] (0.033 - 0.058)	0.18 (0.23)*	0.17 (0.16)*	0.25 (0.32)*
Bilateral Precentral Gyrus	0.467 ± 0.007 (0.414 - 0.514)	0.468 ± 0.007 (0.418 - 0.530)	0459 ± 0.008 (0.386 - 0.538	0.91 (0.77)*	0.84 (0.78)*	0.86 (0.59)*
Bilateral Putamen	0.136 ± 0.003 (0.108 to 0.168)	0.130 ± 0.004 (0.100 to 0.150)	0.126 ± 0.004 (0.098 to 0.155)	0.08 (0.16)*	0.14 (0.15)*	0.07 (0.14)*

Data are presented as mean ± standard error of the mean ± SEM g/mm^3 or median [interquartile range] g/mm^3 for data that is not normally distributed, and range in brackets. Data appear in the raw format, but were analyzed adjusting for age, gender and BMI as covariates, and (age, gender, BMI and un-normalized ICV)*. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests or, if not normally disturbed, Mann Whitney U test and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test or, if not normally distributed, Friedman ANOVA on Ranks with *post hoc* Dunn's test. Below statistically significant P values (<0.05) for the overall ANOVA, the statistically significant pairwise comparisons and the direction of the result are shown using > or <. BMI-M: body mass index matched, BAND: gastric banding, RYGB: gastric bypass.

5.4.1.3 Whole brain analysis

In the whole brain VBM analyses, using threshold free cluster enhancement, there was one cluster in the left temporal region for which grey matter volume was *lower* in the patients who had undergone BAND surgery compared to BMI-matched unoperated controls, after adjusting for age, gender and BMI (Table 5.4). There were 2 clusters in left middle temporal region and left amygdala for which grey matter volume was *lower* in patients who had undergone bariatric surgery (BAND and RYGB groups combined) compared to BMI-matched unoperated controls, after adjusting for age, gender and BMI (Table 5.4).

There were no significant clusters where the BAND and RYGB combined group had *greater* grey matter volume than BMI-matched controls, where grey matter volume BMI-matched controls differed from BAND alone or RYGB alone, or where grey matter volume differed between the BAND and RYGB subjects, adjusting for age, gender and BMI.

Table 5.4 Spatial coordinates of whole brain comparison of GM volume between groups.

Contrast	Number of voxels	TFCE P value	x	y	z	Brain region
BMI-M > BAND	98	0.028	-70	-44	-8	Left middle temporal
BMI-M > BAND and RYGB combined	485	0.004	-64	-42	-6	Left middle temporal
	67	0.038	-24	-4	-16	Left amygdala

Co-ordinates for whole brain analysis using threshold-free cluster enhancement (TFCE) for differences in GM density between BMI-M (n=20), BAND (n=19) and RYGB (n=19), adjusting for age, gender and BMI.

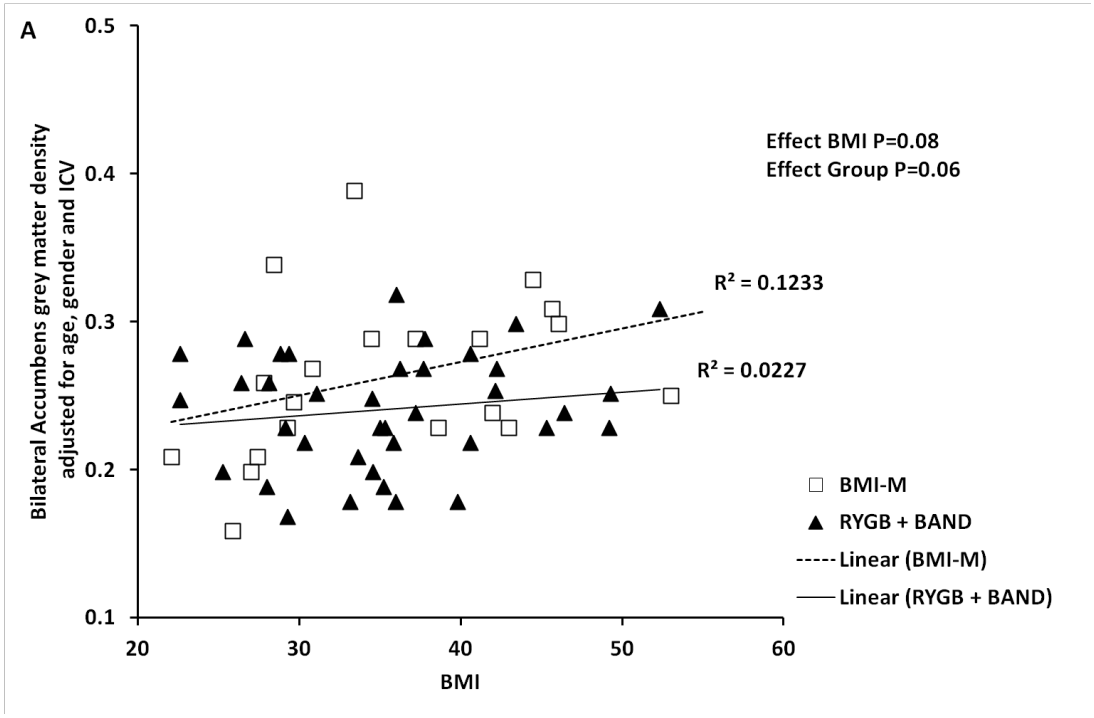
5.4.1.4 Effect of bariatric surgery on influence of BMI on VBM

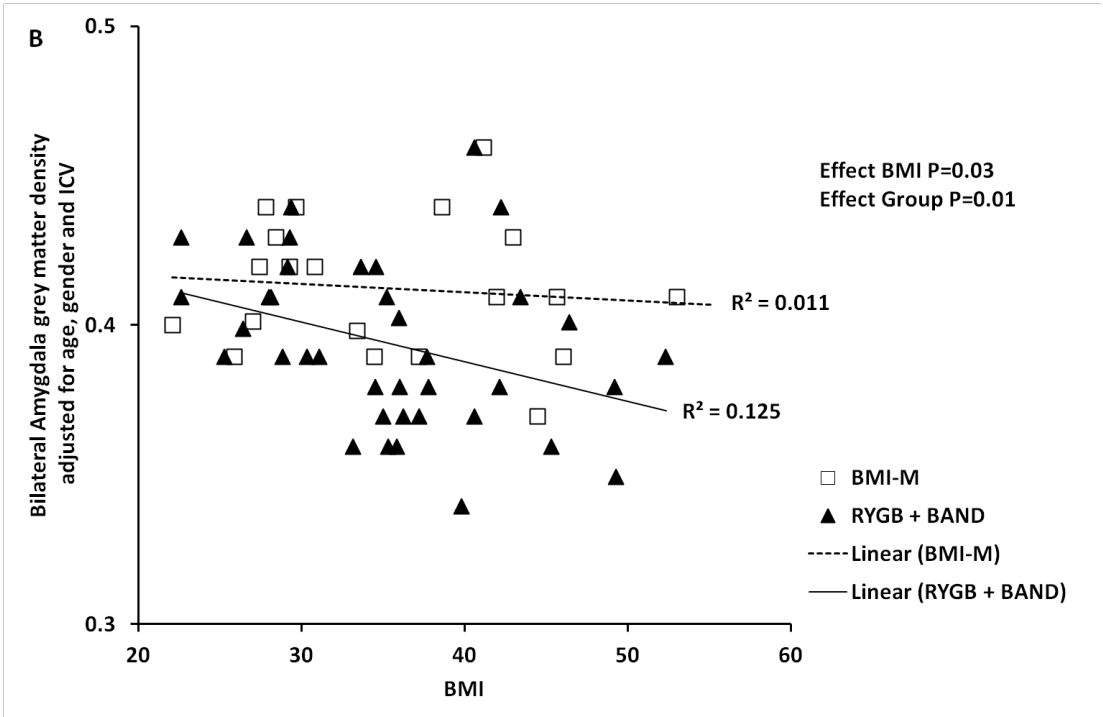
When comparing operated with unoperated groups in the VBM analysis using multiple regression linear analysis, there was no significant interaction effect for the interaction of group and BMI (group x BMI) for the combined RYGB + BAND group vs. unoperated BMI-M group in any of the ROIs, adjusting for age, gender and ICV (Table 5.5).

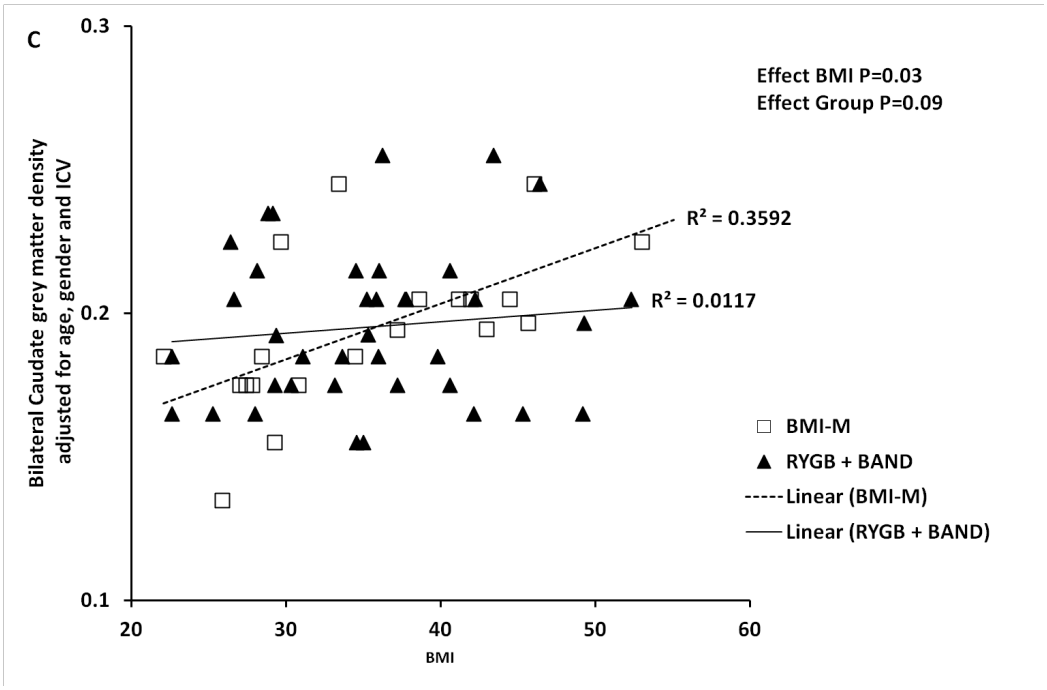
In the analysis of the combined RYGB and BAND group vs. BMI-matched unoperated control subjects, there was a significant overall effect of BMI, independent of group, to reduce grey matter volume in the amygdala (mean ± SEM β -0.0010 ± 0.0005, P=0.03) and increase grey matter volume in the caudate (mean ± SEM β -0.0010 ± 0.0005, P=0.03), with a trend to increase grey matter volume in the nucleus accumbens and reduce grey matter volume in the hippocampus (Table 5. 5, Fig 5.6 A-C). As reported in the ANCOVA, there was a significant effect of group, independent of BMI, such that the operated group had lower grey matter volume in the caudate than the unoperated group (Table 5.5).

When comparing between surgical groups for the VBM analysis, there was no significant interaction effect for the interaction of group and BMI (group x BMI) for the RYGB compared to BAND group in any of the ROIs. There was a significant overall effect of BMI, independent of group, to reduce grey matter volume in the amygdala (mean ± SEM β -0.0013 ± 0.0006, P=0.04) (Table 5.5).

Figure 5.6 Effect of BMI on GM density in obese patients after bariatric surgery and controls.







Relationship between BMI and GM density in (A) nucleus accumbens, (B) amygdala, (C) caudate in BMI-matched controls (BMI-M) (n=20, □and dashed line) and obese patients after gastric bypass (RYGB) or gastric banding (BAND) surgery (n=38, ▲ and solid line), adjusting for age, gender and ICV. n=19-20 per group.

Table 5.5 Effect of BMI, group and BMI*group on GM density between obese patients after bariatric surgery and controls

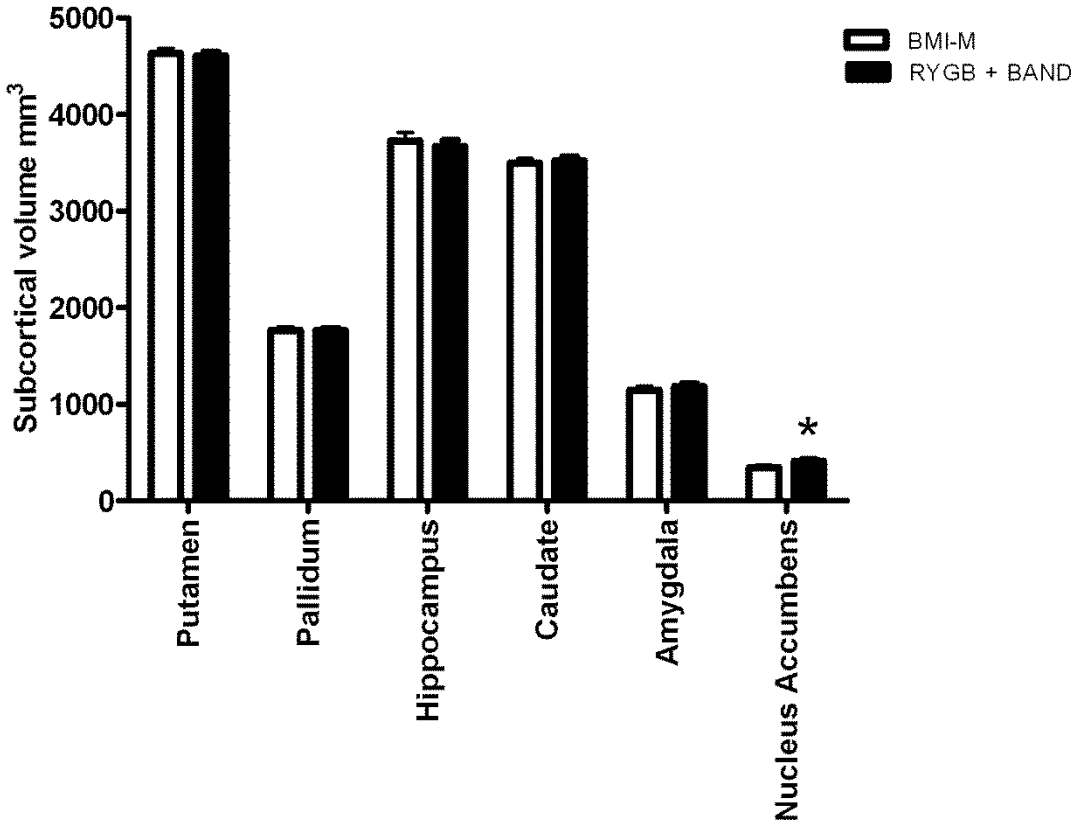
	Model A: age, gender, ICV, BMI, group, BMI*group		Model B: age, gender, ICV, BMI, group							
	BAND vs. RYGB	BMI-M vs. BAND +RYGB	BAND vs. RYGB				BMI-M vs. BAND+RYGB			
	BMI*group	BMI*group	BMI		BAND – RYGB Group		BMI		Operated – Unopterated Group	
	P	P	β ± SEM	P	β ± SEM	P	β ± SEM	P	β ± SEM	P
N. Accumbens	0.23	0.35	0.0011 ± 0.0008	0.19	-0.012 ± 0.012	0.36	0.0014 ± 0.0008	0.08	0.025 ± 0.013	0.06
Amygdala	0.15	0.24	-0.0013 ± 0.0006	**0.04**	0.008 ± 0.009	0.40	-0.0010 ± 0.0005	**0.03**	-0.019 ± 0.007	**0.01**
Caudate	0.61	0.09	0.0006 ± 0.0006	0.29	0.011 ± 0.009	0.21	0.0010 ± 0.0005	**0.03**	-0.001 ± 0.008	0.89
Hippocampus	0.24	0.50	-0.0013 ± 0.0007	0.06	0.004 ± 0.010	0.67	-0.0011 ± 0.0005	0.06	-0.013 ± 0.009	0.16
Insula	0.19	0.40	0.0001 ± 0.0009	0.87	0.001 ± 0.013	0.96	-0.0000 ± 0.0006	0.95	-0.003 ± 0.010	0.75
Pallidum	0.32	0.13	-0.0001 ± 0.0002	0.70	0.004 ± 0.003	0.15	0.0000 ± 0.0001	0.95	-0.002 ± 0.002	0.32
Precentral Gyrus	0.80	0.41	0.0004 ± 0.0006	0.58	0.003 ± 0.009	0.78	0.0007 ± 0.0005	0.13	-0.004 ± 0.008	0.59
Putamen	0.42	0.53	0.0003 ± 0.0003	0.39	0.007 ± 0.005	0.15	0.0004 ± 0.0003	0.18	-0.007 ± 0.004	0.14

Statistical results from multiple linear regression analysis. GM volume was adjusted for age, gender and ICV. There was no effect of the interaction of BMI and group (BMI*group) on the GM density, adjusted for age, gender and ICV. The effect of group and BMI on GM density adjusted for age, gender and ICV was calculated excluding BMI*group from the model. Values refer to statistical significance (P value) for effect of: (BMI*group), and β± SEM and P-value of BMI and group on GM density adjusted for age, gender and ICV**.**

5.4.2 Subcortical volume (FIRST)

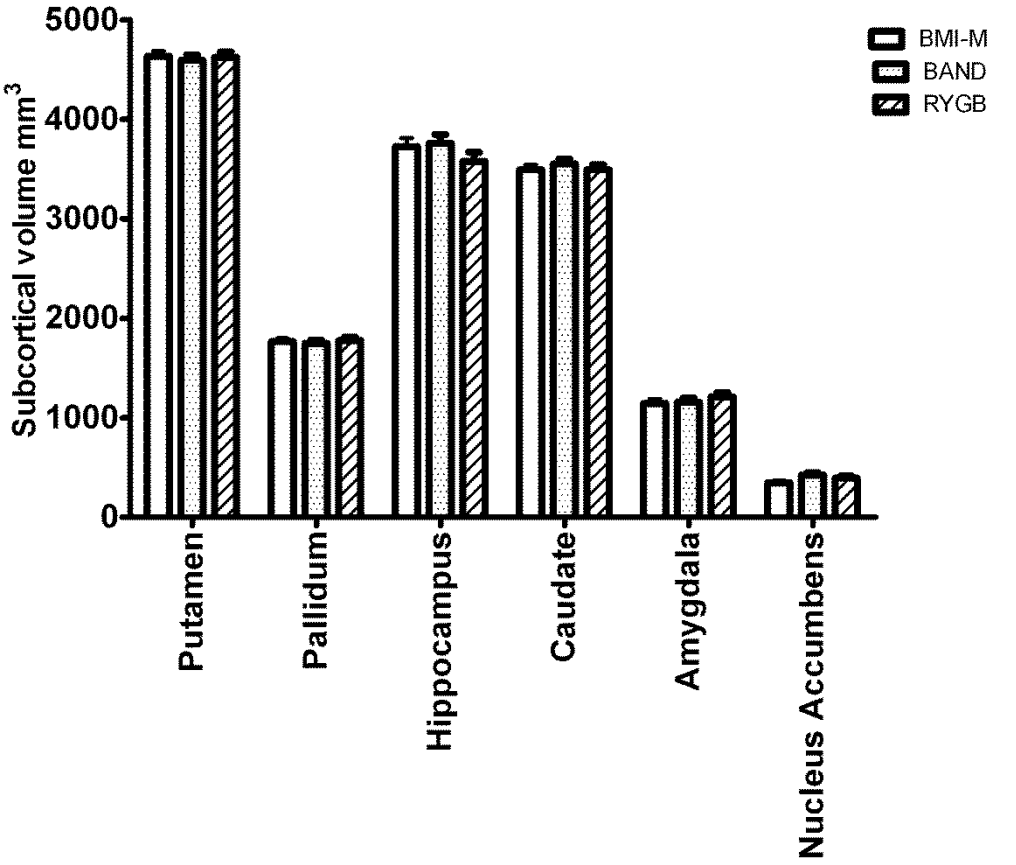
Subcortical volumetric measurements of *a priori* selected anatomical structures were carried out using FIRST. Using this measurement technique, the volume of the bilateral nucleus accumbens was *higher* in the operated group (RYGB and BAND combined) compared to unoperated BMI-matched controls, after adjusting for age, gender, BMI and ICV (Table 5.6, Fig. 5.7). There were no significant differences between the 3 groups in the subcortical volumetric analysis of any of the aforementioned structures (Table 5.6, Fig. 5.8).

Figure 5.7 Comparison of sub-cortical volume between obese patients after bariatric surgery and controls



Comparison of GM volume using FIRST, in bilateral *a priori* regions of interest, between BMI-matched controls (BMI-M) (n=20, white) and obese patients who have undergone gastric bypass (RYGB) or gastric banding (BAND) surgery (n=38, black), adjusting for age, gender, BMI and ICV. Data are presented as mean ± SEM. *P<0.05 vs. BMI-M.

Figure 5.8 Comparison of sub-cortical volume between obese patients after gastric bypass, gastric banding surgery and controls



Comparison of GM volume using FIRST, in bilateral *a priori* regions of interest, between BMI-matched controls (BMI-M, white) and obese patients who have undergone gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery, adjusting for age, gender, BMI and ICV. Data are presented as mean ± SEM. n=19-20 per group.

Table 5.6 Subcortical volumetric region of interest analysis using FIRST

	BMI – M	BAND	RYGB	BAND vs. RYGB vs. BMI-M P	BAND vs. RYGB P	BAND+RYGB vs. BMI-M P
n	**20**	**19**	**19**			
Bilateral Accumbens	338.1 ± 25.5 (97.0 - 476.2)	435.1 ± 21.0 (293.2 - 660.2)	389.6 ± 25.1 (186.2 - 570.6)	0.07	0.27	0.04
Bilateral Amygdala	1131.4 ± 48.5 (802.6 - 1653.2)	1171.8 ± 42.6 (857.5 - 1474.5)	1208.2 ± 55.4 (871.7 - 1543.0)	0.53	0.37	0.48
Bilateral Caudate	3472.5 ± 111.8 (2346.7 - 4370.1)	3603.8 ± 112.7 (2831.8 - 4505.1)	3463.3 ± 91.3 (2779.6 - 4241.4)	0.66	0.41	0.66
Bilateral Hippocampus	3714.8 ± 86.6 (3035.4 - 4624.8)	3774.4 ± 105.9 (3109.2 - 4822.6)	3574.8 ± 114.3 (2319.8 - 4440.2)	0.33	0.22	0.61
Bilateral Pallidum	1741.6 ± 52.8 (1427.0 - 2545.0)	1762.1 ± 52.6 (1463.9 - 2384.6)	1790.2 ± 46.3 (1341.6 - 2116.8)	0.84	0.54	0.92
Bilateral Putamen	4556.9 ± 104.3 (3682.4 - 5399.5)	4653.2 ± 141.0 (3753.6 - 5806.1)	4642.6 ± 139.3 (3740.4 -5875.1)	0.87	0.66	0.71
Bilateral Thalamus	7778.5 ± 204.9 (5879.9 - 9880.8)	7734.9 ± 185.5 (6533.8 - 9534.9)	7954.9 ± 167.8 (6294.9 - 9031.8)	0.29	0.12	0.99

Data are presented as mean ± SEM mm^3, and range in brackets. Data was corrected for intracranial volume (ICV), age, gender and BMI. Data appear in the raw format, but were analyzed adjusting for as covariates. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test. Below statistically significant P values (<0.05) for the overall ANOVA, the statistically significant pairwise comparisons and the direction of the result are shown using > or <. BMI-M: body mass index matched, BAND: gastric banding, RYGB: gastric bypass.

5.4.3 DTI results

5.4.3.1 Participant characteristics

There were no significant differences between the three groups in age, gender ratio, ethnic background distribution, current BMI, percentage body fat or prevalence of BED. The two surgical groups had similar pre-operative BMI and pre-operative prevalence of BED. The RYGB group had more obesity-associated co-morbidities pre-operatively, but not post-operatively, compared to the BAND group (Table 5.7).

Table 5.7 Participant demographics at time of DTI scans

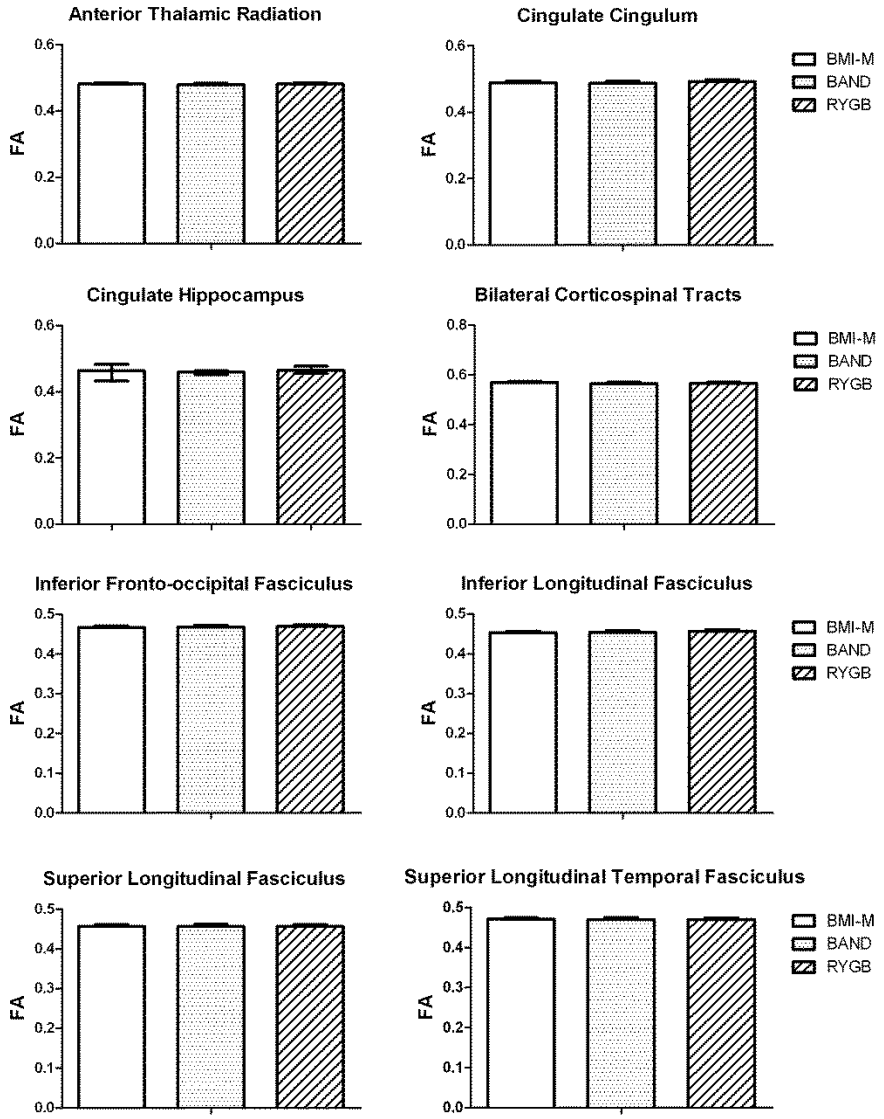
	BMI-M	BAND	RYGB	P [a]
n	17	12	17	
Age (years)	39.7 ± 2.5 (20.0 - 55.0)	40.9 ± 3.4 (22.0 - 59.0)	44.9 ± 2.1 (23.0 - 59.0)	0.52
Gender (Male : Female)	3:14	1:11	2:15	0.87
Ethnicity: European Caucasians, n (%)	8 (47%)	10 (83%)	13 (76%)	0.10
Pre-operative BMI (kg/m^2)	n/a	49.4 ± 3.8 (36.5 – 86.2)	53.4 ± 3.4 (34.6 – 88.1)	0.45
Current BMI (kg/m^2)	30.8 [27.8 - 42.0] (24.7 - 55.6)	34.6 [29.0 - 37.3] (24.8 - 45.7)	36.6 [30.5 - 39.2] (23.6 - 54.2)	0.64
Current Height (m)	1.65 ± 0.02 (1.49 - 1.78)	1.68 ± 0.03 (1.53 - 1.79)	1.66 ± 0.02 (1.52 - 1.85)	0.69
Current Weight (kg)	87.0 [73.9 – 116.8] (65.5 - 162.5)	92.5 [86.8 - 104.0] (75.2 - 117.1)	98.7 [86.7 - 115.6] (64.2 - 144.0)	0.48
Current body fat (%)	41.2 [33.6 - 49.1] (26.0 - 54.0)	43.3 [37.4 -45.5] (21.7 - 53.2)	43.3 [36.7 - 49.3] (16.8 - 68.2)	0.96
Weight loss (% of pre-operative weight)	n/a	25.3 ± 3.4 (10.0 - 52.0)	29.0 ± 1.5 (16.3 - 40.0)	0.34
Pre-operative obesity co-morbidity score	n/a	5.0 [2.8 - 6.0] (1.0 - 9.0)	10.0 [6.5 - 12.0] (3.0 - 19.0)	0.002
Current obesity co-morbidity score	0.0 [0.0 – 3.5] (0.0 - 18.0)	1.5 [0.0 -2.0] (0.0 - 4.0)	1.0 [0.0 - 3.0] (0.0 - 10.0)	0.79
Pre-operative BED	2 (12%)	4 (33%)	3 (18%)	0.33
Post-operative BED		2 (17%)	1 (6%)	0.37

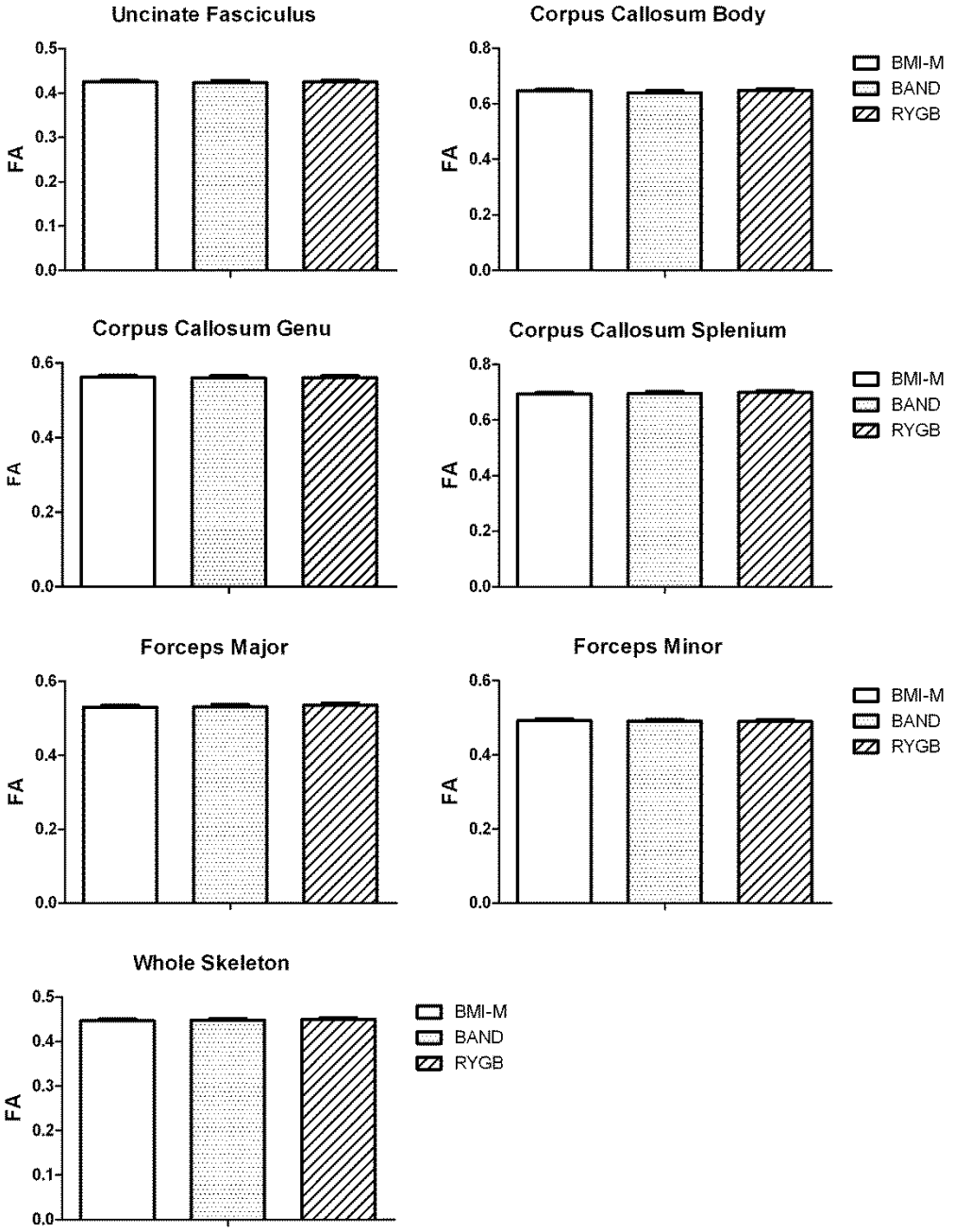
Data are presented as mean ± standard error of the mean ± SEM, and range in brackets. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests or, if not normally disturbed, Mann Whitney U test and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test or, if not normally distributed, Friedman ANOVA on Ranks with *post hoc* Dunn's test. Abbreviations: BAND: gastric banding, BED: binge eating disorder; DM: type 2 diabetes mellitus, DTI: Diffusion Tensor Imaging; n/a not applicable; RYGB: gastric bypass; VBM: voxel-based morphometry

5.4.3.2 Region of interest analysis

There was no significant difference of mean FA or MD in the any of the *a priori* white matter ROIs or the whole white matter skeleton between obese patients who had undergone RYGB compared to BAND surgery, or between operated patients (RYGB and BAND combined) compared to unoperated BMI-matched controls, after adjusting for age, gender and BMI (Table 5.7 and 5.8, Fig 5.9 and 5.10).

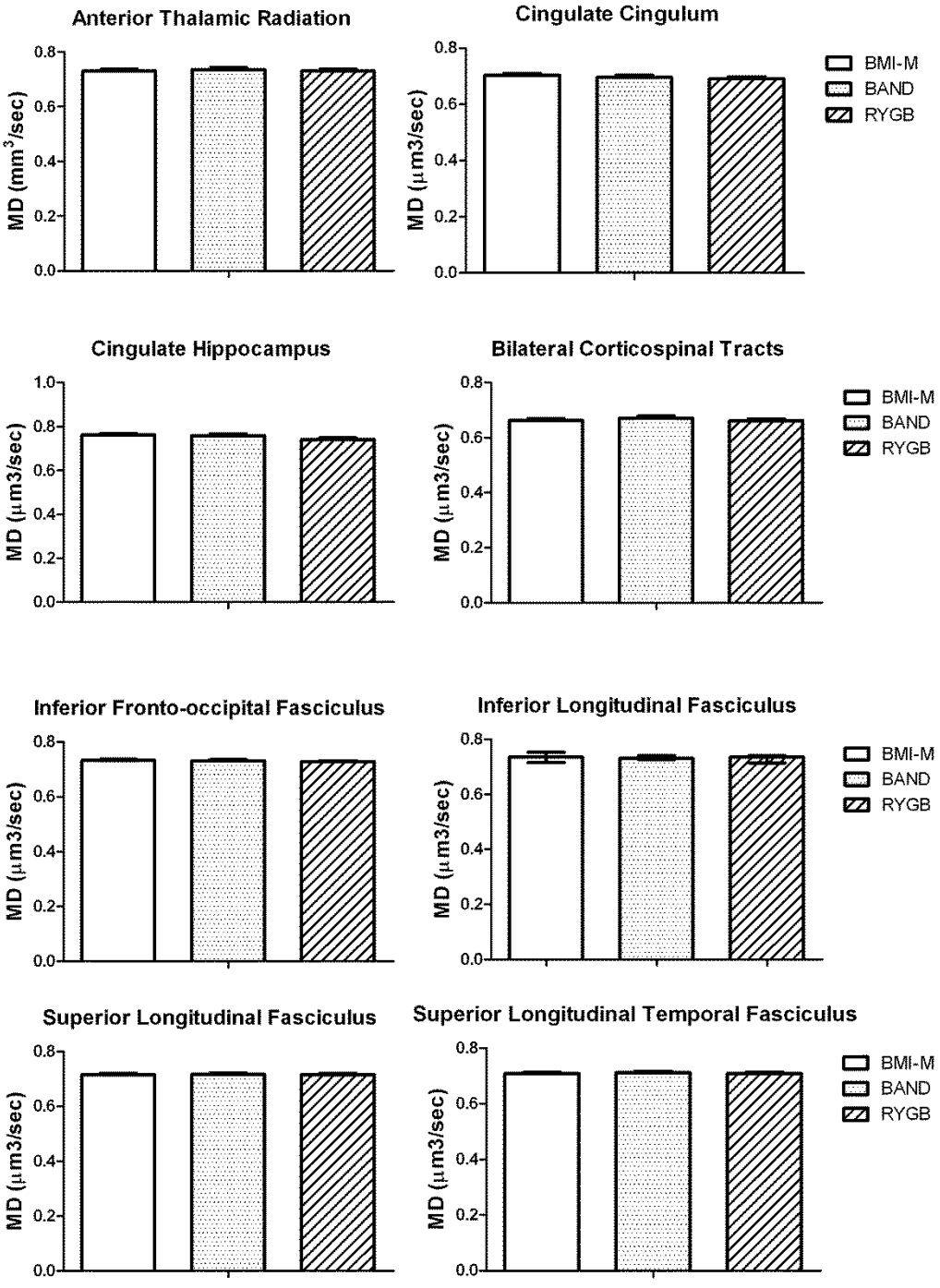
Figure 5.9 Comparison of white matter tract integrity fractional anisotropy between obese patients after gastric bypass, gastric banding surgery and controls

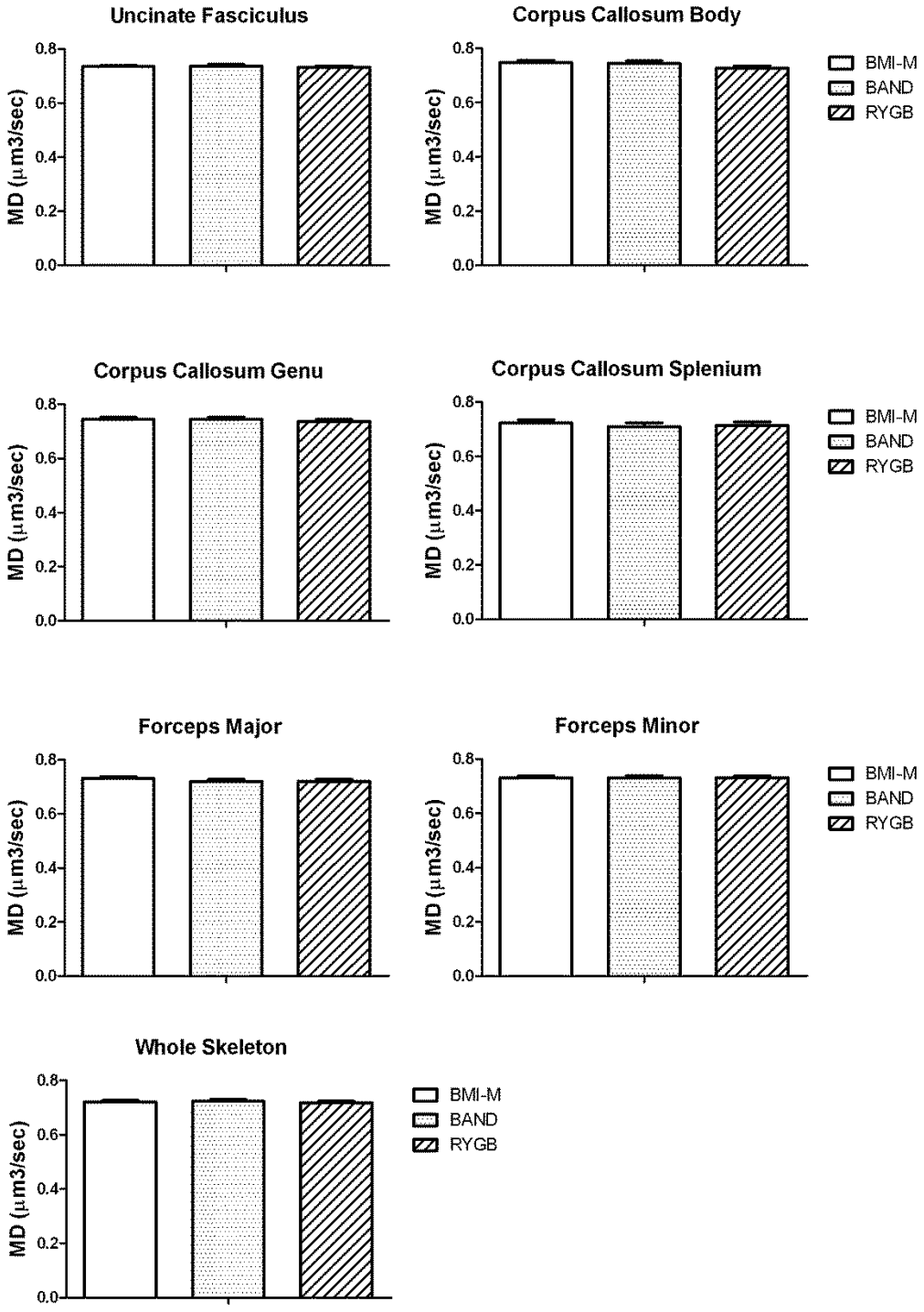




Comparison of bilateral fractional anisotropy (FA) in *a priori* white matter tracts and average of whole white matter skeleton, between BMI-matched controls (BMI-M, white), and obese patients who have undergone gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery, adjusting for age, gender and BMI. Data are presented as mean ± SEM, or median ± interquartile range for non-parametric data (only cingulate hippocampus). n=12-17 per group.

Figure 5.10 Comparison of white matter tract integrity mean diffusivity between obese patients after gastric bypass, gastric banding surgery and controls.





Comparison of mean diffusivity (MD), in *a priori* white matter tracts and average of whole white matter skeleton, between BMI-matched controls (BMI-M, white), obese patients who have undergone gastric banding (BAND, dotted) and gastric bypass (RYGB, striped) surgery, adjusting for age, gender and BMI. Data are presented as mean ± SEM, or median ± interquartile range for non-parametric data (only inferior longitudinal fasiculus). n=12-17 per group.

Table 5.8 Fractional anisotropy region of interest analysis

	BMI – M	BAND	RYGB	BMI-M vs. BAND vs. RYGB Adjusted P	BAND vs. RYGB Adjusted P	BAND + RYGB vs. BMI-M Adjusted P
n	17	12	17			
Bilateral Anterior Thalamic Radiation	0.483 ± 0.005 (0.460 to 0.528)	0.480 ± 0.004 (0.458 to 0.497)	0.478 ± 0.004 (0.446 to 0.517)	0.94	0.59	0.90
Bilateral Cingulum Cingulate	0.492 ± 0.006 (0.451 to 0.544)	0.488 ± 0.006 (0.454 to 0.519)	0.489 ± 0.006 (0.439 to 0.561)	0.84	0.49	0.85
Bilateral Cingulum Hippocampus	0.461 [0.435-0.479] (0.407 to 0.511)	0.459 [0.456 -0.4640] (0.444 to 0.475)	0.465 [0.456-0.473] (0.416 to 0.532)	0.56	0.36	0.34
Bilateral Corticospinal Tract	0.572 ± 0.005 (0.540 to 0.614)	0.566 ± 0.003 (0.550 to 0.588)	0.564 ± 0.004 (0.538 to 0.591)	0.79	0.99	0.49
Bilateral Inferior Fronto-occipital Fasciculus	0.470 ± 0.005 (0.441 to 0.519)	0.468 ± 0.004 (0.444 to 0.486)	0.467 ± 0.005 (0.429 to 0.504)	0.90	0.72	0.75
Bilateral Inferior Longitudinal fasciculus	0.455 ± 0.005 (0.424 to 0.501)	0.454 ± 0.004 (0.427 to 0.469)	0.455 ± 0.004 (0.418 to 0.483)	0.76	0.53	0.96
Bilateral Superior Longitudinal Fasciculus	0.459 ± 0.005 (0.427 to 0.510)	0.457 ± 0.003 (0.438 to 0.471)	0.455 ± 0.004 (0.416 to 0.479)	0.99	0.98	0.94
Bilateral Superior Longitudinal Temporal	0.473 ± 0.006 (0.434 to 0.524)	0.470 ± 0.003 (0.447 to 0.485)	0468 ± 0.005 (0.420 to 0.496)	0.99	0.98	0.87
Bilateral Uncinate Fasciculus	0.428 ± 0.005 (0.395 to 0.473)	0.423 ± 0.004 (0.400 to 0.446)	0.423 ± 0.004 (0.387 to 0.454)	0.91	0.75	0.78
Corpus Callosum Body	0.648 ± 0.006 (0.614 to 0.693)	0.643 ± 0.008 (0.597 to 0.678)	0.644 ± 0.007 (0.565 to 0.705)	0.73	0.43	0.88
Corpus Callosum Genu	0.565 ± 0.006 (0.531 to 0.616)	0.561 ± 0.006 (0.524 to 0.586)	0.556 ± 0.006 (0.509 to 0.612)	0.96	0.93	0.78

Corpus Callosum Splenium	0.695 ± 0.005 (0.655 to 0.730)	0.697 ± 0.006 (0.666 to 0.735)	0.698 ± 0.006 (0.646 to 0.776)	0.66	0.60	0.44
Forceps Major	0.532 ± 0.005 (0.495 to 0.578)	0.532 ± 0.004 (0.507 to 0.551)	0.533 ± 0.006 (0.476 to 0.566)	0.70	0.48	0.54
Forceps Minor	0.495 ± 0.006 (0.468 to 0.546)	0.491 ± 0.005 (0.456 to 0.512)	0.487 ± 0.005 (0.447 to 0.533)	0.97	0.98	0.83
Whole Skeleton	0.449 ± 0.004 (0.426 to 0.492)	0.448 ± 0.003 (0.431 to 0.462)	0.447 ± 0.004 (0.413 to 0.482)	0.90	0.63	0.76

Fractional anisotropy was compared between groups, adjusting for age, gender and BMI. Data are presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and range in brackets. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests or, if not normally disturbed, Mann Whitney U test and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test or, if not normally distributed, Friedman ANOVA on Ranks with *post hoc* Dunn's test. Below statistically significant P values (<0.05) for the overall ANOVA, the statistically significant pairwise comparisons and the direction of the result are shown using > or <. BMI-M: body mass index matched, BAND: gastric banding, RYGB: gastric bypass.

Table 5.9 Mean diffusivity region of interest analysis

	BMI – M	BAND	RYGB	BMI-M vs. BAND vs. RYGB Adjusted P	BAND vs. RYGB Adjusted P	BAND+RYGB vs. BMI-M Adjusted P
n	**17**	**12**	**17**			
Bilateral Anterior Thalamic Radiation	0.730 ± 0.006 (0.686 - 0.774)	0.735 ± 0.006 (0.712 -0.762)	0.733 ± 0.007 (0.682 - 0.770)	0.79	0.66	0.72
Bilateral Cingulum Cingulate	0.702 ± 0.005 (0.676 - 0.744)	0.695 ± 0.007 (0.657 - 0.725)	0.693 ± 0.007 (0.617 - 0.736)	0.33	0.56	0.17
Bilateral Cingulum Hippocampus	0.763 ± 0.006 (0.723 - 0.780)	0.759 ± 0.003 (0.732 - 0.776)	0.739 ± 0.009 (0.639 - 0.780)	0.09	0.13	0.15
Bilateral Corticospinal Tract	0.664 ± 0.007 (0.627 - 0.725)	0.671 ± 0.005 (0.650 - 0.708)	0.662 ± 0.006 (0.614 - 0.708)	0.63	0.39	0.83
Bilateral Inferior Fron-- occipital Fasciculus	0.732 ± 0.005 (0.702 - 0.768)	0.731 ± 0.003 (0.715 - 0.748)	0.729 ± 0.005 (0.695 - 0.771)	0.79	0.90	0.56
Bilateral Inferior Longitudinal fasciculus	0.736 [0.715-0.752] (0.708 - 0.791)	0.731 [0.727 - 0.741] (0.692 - 0.740)	0.736 [0.716-0.740] (0.700 - 0.768)	0.73	0.87	0.58
Bilateral Superior Longitudinal Fasciculus	0.715 ± 0.005 (0.685 - 0.754)	0.715 ± 0.004 (0.692- 0.740)	0.716 ± 0.004 (0.686 - 0.749)	0.99	0.84	0.94
Bilateral Superior Longitudinal Temporal	0.709 ± 0.004 (0.681 - 0.748)	0.712 ± 0.004 (0.688 - 0.732)	0.710 ± 0.004 (0.676 - 0.750)	0.92	0.93	0.80
Bilateral Uncinate Fasciculus	0.734 ± 0.005 (0.701 - 0.765)	0.738 ± 0.005 (0.700 - 0.763)	0.734 ± 0.006 (0.691 - 0.783)	0.88	0.76	0.94
Corpus Callosum Body	0.746 ± 0.007 (0.689 - 0.799)	0.743 ± 0.009 (0.693 - 0.788)	0.731 ± 0.009 (0.664 - 0.796)	0.16	0.16	0.19
Corpus Callosum Genu	0.744 ± 0.006 (0.697 - 0.785)	0.744 ± 0.006 (0.722 - 0.794)	0.740 ± 0.008 (0.671 - 0.807)	0.69	0.59	0.57

Corpus Callosum Splenium	0.723 ± 0.009 (0.621 - 0.775)	0.707 ± 0.014 (0.607 - 0.783)	0.716 ± 0.013 (0.561 - 0.786)	0.74	0.99	0.79
Forceps Major	0.730 ± 0.007 (0.662 - 0.766)	0.717 ± 0.007 (0.677 - 0.744)	0.722 ± 0.006 (0.662 - 0.766)	0.41	0.92	0.18
Forceps Minor	0.730 ± 0.005 (0.689 - 0.764)	0.732 ± 0.005 (0.702 - 0.773)	0.732 ± 0.007 (0.667 - 0.798)	0.99	0.96	0.96
Whole Skeleton	0.720 ± 0.005 (0.689 - 0.755)	0.723 ± 0.004 (0.707 - 0.740)	0.719 ± 0.005 (0.673 - 0.754)	0.76	0.57	0.95

Data are presented as mean ± standard error of the mean ± SEM μm^3/sec or median [interquartile range] μm^3/sec for data that is not normally distributed, and range in brackets. Data appear in the raw format, but were analyzed adjusting for age, gender and BMI as covariates. Normality was assessed using Kolmogarov-Smirnov test and variance with Levene's test. Comparisons between 2 groups used Student's unpaired t-tests or, if not normally disturbed, Mann Whitney U test and between 3 groups used one-way ANOVA with *post hoc* Fisher's LSD test or, if not normally distributed, Friedman ANOVA on Ranks with *post hoc* Dunn's test. Below statistically significant P values (<0.05) for the overall ANOVA, the statistically significant pairwise comparisons and the direction of the result are shown using > or <. BMI-M: body mass index matched, BAND: gastric banding, RYGB: gastric bypass.

5.4.3.3 Whole brain analysis

There were no significant differences in FA or MD in pairwise comparison between the RYGB and BAND, RYGB and BMI-matched control or BAND and BMI-matched control groups, after adjusting for age, gender and BMI, using voxelwise analysis of the whole brain corrected for multiple comparisons using TFCE. Similarly there were no differences in FA or MD across the whole brain between operated patients (RYGB and BAND combined) compared to BMI-matched unoperated controls.

5.4.3.4 Effect of bariatric surgery on influence of BMI on white matter tracts

When comparing between operated and unoperated groups for the FA and MD analysis using multiple regression linear analysis, there was no significant interaction effect of group and BMI (group x BMI) for the combined RYGB + BAND group compared to the unoperated BMI-M group in any of the ROIs, adjusting for age and gender.

In the combined analysis of operated (RYGB and BAND combined) compared to unoperated BMI-M group, there was no significant overall effect of BMI, independent of group, on FA or MD in any of the ROIs, adjusting for age and gender.

When comparing between surgical groups for the FA and MD analysis, there was no significant interaction effect of (group x BMI) for the RYGB compared to BAND group in any of the ROIs. There was also no significant overall effect of BMI, independent of group, on FA or MD.

5.5 Discussion

Using VBM, this study found that patients who have undergone RYGB and BAND surgery for obesity had significantly lower grey matter volume in sub-cortical brain regions than BMI-matched unoperated controls: for the amygdala on both whole brain and ROI analysis, and for the nucleus accumbens and hippocampus in ROI analysis only. However only the effect in the amygdala survived adjustment for ICV, in addition to age, gender and BMI. Using FIRST segmentation, the nucleus accumbens was significantly larger in the operated compared to unoperated groups. There were no significant differences in any of the measures of grey matter volume between the operated groups, i.e. RYGB compared to BAND group.

In VBM analysis, BMI, independent of group, correlated positively with caudate grey matter volume, and negatively with amygdala volume, with a trend for positive correlation with nucleus accumbens volume and negative correlation with hippocampus volume. The results of the Group's analysis of a larger cohort unoperated subjects across a wider range of BMI had previously shown similar directions of association with obesity or correlation with BMI for nucleus accumbens and caudate. However that analysis had shown opposite positive associations of obesity and/or BMI with amygdala and hippocampus grey matter volume, which are difficult to interpret.

There were also no significant differences between operated and unoperated subjects or between the 2 surgical groups in white matter integrity measured using DTI (mean FA or MD).

5.5.1 Amygdala

The amygdala is known to be involved in the processing of emotional responses to rewarding stimuli (Murray 2007). It appears to be particularly important in mediating

unconscious biases and preferences and making links between cues and the current value of reward, by incorporating the affective associations of the stimuli in a Pavlovian manner. Both positive and negative affective associations are processed in the amygdala. For instance, the amygdala plays an important role in the development of conditioned taste aversion (Yamamoto 1993), but also plays a role in positive reinforcement (Paton et al. 2006).

Early life emotional deprivation leads to increased amygdala volume (Mehta et al. 2009), and dysfunction in the amygdala has been implicated in illnesses of emotional regulation, such as depression (Kennedy et al. 1997). For instance, in depression, amygdala metabolism is positively associated with the severity of illness and reduces in response to treatment (Drevets 1999). A recent study found that mindfulness is associated with reduced amygdala volume (Taren et al. 2013). Mindfulness is a psychological attribute, which can be innate or acquired, which leads to increased awareness of thoughts, emotions and surroundings, whilst maintaining an attitude of curiosity, openness, and acceptance. Treatments incorporating mindfulness training have been shown to reduce stress reactivity, anxiety (Roemer et al. 2007) and disordered eating (Kristeller et al. 2011).

As seen in Figure 5.1 (and Table 5.10) and in one other study (Orsi et al. 2011) amygdala volume is increased in obesity or with raised BMI. The finding of reduced amygdala grey matter volume in the bariatric surgery groups, compared to the unoperated obese in this study therefore suggests that bariatric surgery may have a restorative role in this area of the brain, either through weight loss itself (for example by reduction in inflammation), or through a change in behaviour. How this relates to its role in emotional processing of reward is unclear, but it is possible that changes in emotional processing of food-related cues seen after bariatric surgery (and particularly RYGB), may result in a change in grey matter volume

or structure in this area. However the cross-sectional analysis of this study makes further interpretation of these findings difficult. In addition, the lack of difference between RYGB and BAND in grey matter volume of the amygdala, suggests that the finding of lower BOLD response to food pictures in RYGB compared to BAND patients in Chapter 3 is not due to structural differences between the groups.

In contradiction to the conclusion from the larger cohort analysis that raised BMI is associated with increased amygdala volume, BMI negatively correlated with amygdala volume independent of group (unoperated or operated) (See Figure 5.4B). This may reflect the smaller sample number in this analysis compared to the larger unoperated cohort, or a different effect of BMI in the surgical group as the negative correlation appeared most pronounced in the surgical group, although there was no significant group x BMI interaction.

Table 5.10 Summary table (Results Chapter 5 and previous Group study)

	OB vs. NW (previous analysis)	**BAND vs. RYGB**	**BAND+RYGB vs. BMI-M**
Eating behaviour questionnaire data	↑ BDI-II ↑ DEBQ restraint ↑ DEBQ emotional ↑ EPQ-R neuroticism	↑ EDE-Q restraint, ↑ EDE-Q weight concerns ↑ EDE-Q shape concerns	
GM density (whole brain), adjusted for age, gender, BMI	↑R caudate, putamen, amygdala, NAcc, cerebellum, L PCG, L middle temporal, L parahipp gyrus, L mid frontal gyrus ↓ R occipital, R inferior temporal, cerebellum, R post central gyrus	↔	↓ L middle temporal lobe, L amygdala
GM density (ROI), adjusted for age, gender, BMI		↔	↓ amygdala, NAcc, hippocampus
GM density (ROI), adjusted for age, gender, BMI, ICV	↑ NAcc, amygdala, caudate, pallidum, L PCG, putamen	↔	↓ amygdala
Subcortical volume adjusted for age, gender, ICV	↓ R NAcc	↔	↑NAcc
White matter integrity FA adjusted for age, gender, BMI		↔	↔
White matter integrity MD adjusted for age, gender, BMI		↔	↔

Summary table of eating behaviour questionnaire data and VBM results of studies carried out in our group. BMI-M: body mass index matched, BAND: gastric banding, EDE-Q: Eating Disorders Eamination-Questionnaire, L: left, NAcc: nucleus accumbens, NW: normal-weight, OB: obese, PCG: pre-central gyrus, R: right, RYGB: gastric bypass.

5.5.2 Nucleus accumbens

The nucleus accumbens is known to be important in the processing of reward, particularly related to food, sex and addictive drugs, including motivation, wanting, expectancy and novelty. It is also involved in reinforcement learning, mediated by dopaminergic and opioid systems (Hernandez et al. 1988; Pecina et al. 2000; Sabatinelli et al. 2007; Demos et al. 2012; Koch et al. 2013).

Nucleus accumbens connectivity with the precuneus and supplementary sensorimotor area mediates sensation-seeking in those with a high risk of addiction (family history of alcoholism) (Weiland et al. 2013). In obesity, the nucleus accumbens has increased connectivity with the OFC (Stoeckel et al. 2009) and nucleus accumbens activation to high-calorie food pictures correlated with percentage weight loss after a 12 week weight loss programme (Murdaugh et al. 2012). Nucleus accumbens volume decreases with decreased reward sensitivity over time, measured by the BAS questionnaire in adolescents (Urosevic et al. 2012), suggesting that the functional role of nucleus accumbens in regulating reward has volumetric correlates.

Nucleus accumbens volume is also increased in anxiety (Kuhn et al. 2011), decreases in line with obsessive traits (Narayanaswamy et al. 2013) and nucleus accumbens volume and morphology is altered in psychopathy (Boccardi et al. 2013), suggesting that the structure of this area is susceptible to psychological traits and states changes.

In the study referred to in Section 5.1, conducted within our Group (unpublished), obesity (see Figure 5.1 and Table 5.10) was associated with increased nucleus accumbens volume/density using VBM in a larger cohort, for which a trend was also found in this study using the combined unoperated and operated cohort. The larger cohort study study also

found reduced nucleus accumbens size using the FIRST technique. Another study has also found BMI to be positively correlated with nucleus accumbens volume (Horstmann et al. 2011).

In the current study, VBM results show reduced grey matter volume in nucleus accumbens in obese patients who had undergone bariatric surgery compared to BMI-matched unoperated patients. This suggests, similarly to the amygdala results, that obesity related changes have been reversed by bariatric surgery. However again the cross-sectional analysis of this study makes further interpretation of these findings difficult. This may be related to changes in behavioural correlates such as altered dietary restraint or external eating, although this was not tested.

Of note, nucleus accumbens (and hippocampus) volume differences between the operated and unoperated groups were abolished once ICV was included as a confounding covariate. It is not clear why this occurred, but may be related to reduction in the degrees of freedom by introducing a fourth covariate. Although many studies make adjustment for ICV or global grey matter volumes, some authors suggest that the sensitivity of detecting regional differences in grey matter volume may be reduced by adjusting for ICV, rather than increased (Ridgway et al. 2008).

FIRST results of subcortical volume, showed reduced nucleus accumbens size in obese patients compared to normal weight controls in the unpublished cohort study by our Group. The current analysis found increased nucleus accumbens size in the operated compared to unoperated obese group. Again this suggests a reversal of obesity related changes by bariatric surgery. The difference in direction between reduced nucleus accumbens density as measured by VBM and increased volume as measured by FIRST is difficult to interpret, but

possibly relates to methodological differences, perhaps with issues concerning segmentation, and the balance between increased cell number, synaptic density and intercellular matrix. The fact that the direction of difference between groups is consistent according to technique employed is however encouraging.

5.5.3. Hippocampus

The hippocampus is particularly important in learning and the consolidation of memory. It has been implicated as part of the system involved in reward-based learning. Alzheimer's disease has been consistently linked with reduced hippocampal volume as a result of atrophy (Ferreira et al. 2011). Long-standing depression (McKinnon et al. 2009), childhood maltreatment (Chaney et al. 2013) and diabetes (McIntyre et al. 2010; Cherbuin et al. 2012; Hempel et al. 2012) have all been associated with reduced grey matter volume in the hippocampus.

In rats, hippocampal volume has been shown to be lower in animals with a high, compared to low risk of addiction (Clinton et al. 2011), and in adolescents performance on the delay discounting task (a measure of impulsivity) correlated with white matter density in the hippocampus (Yu 2012). Hippocampus grey matter volumes were higher in obese compared to normal weight adolescents in obesity (Moreno-Lopez et al. 2012).

In our Group's large cohort analysis of unoperated subjects, hippocampal volumes were increased in obese compared to normal weight subjects, but the literature is contradictory in that most other studies of non-elderly adults found no differences between obese and normal weight subjects in hippocampal volume (Pannacciulli et al. 2006; Haltia et al. 2007; Gunstad et al. 2008) and no correlation in this area with BMI (Ward et al. 2005; Pannacciulli et al. 2006; Haltia et al. 2007; Gunstad et al. 2008; Horstmann et al. 2011; Taki et al. 2012)

(see Table 1.3), and one study found reduced hippocampal volume in obesity (Kurth et al. 2012).

Fitness was associated with increased hippocampal volumes in children, which correlated with better cognitive performance (Chaddock et al. 2010). The same was true for adults with Alzheimer's dementia (Fotuhi et al. 2012), suggesting a neuroprotective role of exercise in this area of the brain.

In the current study, hippocampal grey matter volume was lower in the operated compared to unoperated obese patients. Given the cross-sectional nature of this study and the contradictory findings of the influence of obesity on hippocampus grey matter volume from previous studies (as discussed above), it is difficult to clarify whether these findings are as a result of weight loss or a persistent effect of the pre-operative higher BMI and higher prevalence of diabetes. In keeping with the potential effect of weight loss per se on hippocampal volume, one study found that weight gain (or less weight loss) over a 5-year period was associated with a smaller decline only in hippocampal volume by the second visit (Bobb et al. 2012).

There have been no studies investigating the effect of weight loss on grey matter or white matter integrity in normal weight or obese individuals. One study, using an older, cruder method of brain volume calculation than VBM, found that weight loss did not result in brain mass loss in obese or lean women (Peters et al. 2011). On the other hand one study has shown a negative association between grey matter volume in frontal gyri and *increase* in weight over time (Yokum et al. 2011). There are no previous studies investigating structural brain changes after bariatric surgery or comparing RYGB and BAND surgery from this point of view.

5.5.4 White matter integrity

The literature is more consistent when it comes to DTI studies of white matter tract integrity. These indicate that there tends to be reduced white matter integrity as evidenced by reduced FA and increased MD in obesity. Again a link between microstructural damage affecting reward processing has been suggested, since an increased presence of inflammatory markers and increased MD in the amygdala was found in obese but not lean patients (Cazettes et al. 2011).

High FA in the uncinate fasciculus white matter tract (indicating higher integrity) is positively correlated with greater ventral striatum activation to monetary loss (compared to gain) in healthy subjects (Camara et al. 2010). This tract is involved in cognition and emotion linking the OFC, PFC, striatum and amygdala (Hasan et al. 2009). Increased integrity of white matter tracts in reward areas has also been positively correlated with monetary reward cue reactivity of the ventral striatum during fMRI tasks (Koch et al. 2013). This suggests that white matter structure may be altered by psychological correlates of reward-related behaviour, and by extenstion, eating behavior. Again there have been no previous studies investigating the effect of bariatric surgery on white matter integrity, but the current cross-sectional study has found no effect of surgery or different types of surgery on white matter integrity.

5.5.5. Strengths and limitations

The cross-sectional nature of the study limits interpretation, whereas longitudinal studies would be better able to give an indication of directionality and to control for the effect of weight loss itself.

Despite significant between group differences detected in the operated vs. non-operated comparison of grey matter volume, these may have been missed in the comparison between surgical groups due to a Type 1 error.

Similarly, apparent negative findings in the DTI part of the study should also be interpreted with caution, due to low numbers, particularly in the BAND group. The lack of significant differences in white matter integrity between the RYGB and BAND groups does agree with the VBM findings, providing some supporting evidence that differences in brain structure between the groups are unlikely to account for observed differences in BOLD signal to food pictures in reward areas of the brain seen in Study 1.

The finding of reduced grey matter volume in patients that have undergone RYGB or BAND surgery for obesity is novel. However, the lack of consistency in the previous studies as to the effect of obesity on grey matter volumes in the hippocampus, nucleus accumbens and amygdala amongst others, makes these results difficult to interpret. The lack of difference between the two types of surgery (RYGB vs. BAND) implies that the effects seen may be due to weight loss per se, rather than any specific effects of each type of surgery. It does however mean that the differences in BOLD activation in amygdala and OFC during evaluation of food cues between the 2 surgical groups reported in Chapter 3 are unlikely to be explained by structural differences in these areas.

The fact that the operated group were BMI-matched to the unoperated control group, and yet still had higher grey matter volumes in amygdala, hippocampus and nucleus accumbens also suggests that these findings are independent of BMI.

VBM and FIRST have the advantage over older methods of volumetric assessment in that they are not operator dependent for accuracy and VBM can also be carried out on a voxel-wide basis. Interpretation of VBM findings is however limited by the fact that not only atrophy or thickening of the grey matter, but also differences in the morphology of the gyri and sulci can alter results. For instance, differences in the folding pattern of the sulcus in one group could result in a difference in apparent grey matter volume. Furthermore, transformation of images to a standardized space, in order to be able to accurately compare on a voxel-wise level (important if findings are to be generalised), can in some cases reduce the accuracy of interpretation. Aligning them too perfectly or not perfectly enough will reduce the ability to detect any differences between groups (Ashburner et al. 2001; Bookstein 2001).

Although correction for multiple comparisons was made at whole brain level, no correction was made for the number or ROIs and therefore the possibility of false positive between group differences cannot be discounted.

5.6 Conclusion

In conclusion, the finding of lower grey matter volume in the nucleus accumbens, amygdala and hippocampus in patients that have lost weight through bariatric surgery compared to unoperated patients of similar BMI is novel. Future longitudinal studies, utilising a control group for non-surgical weight loss are required to confirm whether a reduction in grey matter volume is a result of the surgery, and whether this finding is a result of weight loss per se, or specific to the effects of bariatric surgery. The lack of difference in grey matter volume and white matter integrity between groups should be interpreted with caution due to low numbers, but suggests that the differences between groups observed in Chapter 3 are not the result of differences in brain microstructure between the groups.

CHAPTER 6: CONCLUSIONS

6.1 Summary of main findings (Table 6.1)

Taken together, these studies suggest that bariatric surgery for obesity can alter both brain function and anatomy, particularly in areas related to food reward processing, and hence eating behaviour. This is the first study to comprehensively investigate how RYGB and BAND surgery differentially affect the gut-brain axis of food reward.

1. After RYGB, obese patients have a markedly different gut-brain-hedonic response to food than after BAND surgery in the fasted state. RYGB patients have lower activation in several brain regions to food, particularly high-calorie foods, including the OFC, amygdala, caudate nucleus, nucleus accumbens and hippocampus, key areas involved in reward, emotion, memory and cognitive responses. This is the first time such a comparison has been made and these findings are therefore novel.
2. This was associated with a more beneficial profile of food preference, food intake and subjective ratings of high-calorie food palatability in RYGB patients. RYGB subjects consume less energy from dietary fat and find ice cream less palatable than after BAND. They rate high-calorie food pictures as less appealing and have healthier eating behavior such as lower dietary restraint and lower external eating than the BAND and/or BMI-matched unoperated controls.
3. These differences in food hedonics are not related to differences in hunger or psychological traits between the surgical groups.
4. As expected, and suggestive of potential mediators of the lower hedonic appeal of food in RYGB, post-prandial GLP-1, PYY and bile acids were elevated in the RYGB compared to BAND patients but there were no difference in acyl ghrelin levels. RYGB patients also reported more dumping symptoms in the first three months after RYGB compared to BAND surgery, and experienced greater post-prandial nausea on the day of scanning.

5. In support of the role of gut hormones in mediating these differences in food hedonics, this is the first study to find that reversal of the post-prandial anorexigenic gut hormone response in RYGB by administration of Octreotide, reduces nucleus accumbens activation to low-calorie food pictures and reduces food appeal in RYGB but not BAND subjects. These preliminary results warrant further investigation with larger numbers of subjects.
6. These differences in food hedonics were not explained by differences in grey matter density or volume or white matter tract integrity between the RYGB and BAND groups.
7. However, the novel finding of reduced grey matter volume in the amygdala in RYGB and BAND subjects compared to BMI-matched unoperated controls adding to previous findings of our Group of increased grey matter volume in the amygdala in obese patients, and suggests that surgically-induced weight loss itself may have had an effect on the brain structure across both groups.

Table 6.1 Summary of results

	RYGB vs. BAND	**RYGB vs. BMI-M**	**BAND vs. BMI-M**	**SURG vs. BMI-M**	**RYGB: Fed-Octreotide vs. Fed-Saline**	**BAND: Fed-Octreotide vs. Fed-Saline**
Functional neuroimaging						
fMRI food pictures whole brain	HC: ↓OFC, NAcc, subcallosal cortex, putamen, caudate, hippo, cingulate and paracingulate gyri LC: ↓OFC, subcallosal cortex					
fMRI food pictures ROI	FOOD:↓ combined ROIs, OFC, amygdala HC: ↓combined ROIs LC: ↓OFC	FOOD: ↓amygdala	↔		FOOD: ↓Nacc, trend combined ROIs LC: ↓Nacc, trend combined ROIs	↔
Structural neuroimaging						
GMD whole brain (VBM adj. Age, gender, BMI)	↔	↔	↓ temporal	↓ temporal, amygdala		
GMD ROI (VBM adj. age, gender, BMI, ICV)	↔	↓ amygdala	↔	↓ amygdala		
GMD ROI (VBM adj. age, gender, BMI)	↔	↓ amygdala	↓ amygdala	↓hippo, amygdala, NAcc		
GMV (FIRST adj. age, gender, BMI, ICV)	↔	↔	↔	↑NAcc		

WM FA or MD whole brain (DTI)	↔	↔	↔	↔		
EM FA or MD ROI (DTI)	↔	↔	↔	↔		
Eating behaviour						
Food appeal scores	↓HC	↓FOOD and HC	↔		↓FOOD	↔
Food liking/wanting scores	↓liking high fat and low fat savoury ↓wanting high fat and low fat savoury					
Eating behaviour questionnaires	↓ EDE-Q restraint ↓ EDE-Q weight concerns ↓ EDE-Q shape concerns	↓ EDE-Q restraint ↓ EDE-Q weight concerns ↓DEBQ external eating	↔			
Ice cream intake and palatability	↔ intake ↓ palatability				↔ intake ↔ palatability	↔ intake ↔ palatability
Food diaries	↓ % fat intake					
Dumping scores	↑					
Mediators						
Gut hormones	↑ post-prandial PYY ↑ post-prandial GLP-1 ↔ pre- and post-prandial acyl ghrelin	↑ pre-prandial PYY ↔ pre-prandial GLP-1 ↔ pre-prandial acyl ghrelin	↔ pre-prandial PYY ↔ pre-prandial GLP-1 ↔ pre-prandial acyl ghrelin			

Bile acids	↑ pre- and post-prandial bile acids	↔ pre-prandial bile acids	↔ pre-prandial bile acids			
VAS appetite	↔	↓ hunger, volume, fullness pleasantness to eat	↓ hunger, volume, pleasantness to eat		↔	↔

Abbreviations: BAND: gastric banding, BMI: body mass index, BMI-M: BMI-matched, DEBQ: Dutch eating behaviour questionnaire, DTI: diffusion tensor imaging, EDE-Q : Eating disorders examination questionnaire, FA: fractional anisotropy, fMRI: functional MRI, FOOD: high-calorie and low-calorie food combined, GLP-1: glucagon like peptide-1,GMD: grey matter density, GMV: grey matter volume, HC: high-calorie, hippo: hippocampus, ICV: intracranial volume, LC: low-calorie, MD: mean diffusivity, NAcc: nucleus accumbens, OFC: orbitofrontal cortex, ROI: region of interest, RYGB: gastric bypass, SURG: bariatric surgery (RYGB and BAND combined) VAS: visual analogue scale, VBM: voxel-based morphometry, WM: white matter
Shaded areas refer to comparisons not performed
ARROWS: refer to direction of result of test group or condition compared to control

6.2 Food hedonics and dietary behaviour

This study is in agreement with longitudinal studies showing decreased reward system activation to food cues in obese subjects after RYGB (Ochner et al. 2010; Ochner et al. 2012; Ochner et al. 2012) (see Section 3.4.2). Taken together, these results demonstrate that RYGB distinctly influences food hedonics via alteration of food reward systems in the brain, and that this is not simply as a result of weight loss. So far longitudinal studies of the same subjects in the fed state have yielded surprising, but inconclusive preliminary results, suggesting that these alterations in food reward may only be evident in the fasted, and not the fed state. No previous studies have examined the potential mechanism underlying these differences in food reward processing between RYGB and BAND surgery, and hence the preliminary results from Chapter 4, in which reversal of gut hormone responses by administration of Octreotide, led to partial increases in reward activation to and appeal of food, specifically low-calorie foods, are novel and warrant further investigation (see Section 4.5.1 and 4.5.2).

The findings are also in agreement with animal and human studies of RYGB which have demonstrated a shift in preference away from high fat and sugary foods, and reduced work to obtain high calorie food rewards (Zheng et al. 2009; Hajnal et al. 2010; Bueter et al. 2011; le Roux et al. 2011; Mathes et al. 2012; Miras et al. 2012; Stefater et al. 2012) (see Section 3.4.5).

Although the dietary intake findings in Chapter 3 are in agreement with studies comparing RYGB and VSG (Brolin et al. 1994; Olbers et al. 2006), no previous study has directly compared RYGB and BAND in terms of calorie and macronutrient intake. The finding that RYGB consumed about 30% less calories corrected for lean body mass and proportionately less fat than BAND subjects is therefore novel. The fact that ice-cream intake was not different between the RYGB and BAND patients in Chapter 3, and was not reduced in RYGB patients by Octreotide in Chapter 4 is

unexpected and contrary to previous findings (le Roux et al. 2007). Possible reasons for this are discussed in more detail in Section 3.4.10 and Section 4.5.4.

The observed difference in appeal of high calorie foods and palatability of ice cream in Chapter 3 between RYGB and BAND and the reversal of food appeal by Octreotide in RYGB patients is also novel (Kenler et al. 1990; Olbers et al. 2006).

Although previous longitudinal studies suggest improvements in body image following both RYGB and BAND surgery (Dixon et al. 2002; Hrabosky et al. 2006; Sarwer et al. 2010), no previous study has directly compared RYGB and BAND with regards to eating disorder psychopathology and body image satisfaction. The finding of lower dietary restraint and less weight and shape concerns in RYGB compared to BAND subjects, despite similar BMI is therefore novel, but may be explained by the greater weight loss following RYGB compared to BAND. Dietary restraint can be complex to interpret. As a general rule, obesity is associated with particularly rigid dietary restraint, interpreted as an attempt to maintain rigid control over dietary intake. However, in obesity, dietary restraint is often accompanied by dietary disinhibition, or loss of control over dietary intake, resulting in overeating when a fast is broken, or emotional or external cues act as triggers (Polivy et al. 2008). The novel finding that RYGB subjects have lower dietary restraint scores, and a tendency toward lower external eating than BAND subjects suggests a therefore a healthier eating profile is achieved by RYGB than BAND, although the reasons for this remain speculative.

6.3 Hunger and satiety

Differences in food hedonics between the groups they were not explained by differences in hunger and therefore appear to be isolated to non-homeostatic systems. However Octreotide did reduce feelings of satiety in the RYGB group. Most studies agree that increasing the palatability or hedonic appeal of food increases the consumption of food, increases subjective ratings of hunger, at least initially after presentation of the food cue, and slows the rate of satiety after consumption (Blundell et al. 2004). However, hunger does not have a symmetrical effect on palatability. Whilst hunger does increase the hedonic response to and appeal of high calorie foods (Goldstone et al. 2009), satiety does not necessarily reduces the hedonic appeal of palatable foods to the same extent (Yeomans et al. 1997). This dissociation has been implicated in the pathogenesis of obesity (Berthoud 2012). The fact that hunger and satiety ratings were equal between the groups in the fasted state, therefore makes the interpretation of the fMRI results of Chapter 3 easier, since the hedonic aspect of appetite is more easily examined independently of hunger.

6.4 Metabolic factors

As in previous studies post-prandial plasma GLP-1 and PYY gut hormone levels, as well as pre-lunch GLP-1 levels, were higher in the RYGB than the BAND group in Chapter 3 (le Roux et al. 2006; Tadross et al. 2009). Although these hormones are known to alter brain reward systems and dopaminergic signaling (Batterham et al. 2007; De Silva et al. 2011; Skibicka et al. 2011) and increased levels are associated with shifts in food preference toward healthier choices, (Martin et al. 2009; Miras et al. 2010; Acosta et al. 2011) and reduction in uncontrolled and emotional eating after RYGB (Bryant et al. 2012), their role in altering BOLD signal in RYGB and BAND patients has not been examined before. The finding therefore that BOLD signal response to food pictures is increased in RYGB patients after administration of Octreotide is novel and further

substantiates the potential role of acute exaggerated post-prandial PYY and GLP-1 release changes in altering food hedonics in RYGB patients.

Bile acids were also found to be elevated in the RYGB but not BAND group, suggesting a further possible mechanism for reduced food reward in this group, since they too reduce food intake, potentially mediated by improved glucose metabolism, modulation of gut hormone secretion and direct or indirect action on FGF19 or bile acid receptors in the brain (Ryan et al. 2013) (see Section 1.3.8). However more detailed exploration of their role as a potential mediator in food reward in RYGB was not tested by this paradigm and Octreotide does not appear to suppress bile acid secretion (Sahin et al. 1999), although results of plasma bile acid assays for Chapter 4 were not available.

Other factors not measured by this paradigm may have also been shown to be important in altering eating behaviour after RYGB (see Fig. 6.1). For instance oxyntomodulin, another anorexogenic gut hormone, has also been shown to be increased after RYGB and not BAND surgery, to cross the blood-brain barrier and to exert its affects in the brain via GLP-1 receptors. Similarly other gut hormones such as CCK may have played a role, although less convincing evidence of their contribution to reduced appetite in RYGB has been found (see Section 1.3.6). It is therefore possible that any effects seen on food reward systems may be as a result of oxyntomodulin, or another as yet undiscovered incretin, rather than the hypothesized PYY and GLP-1 changes.

Figure 6.1 Mechanisms of weight loss after bariatric surgery

Table 1 | Mechanisms of weight loss after bariatric surgery*

Parameter	RYGB	AGB	VSG
Food intake	↓	↓	↓
Gastric emptying	↑/↓	↔	↑
Macronutrient malabsorption	Minimal fat malabsorption	NA	NA
Hypothalamic peptide expression levels	NA	NA	↔
Vagal signalling	Implicated	Implicated	NA
Plasma GLP-1 levels	↑	↔	↑
Plasma PYY levels	↑	↔	↑
Plasma ghrelin levels	↑/↓/↔	↑	↓
Plasma CCK levels	↔	NA	↔
Plasma leptin levels	↓	↓	↓
Plasma bile acid levels	↑	↔	↑
Gut microbiota	Altered	NA	NA
Energy expenditure	↑/↓/↔	NA	↔
Food preferences	↓ consumption of fat and sugar	↔ or ↑ consumption of fat and sugar	↔ or ↓ consumption of fat and sugar
Meal frequency	↑	↓/↔	NA
Food reward	↓	↔/↑	↔/↓
Condition taste aversion	Demonstrated for fat	NA	Demonstrated for fat

*Evidence was obtained from both human and animal studies, mechanisms underlying the most common bariatric surgical procedures are listed. Abbreviations: ↑, increase; ↓, decrease; ↔, no change; AGB, adjustable gastric banding; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; NA, no available evidence; PYY, peptide YY; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

AGB=BAND, VSG=Sleeve gastrectomy (with copyright permission, (Miras et al. 2013)

The role of orexigenic ghrelin which has stimulatory effects on food hedonics and reward system activation to food cues (Malik et al. 2008; Goldstone et al. 2010; Skibicka et al. 2011) in modulating appetite changes after RYGB, is not clear and evidence is contradictory (see Section 1.3.6.3). However, in Chapter 3 there was no difference in plasma acyl ghrelin between surgical groups. Some studies have found reduced fasting and/or post-prandial ghrelin levels after RYGB compared to before surgery or to unoperated controls. This finding is however not universal, related to differences in surgical techniques, assay of total vs. active acyl ghrelin, problems with handling and storage of plasma samples (Cummings et al. 2004; Pournaras et al. 2009; Stefater et al. 2012). Likewise, the effect of reduced leptin levels after RYGB and BAND surgery is not known, although would be expected to increase appetite and potentially food reward in both groups.

PET and fMRI studies suggest that peripheral insulin resistance is associated with central insulin resistance, including in areas of the brain associated with food reward processing (Anthony et al. 2006{Kullmann, 2012; Kullmann et al. 2012). In this particular study insulin resistance had a tendency to be lower in the RYGB group, who also had a greater prevalence of past T2DM. It is difficult to interpret how these factors may have influenced the results, but possible that reduced activation to food pictures in the RYGB group may be due to greater insulin sensitivity in this group.

Further factors not measured by this study potentially differentially influence weight loss after RYGB compared to BAND. For instance, altered nutrient sensing in the portal vein, altered gut microbiota, relatively increased resting energy expenditure and increased meal frequency may all play a role in changing appetite after RYGB, as may altered vagal signaling seen in BAND surgery (see Fig 6.2).

6.5 Altered grey matter volume after obesity surgery

This is the first study to examine brain volume differences between different types of bariatric surgery, or to compare BMI-matched controls to bariatric surgery subjects. There were no structural differences in grey and white matter volume between obese subjects who had undergone RYGB or BAND surgery. Some, but not all studies of grey matter volume in obesity, suggest that obesity is associated with higher grey matter volume in the OFC, amygdala, nucleus accumbens, caudate and putamen, possibly associated with inflammatory changes in the brain as a result of obesity. This appears to particularly true of non-elderly adults, whereas higher BMI in older adults appears to be associated with increased atrophy in most areas of the brain. Lower grey matter volume was seen in the amygdala, nucleus accumbens and hippocampus in bariatric surgery subjects compared to BMI-matched unoperated controls, suggesting a potential reparative effect of bariatric surgery on grey matter volume.

The implications of these findings are two-fold. They suggest firstly that our fMRI findings of differences in food reward processing between RYGB and BAND subjects are not associated with structural differences in reward areas of the brain. Secondly, it suggests that weight loss itself may result in reduced grey matter volume. This is supported by longitudinal evidence of a decline in white matter volume in obese subjects after weight loss (Haltia et al. 2007).

Ours is also the first study to examine differences in white matter integrity between obese subjects after RYGB or BAND surgery. No differences were found between groups, although this may be due to a Type 1 error, since the BAND group numbers were low (n=12).

6.6 Strengths and limitations

The cross-sectional nature of the study is the greatest hindrance to drawing inferences from the results, and longitudinal studies are required to confirm the differences between groups and examine causality and mechanism of the findings.

Furthermore, although many pre- and post-operative confounding variables were similar between surgical groups, patient allocation to surgery was not randomized. Nevertheless, clinical practice within the centre where recruitment took place, does not differ from other bariatric centers in that the choice of surgical procedures is not influenced by pre-operative food hedonics. If anything subjects who chose RYGB surgery tended to be heavier pre-operatively and therefore less likely to have healthier food hedonics than the BAND subjects. However they did have higher rates of pre-operative T2DM, which may have had some effect on the results (see Section 3.4.10 for further discussion).

The choice of operation is also not influenced by clinicians working in the centre. Although an early study had suggested that sweet eating subjects may do less well after another restrictive procedure, the vertical banded gastroplasty, it has never been the practice of the majority of centers including our own to deter such subjects from BAND, and furthermore subsequent research has refuted this earlier finding (Hudson et al. 2002).

The study was fairly robust in its exclusion criteria. In this way care was taken to reduce the number of confounding variables that may have been present. Furthermore the effect of the stage of menstrual cycle on BOLD response to food was controlled for by only scanning female pre-menopausal subjects in the follicular phase, although recent restriction to the luteal phase may have been better (Alonso-Alonso et al. 2011). Attempts were also made to reduce the influence of variability in possible confounders on BOLD response to food by standardizing pre-visit protocol. Participants were asked to eat at the same time in the evening, to have a good night's rest and to abstain from alcohol and exercise the day before the study visit. In addition, participants were all scanned at the same time of day and on each visit, to reduce the effect of diurnal variation in BOLD response to food.

This is the first study to make use of such a comprehensive array of techniques to assess hedonic response to food in RYGB and BAND patients, as an adjunct to fMRI scanning, including profiling of psychological and eating behavior, measurement of physiological markers, including gut hormones, and ratings of post-ingestive effects of food intake. This is a relative strength of the study. However it does mean that a large number of statistical analyses were carried out, and the results could be inflated by chance findings due to the large number of multiple comparisons and cross-correlations. No correction was made for this. However these were mainly secondary and complementary findings, whereas in the case of the primary findings, that of BOLD signal differences, correction was made for issues of multiple comparison arising from the large number

of voxels being analyzed. The use of fMRI in particular offers the advantage of capturing a biological measurement of the possible mechanism for the other behavioural measures used.

The use of the food evaluation task during fMRI is novel in this group of patients. The agreement of the appeal data with the fMRI findings lends weight to the assumption that those differences observed on a neural level, may translate into actual behaviour and improves the chance that subjects were paying attention to the pictures whilst in the scanner. Furthermore, conscious evaluation of a test meal's palatability showed similar differences between the RYGB and BAND group, comparable to and correlating with the fMRI data, even though actual intake did not differ between the groups, suggesting that in this study, fMRI holds some validity in terms of its association with perceived food reward.

fMRI is increasingly being used to investigate particularly non-homeostatic control of eating behaviour in the brain. The sample size of scanned subjects, although small by general standards, in both Chapter 3 and 4 is comparable to other phenotyping studies after bariatric surgery (le Roux et al. 2006; Laferrere et al. 2011), and fMRI studies investigating food reward (Fletcher et al. 2010; De Silva et al. 2011). The possibility that a type 1 error may have occurred for some of the results cannot be excluded. The study employed a number of complimentary behavioral measures and notably their results were in the same direction as the primary fMRI end point. Similarly, the numbers included in the VBM and DTI study may have been too small to detect differences between groups.

In our fMRI paradigm we studied the differences in BOLD activation to food pictures between surgical groups, rather than food receipt itself. However, such fMRI paradigms with food pictures have been widely used to study human eating behavior (Carnell et al. 2012). Additionally fMRI responses to food pictures, anticipation of food receipt and actual food receipt all increase during

food restriction (Stice et al. 2013). The use of food pictures also allows exposure, albeit visual, of the subjects to more complex, real-life food stimuli than can be achieved with the restricted nature of tastants used in fMRI experiments such as milkshakes. Our use of active evaluation of the pictures during the fMRI task may have enhanced our ability to detect differences in the OFC response (Bender et al. 2009). Indeed our behavioural analysis did demonstrate that subjects after RYGB found ice-cream less palatable than after BAND, and so it will be of interest to next examine differences in fMRI responses to taste of such high fat/sweet foods between the two surgical groups.

It was a surprising finding that there was not lower consumption of ice cream in the RYGB compared to BAND group. A possible explanation is that the test meal was not specifically designed to examine food preference, as subjects were not given a choice of foods of different caloric density. Use of a food choice paradigm in the test meal may have found more complimentary results to the fMRI data. However, analysis of macronutrient intake outside of the laboratory did reveal lower total caloric and particularly fat intake after RYGB compared to BAND.

At this stage it is not possible to confirm which, if any, of the associated changes in gut physiology are responsible for our finding of lower food hedonics in RYGB subjects. The results from Chapter 4, although preliminary and suggestive of a possible mechanism are not sufficiently robust to either confirm or refute the hypothesis that exaggerated postprandial plasma PYY and GLP-1 rises are a key component in attenuated brain reward responses to food in RYGB. Further studies are therefore needed to explore possible underlying mechanisms underlying the findings.

6.7 Future studies

6.7.1 Longitudinal studies

Having established a difference in hedonic response to food between obese patients who undergo RYGB and BAND surgery, future studies should aim to confirm the directionality of hedonic response alteration after RYGB and BAND surgery, ideally through longitudinal studies. Although it seems likely that hedonic response is reduced after surgery in RYGB, as seen in other longitdunal studies, the difference observed in Chapter 3 between the two types of surgery may also be due to *increased* hedonic response after BAND.

BAND surgery leads to restriction of food that can be eaten at a given time, and hence certain foods are difficult to eat, and avoided. For example, meat that is not minced, vegetables and salad and bread products are often avoided. On the other hand, ice cream, dairy products, sweet drinks and 'melting' foods such as crisps and chocolate are easily tolerated. It is not difficult to imagine therefore that within the limited repertoire of foods easily tolerated after BAND insertion, that calorie dense, palatable foods might actually increase in their appeal. Relatively little is known about how food preference changes after restrictive procedures. Sugarman's early study suggested that sweet eaters in particular may not benefit from restrictive surgery (Sugerman et al. 1987), but due to methodological limitations of the study in question, and subsequent studies refuting this finding in BAND surgery (Hudson et al. 2002), this view is not held in the wider bariatric surgery community. Other studies have also suggested that restrictive procedures may actually result in an increase in sweetened dairy products (Kenler et al. 1990).

Thus far longitudinal studies of hedonic response to food in RYGB surgery have also not examined gut hormone responses in conjunction. Replication of Chapter 3, longitudinally in RYGB and BAND patients would allow comparison of the directionality of hedonic fMRI response and its association with gut hormone responses. Furthermore if a correlation between weight loss

over time and fMRI response to food was found, this would add weight to the hypothesis that the hedonic response is a key factor in the mechanism underlying RYGB's success.

6.7.2 Comparison with lean controls

A control group of lean subjects, matched for age, gender, ethnicity and possibly also socio-economic class and education level would be difficult to achieve due to the association of obesity with lower socio-economic class and lower education levels (WHO 2005). However, if comparison with a lean control group were to be valid, it would be essential that they were matched for these confounding variables. The addition of a lean control group in a longitudinal study would allow examination of whether RYGB was in fact returning hedonic responses to that of a healthy weight individual, despite possibly remaining in an overweight/obese category. This is especially interesting, given that many other risk factors such as cardiovascular and T2DM risk, return to that of a healthy weight individual despite persisting obesity.

Evidence suggests that hedonic responses to food differ between lean and obese individuals, and that lean individuals at risk for obesity may also have a higher hedonic response to food, and reduced cognitive control in response to food, although the literature on this subject is very varied, (see Section 1.7.3.2, Table 1.1 and 1.2).

6.7.3 Comparison of RYGB and other weight loss mechanisms

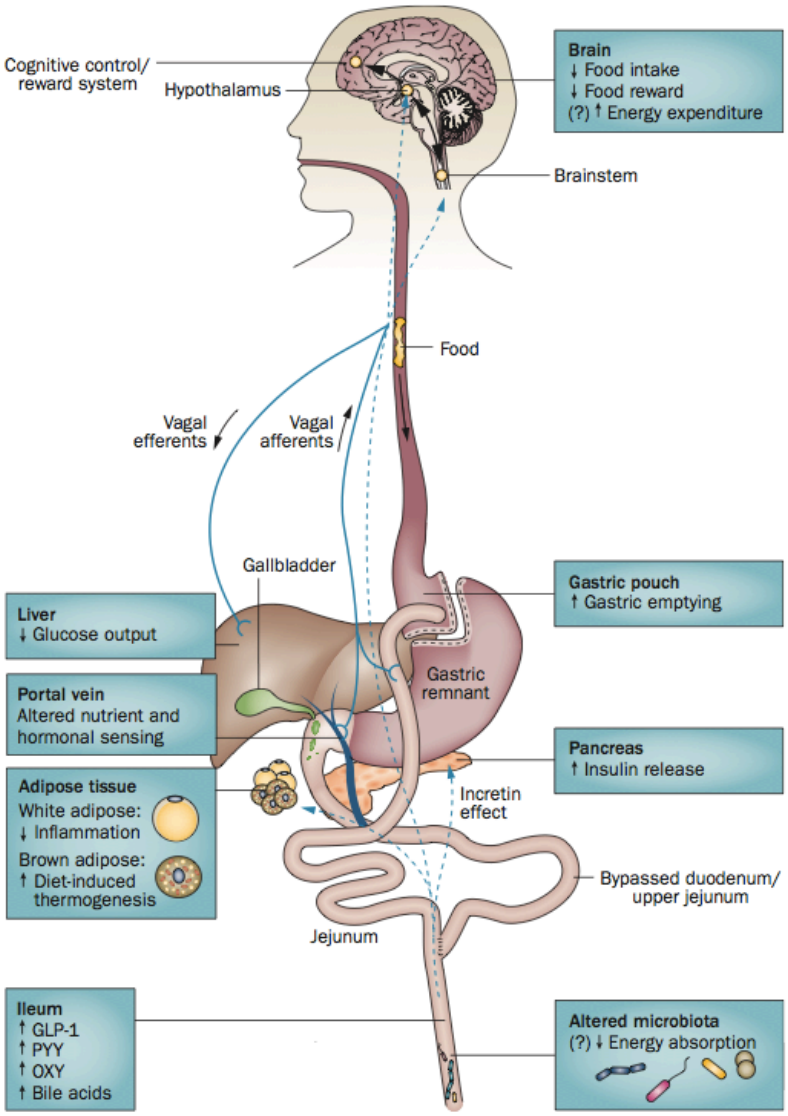
fMRI is a useful tool to investigate the hedonic response to food in that it allows examination of a biological substrate of behaviour. This has implications for the examination of other existing and current treatments for obesity. There have already been fMRI studies examining brain food reward system responses following administration of drugs designed to reduce food intake (Fletcher et al. 2010; Horder et al. 2010). fMRI in this way acts as a useful proxy measure for

success, and particularly if comparison is made with RYGB, the most successful established treatment of obesity to date.

Comparison with low-calorie dieting and other dietary manipulations, as well as other surgical procedures, such as sleeve gastrectomy and the endobarrier, will also provide insights into the mechanisms of these as far as food hedonics go. RYGB is a complex procedure with multiple possible synergistic or additive mechanisms which may all play a role in reducing the hedonic response to food (see Fig. 6.2).

Since procedures such as the endobarrier and sleeve gastrectomy have potentially simpler underlying mechanisms which essentially isolate one part of the anatomical manipulation in RYGB, these in themselves will provide insights into how RYGB potentially works. In other words, the effect on food hedonics of early delivery of food to the distal ileum and reduction in stomach volume (as in sleeve gastrectomy) or the effect of food bypassing the duodenum and proximal ileum (as in the endobarrier) can independently be tested.

Figure 6.2 Mechanisms of reduced food intake in RYGB



(with copyright permission, (Miras et al. 2013)

6.7.4 Further investigation of post-prandial gut hormone response reversal in RYGB

The preliminary findings of Chapter 4 will be improved by the addition of further 8-13 subjects in the RYGB arm. This will allow within-subject analysis of the effect of Octreotide administration and will allow the study to be sufficiently powered to either confirm or refute the hypothesis.

Analysis of pilot data from Chapter 4, indicates that n=20 per group with an alpha of 0.05 gives 89% power to determine a mean difference of 0.054 change in BOLD signal in the average reward system (OFC, amygdala, insula, nucleaus accumbens and caudate), 59% power to determine a mean difference of 0.026 change in BOLD signal in the amygdala, and 100% power to determine a mean difference of 0.063 change in BOLD signal in the nucleus accumbens to between Fed-Octreotide and Fed-Saline visit.

In addition, measurement of plasma acyl ghrelin, PYY, GLP-1 and bile acids will add to the ability to interpret the results. For instance it is likely that Octreotide suppresses ghrelin but not bile acids, thereby diluting the effect of PYY and GLP-1 suppression of BOLD response. This would go some way to clarifying the potential role of gut hormones as mediators in the reduced hedonic response to food seen after RYGB surgery. There has been one study investigating the effect of gut hormone infusion (PYY and GLP-1) on food hedonics in normal weight individuals, which should be replicated in obese subjects. There appear to be distinct advantages to using combinations of gut hormones to obtain synergistic and additive effects, as evidenced from animal and human studies (Sadry et al. 2013). Similarly the additive role of gut hormone infusions or agonists in increasing the effect of RYGB (or indeed another bariatric operation such as BAND), as suggested by animal studies (Fenske et al. 2012) warrants further investigation in humans, and may have particular relevance for patients where weight regain occurs after RYGB.

6.7.5 Dissociation of food reward and non-food reward in bariatric surgery procedures

It is intriguing to speculate that if RYGB reduces hedonic responses to food, this may be a more generalized effect on non-food reward. This can be tested with alternative paradigms such as those used to measure decision-making, such as food or monetary related Go-NoGo tasks, choice paradigms, progressive ratio tasks, monetary and food incentive delay tasks to test anticipation vs. receipt of food cues or anticipation vs. receipt actual tastants, delay discounting tasks

specifically measuring impulsivity in relation to food, financial evaluation tasks, and the effect of stress sensitivity in longitudinal studies of patients undergoing RYGB (and other bariatric) surgery.

Paradigms that measure more subtle neuropsychological constructs, such as the Stroop test have also been modified to measure attentional bias to food (Food Stroop test). By adapting existing tasks to test food reward and non-food reward separately, these effects can be dissociated, and investigated in obese patients before and after RYGB. This allows generalisability across patient groups, cultures and comparison with drug and alcohol addiction.

On the face of it, a reduced hedonic response to food seems positive, particularly if it is associated with improved eating behaviour. However it does raise the question of whether hedonic responses to other emotion-regulating substances may therefore be heightened. This may have particular relevance to obese patient undergoing RYGB who have a history of drug or alcohol addiction or misuse, or who use the rewarding aspects of food as a way to manage stress, anxiety or depression. Studies have shown that RYGB is associated with increased alcohol and other drug misuse and dependency (King et al. 2012; Conason et al. 2013) and that this finding seems to be specific to RYGB compared to restrictive procedures (VBG) (Ostlund et al. 2013). There is also the suggestion that RYGB may actually increase suicide rates from a cross-sectional study comparing approximately 9000 patients who had undergone RYGB compared to BMI-matched unoperated obese individuals (Adams et al. 2007).

On a psychological level, if food is used to manage affect, removal of the affective response to food, may in fact make an individual more vulnerable to psychological stress, depression and even suicide. If on the other hand, the reduction of the hedonic appeal of food is generalized, then this may have implications for pharmacological targets for treatment of other forms of addiction.

6.7.6 DTI study in larger numbers

The results from Study 3 are inconclusive as far as the DTI results go, and this is most likely due to being underpowered. Longitudinal studies in a larger cohort are indicated to demonstrate whether RYGB or BAND surgery differentially alter white matter tract integrity in reward areas, which might lead to an artefactual difference in hedonic response to food. In addition, since studies examining the longitudinal effect of weight loss in obesity on white and grey matter structure are few, this would add significantly to our understanding of how weight loss impacts on the brain on a structural level.

6.7.7 Functional connectivity

FDT (FMRIB's Diffusion Toolbox) includes probabilistic tractography tools, which allow the user to generate white matter connections between areas of interest. In this way, integrity in the white matter reward-based tracts which link the areas in which differences were seen in the RYGB group, e.g. amygdala and OFC, can be compared between groups, or changes observed longitudinally in the same subjects.

Functional connectivity analyses using independent component analysis (ICA) techniques may give further information about the connectivity between regions of interest, between groups, or as influenced by Octreotide. Connectivity has been shown to be altered in obesity (Stoeckel et al. 2009; Garcia-Garcia et al. 2012; Kullmann et al. 2012) and improved in successful treatment thereof (Weygandt et al. 2013).

In addition, by comparing resting state networks from resting and task fMRI data, inferences can be made about how bariatric surgery or RYGB may alter cognitive networks in the brain which may unconsciously influence eating behaviour. In this way our group has shown that salience

resting state network integrity is increased in obesity and predicts orbitofrontal cortex activation to high-calorie food cues (Starke JA 2013)(see abstract publications).

6.8 Conclusions

These findings emphasize the under-recognized differences in the mechanisms underlying the success of these two surgical treatments of obesity. These can now be expanded to include differences in food reward and hedonics, potentially mediated in part by acute effects of gut hormones in RYGB. This may prompt the development of more personalized approaches to surgical choices that might use pre-operative assessment of food preference and craving and consider how the loss of food reward may impact on an individual. In addition, patients seen after RYGB and BAND surgery can be approached with a greater understanding of how surgery may differently alter their eating behaviour, so that supportive treatment is appropriately targeted.

The preliminary nature of the small study of the effects of Octreotide in reducing food hedonics including activation in the nucleus accumbens to food pictures in RYGB patients, precludes extensive interpretation, especially of the difference between surgical groups, but does support the hypothesis that gut hormones may mediate the hedonic changes in appetite seen after RYGB.

RYGB and BAND surgical treatments for obesity are quite distinct in their mechanisms of weight loss; they both reduce hunger and appear to improve grey matter structure in reward areas, but differentially alter food reward responses. The demonstration that anatomical changes to the gut can have such different effects in the brain highlights the importance of the gut-brain-food hedonic axis in the control of eating behavior and body weight.

Further studies are needed to clarify the role of post-prandial gut hormone responses, as well as other factors such as bile acids and post-ingestive effects of high-fat and sweet foods, as potential mediators for the lower hedonic response to food seen in RYGB. In addition, in depth interrogation of the mechanisms underlying this and other potential mechanisms will accelerate development of efficacious, cheaper, and safer non-surgical treatments for obesity.

CHAPTER 7: REFERENCES

(2012). "Very low calorie diets." Drug Ther Bull **50**(5): 54-57.

Abiles, V., S. Rodriguez-Ruiz, et al. (2010). "Psychological characteristics of morbidly obese candidates for bariatric surgery." Obes Surg **20**(2): 161-167.

Acosta, A., M. D. Hurtado, et al. (2011). "Salivary PYY: a putative bypass to satiety." PLoS One **6**(10): e26137.

Adams, T. D., R. E. Gress, et al. (2007). "Long-term mortality after gastric bypass surgery." N Engl J Med **357**(8): 753-761.

Adams, T. D. and S. C. Hunt (2009). "Cancer and obesity: effect of bariatric surgery." World J Surg **33**(10): 2028-2033.

Adrian, T. E., G. L. Ferri, et al. (1985). "Human distribution and release of a putative new gut hormone, peptide YY." Gastroenterology **89**(5): 1070-1077.

Adrian, T. E., A. P. Savage, et al. (1986). "Peptide YY abnormalities in gastrointestinal diseases." Gastroenterology **90**(2): 379-384.

Aguirre, V., N. Stylopoulos, et al. (2008). "An endoluminal sleeve induces substantial weight loss and normalizes glucose homeostasis in rats with diet-induced obesity." Obesity (Silver Spring) **16**(12): 2585-2592.

Ajala, O., P. English, et al. (2013). "Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes." Am J Clin Nutr **97**(3): 505-516.

Alexander, A. L., J. E. Lee, et al. (2007). "Diffusion tensor imaging of the brain." Neurotherapeutics **4**(3): 316-329.

Alger, S. A., M. D. Schwalberg, et al. (1991). "Effect of a tricyclic antidepressant and opiate antagonist on binge-eating behavior in normoweight bulimic and obese, binge-eating subjects." Am J Clin Nutr **53**(4): 865-871.

Alhadeff, A. L., L. E. Rupprecht, et al. (2012). "GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake." Endocrinology **153**(2): 647-658.

Allen, J. M., M. L. Fitzpatrick, et al. (1984). "Effects of peptide YY and neuropeptide Y on gastric emptying in man." Digestion **30**(4): 255-262.

Allen, P. J., P. Batra, et al. (2012). "Rationale and consequences of reclassifying obesity as an addictive disorder: neurobiology, food environment and social policy perspectives." Physiol Behav **107**(1): 126-137.

Alonso-Alonso, M., F. Ziemke, et al. (2011). "Brain responses to food images during the early and late follicular phase of the menstrual cycle in healthy young women: relation to fasting and feeding." Am J Clin Nutr **94**(2): 377-384.

Anderson, J., M. Jenkinson, et al. (2007). Non-linear-registration, aka Spatial normalisation. F. t. r. TR07JA2.

Anderson, J., M. Jenkinson, et al. (2007). Non-linear optimisation. F. t. r. TR07JA.

Anderson, J. W., E. C. Konz, et al. (2001). "Long-term weight-loss maintenance: a meta-analysis of US studies." Am J Clin Nutr **74**(5): 579-584.

Angrisani, L., P. P. Cutolo, et al. (2009). "Laparoscopic adjustable gastric banding with truncal vagotomy versus laparoscopic adjustable gastric banding alone: interim results of a prospective randomized trial." Surg Obes Relat Dis **5**(4): 435-438.

Anthony, K., L. J. Reed, et al. (2006). "Attenuation of insulin-evoked responses in brain networks controlling appetite and reward in insulin resistance: the cerebral basis for impaired control of food intake in metabolic syndrome?" Diabetes **55**(11): 2986-2992.

Aotani, D., K. Ebihara, et al. (2012). "Functional magnetic resonance imaging analysis of food-related brain activity in patients with lipodystrophy undergoing leptin replacement therapy." J Clin Endocrinol Metab **97**(10): 3663-3671.

Ara, R., L. Blake, et al. (2012). "What is the clinical effectiveness and cost-effectiveness of using drugs in treating obese patients in primary care? A systematic review." Health Technol Assess **16**(5): iii-xiv, 1-195.

Arbisi, P. A., C. J. Billington, et al. (1999). "The effect of naltrexone on taste detection and recognition threshold." Appetite **32**(2): 241-249.

Arnone, D., A. M. McIntosh, et al. (2012). "Magnetic resonance imaging studies in unipolar depression: systematic review and meta-regression analyses." Eur Neuropsychopharmacol **22**(1): 1-16.

Aron, A. R. and R. A. Poldrack (2005). "The cognitive neuroscience of response inhibition: relevance for genetic research in attention-deficit/hyperactivity disorder." Biol Psychiatry **57**(11): 1285-1292.

Arts, J., P. Caenepeel, et al. (2009). "Efficacy of the long-acting repeatable formulation of the somatostatin analogue octreotide in postoperative dumping." Clin Gastroenterol Hepatol **7**(4): 432-437.

Ashburner, J., J. G. Csernansky, et al. (2003). "Computer-assisted imaging to assess brain structure in healthy and diseased brains." Lancet Neurol **2**(2): 79-88.

Ashburner, J. and K. J. Friston (2001). "Why voxel-based morphometry should be used." Neuroimage **14**(6): 1238-1243.

Ashby, D. R., H. E. Ford, et al. (2009). "Sustained appetite improvement in malnourished dialysis patients by daily ghrelin treatment." Kidney Int **76**(2): 199-206.

Assaf, Y. and O. Pasternak (2008). "Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review." J Mol Neurosci **34**(1): 51-61.

Astrup, A., S. Rossner, et al. (2009). "Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study." Lancet **374**(9701): 1606-1616.

Avena, N. M., P. Rada, et al. (2008). "Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake." Neurosci Biobehav Rev **32**(1): 20-39.

Aylwin, S. and Y. Al-Zaman (2008). "Emerging concepts in the medical and surgical treatment of obesity." Front Horm Res **36**: 229-259.

Baicy, K., E. D. London, et al. (2007). "Leptin replacement alters brain response to food cues in genetically leptin-deficient adults." Proc Natl Acad Sci U S A **104**(46): 18276-18279.

Baker, S., G. Jerums, et al. (2009). "Effects and clinical potential of very-low-calorie diets (VLCDs) in type 2 diabetes." Diabetes Res Clin Pract **85**(3): 235-242.

Baldassano, S., A. L. Bellanca, et al. (2012). "Food intake in lean and obese mice after peripheral administration of glucagon-like peptide 2." J Endocrinol **213**(3): 277-284.

Bantle, J. P., S. Ikramuddin, et al. (2007). "Hyperinsulinemic hypoglycemia developing late after gastric bypass." Obes Surg **17**(5): 592-594.

Barnes, M. J., S. D. Primeaux, et al. (2008). "Food deprivation increases the mRNA expression of micro-opioid receptors in the ventral medial hypothalamus and arcuate nucleus." Am J Physiol Regul Integr Comp Physiol **295**(5): R1385-1390.

Barrett-Connor, E. (1991). "Nutrition epidemiology: how do we know what they ate?" Am J Clin Nutr **54**(1 Suppl): 182S-187S.

Bates, C. J., A. Prentice, et al. (1999). "Gender differences in food and nutrient intakes and status indices from the National Diet and Nutrition Survey of people aged 65 years and over." Eur J Clin Nutr **53**(9): 694-699.

Batterham, R. L., M. A. Cohen, et al. (2003). "Inhibition of food intake in obese subjects by peptide YY3-36." N Engl J Med **349**(10): 941-948.

Batterham, R. L., M. A. Cowley, et al. (2002). "Gut hormone PYY(3-36) physiologically inhibits food intake." Nature **418**(6898): 650-654.

Batterham, R. L., D. H. ffytche, et al. (2007). "PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans." Nature **450**(7166): 106-109.

Batterink, L., S. Yokum, et al. (2010). "Body mass correlates inversely with inhibitory control in response to food among adolescent girls: an fMRI study." NeuroImage **52**(4): 1696-1703.

Bays, H. E., B. Laferrere, et al. (2009). "Adiposopathy and bariatric surgery: is 'sick fat' a surgical disease?" Int J Clin Pract **63**(9): 1285-1300.

Beaver, J. D., A. D. Lawrence, et al. (2006). "Individual differences in reward drive predict neural responses to images of food." J Neurosci **26**(19): 5160-5166.

Beck, A. T., R. A. Steer, et al. (1996). "Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients." J Pers Assess **67**(3): 588-597.

Beckmann, C. F., M. Jenkinson, et al. (2003). "General multilevel linear modeling for group analysis in FMRI." NeuroImage **20**(2): 1052-1063.

Beer-Borst, S., S. Hercberg, et al. (2000). "Dietary patterns in six european populations: results from EURALIM, a collaborative European data harmonization and information campaign." Eur J Clin Nutr **54**(3): 253-262.

Bellebaum, C., B. Koch, et al. (2008). "Focal basal ganglia lesions are associated with impairments in reward-based reversal learning." Brain **131**(Pt 3): 829-841.

Bello, N. T. and A. Hajnal (2010). "Dopamine and binge eating behaviors." Pharmacol Biochem Behav **97**(1): 25-33.

Bender, G., M. G. Veldhuizen, et al. (2009). "Neural correlates of evaluative compared with passive tasting." Eur J Neurosci **30**(2): 327-338.

Berg, K. C., C. B. Peterson, et al. (2012). "Psychometric evaluation of the eating disorder examination and eating disorder examination-questionnaire: a systematic review of the literature." Int J Eat Disord **45**(3): 428-438.

Bermudez-Silva, F. J., P. Cardinal, et al. (2012). "The role of the endocannabinoid system in the neuroendocrine regulation of energy balance." J Psychopharmacol **26**(1): 114-124.

Berridge, K. C., T. E. Robinson, et al. (2009). "Dissecting components of reward: 'liking', 'wanting', and learning." Curr Opin Pharmacol **9**(1): 65-73.

Berrington de Gonzalez, A., P. Hartge, et al. (2010). "Body-mass index and mortality among 1.46 million white adults." N Engl J Med **363**(23): 2211-2219.

Berthoud, H. R. (2012). "The neurobiology of food intake in an obesogenic environment." Proc Nutr Soc **71**(4): 478-487.

Blundell, J., C. de Graaf, et al. (2010). "Appetite control: methodological aspects of the evaluation of foods." Obes Rev **11**(3): 251-270.

Blundell, J. E. and G. Finlayson (2004). "Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption?" Physiol Behav **82**(1): 21-25.

Bobb, J. F., B. S. Schwartz, et al. (2012). "Cross-sectional and longitudinal association of body mass index and brain volume." Hum Brain Mapp.

Boccardi, M., M. Bocchetta, et al. (2013). "Atypical nucleus accumbens morphology in psychopathy: another limbic piece in the puzzle." Int J Law Psychiatry **36**(2): 157-167.

Bohon, C. and E. Stice (2012). "Negative affect and neural response to palatable food intake in bulimia nervosa." Appetite **58**(3): 964-970.

Bohon, C., E. Stice, et al. (2009). "f." Int J Eat Disord **42**(3): 210-221.

Bond, D. S., S. Phelan, et al. (2009). "Weight-loss maintenance in successful weight losers: surgical vs non-surgical methods." Int J Obes (Lond) **33**(1): 173-180.

Booij, J. and T. van Amelsvoort (2012). "Imaging as tool to investigate psychoses and antipsychotics." Handb Exp Pharmacol(212): 299-337.

Bookstein, F. L. (2001). ""Voxel-based morphometry" should not be used with imperfectly registered images." Neuroimage **14**(6): 1454-1462.

Bora, E., M. Yucel, et al. (2012). "White matter microstructure in opiate addiction." Addict Biol **17**(1): 141-148.

Borg, C. M., C. W. le Roux, et al. (2006). "Progressive rise in gut hormone levels after Roux-en-Y gastric bypass suggests gut adaptation and explains altered satiety." Br J Surg **93**(2): 210-215.

Bose, M., S. Machineni, et al. (2010). "Superior appetite hormone profile after equivalent weight loss by gastric bypass compared to gastric banding." Obesity (Silver Spring) **18**(6): 1085-1091.

Bose, M., J. Teixeira, et al. (2010). "Weight loss and incretin responsiveness improve glucose control independently after gastric bypass surgery." J Diabetes **2**(1): 47-55.

Botkin, C. D. and M. M. Osman (2007). "Prevalence, Challenges, and Solutions for 18F-FDG PET Studies of Obese Patients: A Technologist's Perspective." J Nucl Med Technol **35**(2): 80-83.

Bottini, G., M. Berlingeri, et al. (2012). "GOOD or BAD responder? Behavioural and neuroanatomical markers of clinical response to donepezil in dementia." Behav Neurol **25**(2): 61-72.

Braet, C., L. Claus, et al. (2007). "Impulsivity in overweight children." Eur Child Adolesc Psychiatry **16**(8): 473-483.

Braet, C. and G. Crombez (2003). "Cognitive interference due to food cues in childhood obesity." J Clin Child Adolesc Psychol **32**(1): 32-39.

Braet, C. and T. Van Strien (1997). "Assessment of emotional, externally induced and restrained eating behaviour in nine to twelve-year-old obese and non-obese children." Behav Res Ther **35**(9): 863-873.

Brethauer, S. A., J. P. Hammel, et al. (2009). "Systematic review of sleeve gastrectomy as staging and primary bariatric procedure." Surg Obes Relat Dis **5**(4): 469-475.

Brethauer, S. A., A. D. Pryor, et al. (2009). "Endoluminal procedures for bariatric patients: expectations among bariatric surgeons." Surg Obes Relat Dis **5**(2): 231-236.

Breum, L., M. H. Rasmussen, et al. (2003). "Twenty-four-hour plasma tryptophan concentrations and ratios are below normal in obese subjects and are not normalized by substantial weight reduction." Am J Clin Nutr **77**(5): 1112-1118.

Brolin, R. E., H. A. Kenler, et al. (1992). "Long-limb gastric bypass in the superobese. A prospective randomized study." Ann Surg **215**(4): 387-395.

Brolin, R. E., L. B. LaMarca, et al. (2002). "Malabsorptive gastric bypass in patients with superobesity." J Gastrointest Surg **6**(2): 195-203; discussion 204-195.

Brolin, R. E., L. B. Robertson, et al. (1994). "Weight loss and dietary intake after vertical banded gastroplasty and Roux-en-Y gastric bypass." Ann Surg **220**(6): 782-790.

Brooks, S. J., C. Benedict, et al. (2012). "Late-life obesity is associated with smaller global and regional gray matter volumes: a voxel-based morphometric study." Int J Obes (Lond).

Brooks, S. J., J. Cedernaes, et al. (2013). "Increased prefrontal and parahippocampal activation with reduced dorsolateral prefrontal and insular cortex activation to food images in obesity: a meta-analysis of FMRI studies." PLoS One **8**(4): e60393.

Brown, E. K., E. A. Settle, et al. (1982). "Food intake patterns of gastric bypass patients." J Am Diet Assoc **80**(5): 437-443.

Bruce, A. S., L. M. Holsen, et al. (2010). "Obese children show hyperactivation to food pictures in brain networks linked to motivation, reward and cognitive control." Int J Obes (Lond) **34**(10): 1494-1500.

Bruce, J. M., L. Hancock, et al. (2011). "Changes in brain activation to food pictures after adjustable gastric banding." Surg Obes Relat Dis.

Bruijnzeel, A. W., L. W. Corrie, et al. (2011). "Effects of insulin and leptin in the ventral tegmental area and arcuate hypothalamic nucleus on food intake and brain reward function in female rats." Behav Brain Res **219**(2): 254-264.

Bryant, E. J., N. A. King, et al. (2008). "Disinhibition: its effects on appetite and weight regulation." Obes Rev **9**(5): 409-419.

Bryant, E. J., N. A. King, et al. (2012). "Relationships among tonic and episodic aspects of motivation to eat, gut peptides, and weight before and after bariatric surgery." Surg Obes Relat Dis.

Buchsbaum, B. R., S. Greer, et al. (2005). "Meta-analysis of neuroimaging studies of the Wisconsin card-sorting task and component processes." Hum Brain Mapp **25**(1): 35-45.

Buchwald, H., Y. Avidor, et al. (2004). "Bariatric surgery: a systematic review and meta-analysis." JAMA **292**(14): 1724-1737.

Buchwald, H. and D. M. Oien (2009). "Metabolic/bariatric surgery Worldwide 2008." Obes Surg **19**(12): 1605-1611.

Buchwald, H. and D. M. Oien (2013). "Metabolic/bariatric surgery worldwide 2011." Obes Surg **23**(4): 427-436.

Bueter, M., C. Lowenstein, et al. (2010). "Vagal sparing surgical technique but not stoma size affects body weight loss in rodent model of gastric bypass." Obes Surg **20**(5): 616-622.

Bueter, M., C. Lowenstein, et al. (2010). "Gastric bypass increases energy expenditure in rats." Gastroenterology **138**(5): 1845-1853.

Bueter, M., A. D. Miras, et al. (2011). "Alterations of sucrose preference after Roux-en-Y gastric bypass." Physiol Behav **104**(5): 709-721.

Burch, J., C. McKenna, et al. (2009). "Rimonabant for the treatment of overweight and obese people." Health Technol Assess **13 Suppl 3**: 13-22.

Burge, J. C., J. Z. Schaumburg, et al. (1995). "Changes in patients' taste acuity after Roux-en-Y gastric bypass for clinically severe obesity." J Am Diet Assoc **95**(6): 666-670.

Burrows, T. L., R. J. Martin, et al. (2010). "A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water." J Am Diet Assoc **110**(10): 1501-1510.

Burton, P., H. J. Smit, et al. (2007). "The influence of restrained and external eating patterns on overeating." Appetite **49**(1): 191-197.

Burton, P. R. and W. A. Brown (2011). "The mechanism of weight loss with laparoscopic adjustable gastric banding: induction of satiety not restriction." Int J Obes (Lond) **35 Suppl 3**: S26-30.

Burton, P. R., W. A. Brown, et al. (2010). "Mechanisms of bolus clearance in patients with laparoscopic adjustable gastric bands." Obes Surg **20**(9): 1265-1272.

Burton, P. R., W. A. Brown, et al. (2009). "Effects of gastric band adjustments on intraluminal pressure." Obes Surg **19**(11): 1508-1514.

Burton, P. R., K. Yap, et al. (2010). "Effects of adjustable gastric bands on gastric emptying, supra- and infraband transit and satiety: a randomized double-blind crossover trial using a new technique of band visualization." Obes Surg **20**(12): 1690-1697.

Busetto, L., G. Segato, et al. (2006). "High ghrelin concentration is not a predictor of less weight loss in morbidly obese women treated with laparoscopic adjustable gastric banding." Obes Surg **16**(8): 1068-1074.

Camara, E., A. Rodriguez-Fornells, et al. (2010). "Microstructural brain differences predict functional hemodynamic responses in a reward processing task." J Neurosci **30**(34): 11398-11402.

Campbell, J. E. and D. J. Drucker (2013). "Pharmacology, physiology, and mechanisms of incretin hormone action." Cell Metab **17**(6): 819-837.

Canetti, L., E. M. Berry, et al. (2009). "Psychosocial predictors of weight loss and psychological adjustment following bariatric surgery and a weight-loss program: the mediating role of emotional eating." Int J Eat Disord **42**(2): 109-117.

Cappelleri, J. C., A. G. Bushmakin, et al. (2009). "Evaluating the Power of Food Scale in obese subjects and a general sample of individuals: development and measurement properties." Int J Obes (Lond) **33**(8): 913-922.

Capuron, L., C. Poitou, et al. (2011). "Relationship between adiposity, emotional status and eating behaviour in obese women: role of inflammation." Psychol Med **41**(7): 1517-1528.

Carnell, S., C. Gibson, et al. (2012). "Neuroimaging and obesity: current knowledge and future directions." Obes Rev **13**(1): 43-56.

Carnell, S. and J. Wardle (2008). "Appetite and adiposity in children: evidence for a behavioral susceptibility theory of obesity." Am J Clin Nutr **88**(1): 22-29.

Carnell, S. and J. Wardle (2008). "Appetitive traits and child obesity: measurement, origins and implications for intervention." Proc Nutr Soc **67**(4): 343-355.

Carrasco, F., K. Papapietro, et al. (2007). "Changes in resting energy expenditure and body composition after weight loss following Roux-en-Y gastric bypass." Obes Surg **17**(5): 608-616.

Carver, S. C. and T. L. White (1994). "Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales." J Pers Soc Psychol **67**: 319-333.

Castellanos, E. H., E. Charboneau, et al. (2009). "Obese adults have visual attention bias for food cue images: evidence for altered reward system function." Int J Obes (Lond) **33**(9): 1063-1073.

Cazettes, F., J. I. Cohen, et al. (2011). "Obesity-mediated inflammation may damage the brain circuit that regulates food intake." Brain Res **1373**: 101-109.

Cecil, J., M. Dalton, et al. (2012). "Obesity and eating behaviour in children and adolescents: contribution of common gene polymorphisms." Int Rev Psychiatry **24**(3): 200-210.

Chaddock, L., K. I. Erickson, et al. (2010). "A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children." Brain Res **1358**: 172-183.

Chambers, A. P., L. Jessen, et al. (2011). "Weight-independent changes in blood glucose homeostasis after gastric bypass or vertical sleeve gastrectomy in rats." Gastroenterology **141**(3): 950-958.

Chan, J. L., E. C. Mun, et al. (2006). "Peptide YY levels are elevated after gastric bypass surgery." Obesity (Silver Spring) **14**(2): 194-198.

Chandarana, K., C. Gelegen, et al. (2011). "Diet and gastrointestinal bypass-induced weight loss: the roles of ghrelin and peptide YY." Diabetes **60**(3): 810-818.

Chaney, A., A. Carballedo, et al. (2013). "Effect of childhood maltreatment on brain structure in adult patients with major depressive disorder and healthy participants." J Psychiatry Neurosci **38**(5): 120208.

Chang, G. Q., O. Karatayev, et al. (2007). "Dietary fat stimulates endogenous enkephalin and dynorphin in the paraventricular nucleus: role of circulating triglycerides." Am J Physiol Endocrinol Metab **292**(2): E561-570.

Chang, G. Q., O. Karatayev, et al. (2004). "Circulating triglycerides impact on orexigenic peptides and neuronal activity in hypothalamus." Endocrinology **145**(8): 3904-3912.

Chang, L. J., T. Yarkoni, et al. (2012). "Decoding the Role of the Insula in Human Cognition: Functional Parcellation and Large-Scale Reverse Inference." Cereb Cortex.

Chapman, A. E., G. Kiroff, et al. (2004). "Laparoscopic adjustable gastric banding in the treatment of obesity: a systematic literature review." Surgery **135**(3): 326-351.

Chaput, J. P., C. Leblanc, et al. (2009). "Risk factors for adult overweight and obesity in the Quebec Family Study: have we been barking up the wrong tree?" Obesity (Silver Spring) **17**(10): 1964-1970.

Chaudhri, O. B., J. R. Parkinson, et al. (2006). "Differential hypothalamic neuronal activation following peripheral injection of GLP-1 and oxyntomodulin in mice detected by manganese-enhanced magnetic resonance imaging." Biochem Biophys Res Commun **350**(2): 298-306.

Chaves, G. V., S. E. Pereira, et al. (2007). "Nutritional status of vitamin A in morbid obesity before and after Roux-en-Y gastric bypass." Obes Surg **17**(7): 970-976.

Cheah, Y. S. and S. A. Amiel (2012). "Metabolic neuroimaging of the brain in diabetes mellitus and hypoglycaemia." Nat Rev Endocrinol **8**(10): 588-597.

Chechlacz, M., P. Rotshtein, et al. (2009). "Diabetes dietary management alters responses to food pictures in brain regions associated with motivation and emotion: a functional magnetic resonance imaging study." Diabetologia **52**(3): 524-533.

Chen, J. G., A. Spagnoli, et al. (2012). "Adipogenic differentiation of adipose tissue-derived human mesenchymal stem cells: effect of gastric bypass surgery." Surg Endosc.

Cherbuin, N., P. Sachdev, et al. (2012). "Higher normal fasting plasma glucose is associated with hippocampal atrophy: The PATH Study." Neurology **79**(10): 1019-1026.

Christensen, R., P. K. Kristensen, et al. (2007). "Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials." Lancet **370**(9600): 1706-1713.

Clegg, D. J., L. M. Brown, et al. (2006). "Gonadal hormones determine sensitivity to central leptin and insulin." Diabetes **55**(4): 978-987.

Clinton, S. M., J. D. Stead, et al. (2011). "Developmental underpinnings of differences in rodent novelty-seeking and emotional reactivity." Eur J Neurosci **34**(6): 994-1005.

Cochrane, C. J. and K. P. Ebmeier (2013). "Diffusion tensor imaging in parkinsonian syndromes: a systematic review and meta-analysis." Neurology **80**(9): 857-864.

Cohen, J. I., F. Cazettes, et al. (2011). "Abnormal cholesterol is associated with prefrontal white matter abnormalities among obese adults, a diffusion tensor imaging study." Neuroradiol J **1**(21): 989-997.

Cohen, J. I., K. F. Yates, et al. (2011). "Obesity, orbitofrontal structure and function are associated with food choice: a cross-sectional study." BMJ Open **1**(2): e000175.

Colantuoni, C., J. Schwenker, et al. (2001). "Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain." Neuroreport **12**(16): 3549-3552.

Coletta, M., S. Platek, et al. (2009). "Brain activation in restrained and unrestrained eaters: an fMRI study." J Abnorm Psychol **118**(3): 598-609.

Colquitt, J. L., J. Picot, et al. (2009). "Surgery for obesity." Cochrane Database Syst Rev(2): CD003641.

Comings, D. E. and K. Blum (2000). "Reward deficiency syndrome: genetic aspects of behavioral disorders." Prog Brain Res **126**: 325-341.

Conason, A., J. Teixeira, et al. (2013). "Substance use following bariatric weight loss surgery." JAMA Surg **148**(2): 145-150.

Cools, R., A. C. Roberts, et al. (2008). "Serotoninergic regulation of emotional and behavioural control processes." Trends Cogn Sci **12**(1): 31-40.

Cornier, M. A., A. K. Salzberg, et al. (2009). "The effects of overfeeding on the neuronal response to visual food cues in thin and reduced-obese individuals." PLoS One **4**(7): e6310.

Cornier, M. A., A. K. Salzberg, et al. (2010). "Sex-based differences in the behavioral and neuronal responses to food." Physiol Behav **99**(4): 538-543.

Cornier, M. A., S. S. Von Kaenel, et al. (2007). "Effects of overfeeding on the neuronal response to visual food cues." Am J Clin Nutr **86**(4): 965-971.

Corwin, R. L., N. M. Avena, et al. (2011). "Feeding and reward: perspectives from three rat models of binge eating." Physiol Behav **104**(1): 87-97.

Cottone, P., V. Sabino, et al. (2008). "Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food." Neuropsychopharmacology **33**(3): 524-535.

Coughlin, K., R. M. Bell, et al. (1983). "Preoperative and postoperative assessment of nutrient intakes in patients who have undergone gastric bypass surgery." Arch Surg **118**(7): 813-816.

Coupaye, M., J. L. Bouillot, et al. (2005). "One-year changes in energy expenditure and serum leptin following adjustable gastric banding in obese women." Obes Surg **15**(6): 827-833.

Cousijn, J., R. W. Wiers, et al. (2012). "Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls." Neuroimage **59**(4): 3845-3851.

Cremonini, F., M. Camilleri, et al. (2005). "Effect of somatostatin analog on postprandial satiation in obesity." Obes Res **13**(9): 1572-1579.

Cummings, D. E., J. Overduin, et al. (2004). "Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution." J Clin Endocrinol Metab **89**(6): 2608-2615.

Cummings, D. E., J. Q. Purnell, et al. (2001). "A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans." Diabetes **50**(8): 1714-1719.

Cummings, D. E., D. S. Weigle, et al. (2002). "Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery." N Engl J Med **346**(21): 1623-1630.

Czyzewska, M. and R. Graham (2008). "Implicit and explicit attitudes to high- and low-calorie food in females with different BMI status." Eat Behav **9**(3): 303-312.

D'Alessio, D. A., R. L. Prigeon, et al. (1995). "Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. A physiological role of glucagon-like peptide I." Diabetes **44**(12): 1433-1437.

Danguir, J. (1988). "Food intake in rats is increased by intracerebroventricular infusion of the somatostatin analogue SMS 201-995 and is decreased by somatostatin antiserum." Peptides **9**(1): 211-213.

Das, S. K., S. B. Roberts, et al. (2003). "Long-term changes in energy expenditure and body composition after massive weight loss induced by gastric bypass surgery." Am J Clin Nutr **78**(1): 22-30.

Davids, S., H. Lauffer, et al. (2010). "Increased dorsolateral prefrontal cortex activation in obese children during observation of food stimuli." Int J Obes (Lond) **34**(1): 94-104.

Davis, C. A., R. D. Levitan, et al. (2009). "Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating." Obesity (Silver Spring) **17**(6): 1220-1225.

de Araujo, I. E., J. G. Ferreira, et al. (2012). "The gut-brain dopamine axis: a regulatory system for caloric intake." Physiol Behav **106**(3): 394-399.

de Castro, J. M. (2010). "The control of food intake of free-living humans: putting the pieces back together." Physiol Behav **100**(5): 446-453.

de Jong, J. R., B. van Ramshorst, et al. (2009). "Weight loss after laparoscopic adjustable gastric banding is not caused by altered gastric emptying." Obes Surg **19**(3): 287-292.

De Jonghe, B. C., A. Hajnal, et al. (2005). "Increased oral and decreased intestinal sensitivity to sucrose in obese, prediabetic CCK-A receptor-deficient OLETF rats." Am J Physiol Regul Integr Comp Physiol **288**(1): R292-300.

De Silva, A., V. Salem, et al. (2011). "The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans." Cell Metab **14**(5): 700-706.

De Silva, A., V. Salem, et al. (2012). "The use of functional MRI to study appetite control in the CNS." Exp Diabetes Res **2012**: 764017.

de Weijer, B. A., E. van de Giessen, et al. (2011). "Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects." EJNMMI Res **1**(1): 37.

Decker, G. A., J. M. Swain, et al. (2007). "Gastrointestinal and nutritional complications after bariatric surgery." Am J Gastroenterol **102**(11): 2571-2580; quiz 2581.

Deichmann, R., J. A. Gottfried, et al. (2003). "Optimized EPI for fMRI studies of the orbitofrontal cortex." Neuroimage **19**(2 Pt 1): 430-441.

Deitel, M. (2008). "The change in the dumping syndrome concept." Obes Surg **18**(12): 1622-1624.

Del Parigi, A., K. Chen, et al. (2002). "Sex differences in the human brain's response to hunger and satiation." Am J Clin Nutr **75**(6): 1017-1022.

Del Parigi, A., J. F. Gautier, et al. (2002). "Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography." Ann N Y Acad Sci **967**: 389-397.

Delgado, M. R. and K. C. Dickerson (2012). "Reward-related learning via multiple memory systems." Biol Psychiatry **72**(2): 134-141.

DelParigi, A., K. Chen, et al. (2004). "Persistence of abnormal neural responses to a meal in postobese individuals." Int J Obes Relat Metab Disord **28**(3): 370-377.

DelParigi, A., K. Chen, et al. (2007). "Successful dieters have increased neural activity in cortical areas involved in the control of behavior." Int J Obes (Lond) **31**(3): 440-448.

DelParigi, A., K. Chen, et al. (2005). "Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast." Neuroimage **24**(2): 436-443.

Demos, K. E., T. F. Heatherton, et al. (2012). "Individual differences in nucleus accumbens activity to food and sexual images predict weight gain and sexual behavior." J Neurosci **32**(16): 5549-5552.

Demos, K. E., W. M. Kelley, et al. (2011). "Dietary restraint violations influence reward responses in nucleus accumbens and amygdala." J Cogn Neurosci **23**(8): 1952-1963.

Dewey, M. and R. Heuberger (2011). "Vitamin D and calcium status and appropriate recommendations in bariatric surgery patients." Gastroenterol Nurs **34**(5): 367-374.

Dickson, S. L., R. H. Shirazi, et al. (2012). "The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors." J Neurosci **32**(14): 4812-4820.

Dimitropoulos, A., J. Tkach, et al. (2012). "Greater corticolimbic activation to high-calorie food cues after eating in obese vs. normal-weight adults." Appetite **58**(1): 303-312.

Dixon, A. F., J. B. Dixon, et al. (2005). "Laparoscopic adjustable gastric banding induces prolonged satiety: a randomized blind crossover study." J Clin Endocrinol Metab **90**(2): 813-819.

Dixon, J. B., C. W. le Roux, et al. (2012). "Bariatric surgery for type 2 diabetes." Lancet **379**(9833): 2300-2311.

Dixon, J. B. and P. E. O'Brien (2002). "Changes in comorbidities and improvements in quality of life after LAP-BAND placement." Am J Surg **184**(6B): 51S-54S.

Dixon, J. B., N. E. Straznicky, et al. (2011). "Surgical approaches to the treatment of obesity." Nat Rev Gastroenterol Hepatol **8**(8): 429-437.

Doherty, C. (2001). "Vertical banded gastroplasty." Surg Clin North Am **81**(5): 1097-1112.

Dossat, A. M., N. Lilly, et al. (2011). "Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake." J Neurosci **31**(41): 14453-14457.

Doucet, E., P. Imbeault, et al. (2000). "Appetite after weight loss by energy restriction and a low-fat diet-exercise follow-up." Int J Obes Relat Metab Disord **24**(7): 906-914.

Drevets, W. C. (1999). "Prefrontal cortical-amygdalar metabolism in major depression." Ann N Y Acad Sci **877**: 614-637.

Drewnowski, A., D. D. Krahn, et al. (1992). "Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement." Physiol Behav **51**(2): 371-379.

Drewnowski, A., D. D. Krahn, et al. (1995). "Naloxone, an opiate blocker, reduces the consumption of sweet high-fat foods in obese and lean female binge eaters." Am J Clin Nutr **61**(6): 1206-1212.

Drewnowski, A., C. Kurth, et al. (1992). "Food preferences in human obesity: carbohydrates versus fats." Appetite **18**(3): 207-221.

Driscoll, I., M. A. Beydoun, et al. (2012). "Midlife obesity and trajectories of brain volume changes in older adults." Hum Brain Mapp **33**(9): 2204-2210.

Druce, M. R., N. M. Neary, et al. (2006). "Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers." Int J Obes (Lond) **30**(2): 293-296.

Druce, M. R., A. M. Wren, et al. (2005). "Ghrelin increases food intake in obese as well as lean subjects." Int J Obes (Lond) **29**(9): 1130-1136.

Dunn, J. P., R. L. Cowan, et al. (2010). "Decreased dopamine type 2 receptor availability after bariatric surgery: preliminary findings." Brain Res **1350**: 123-130.

Dunn, J. P., R. M. Kessler, et al. (2012). "Relationship of dopamine type 2 receptor binding potential with fasting neuroendocrine hormones and insulin sensitivity in human obesity." Diabetes Care **35**(5): 1105-1111.

Echo, J. A., N. Lamonte, et al. (2002). "Alterations in food intake elicited by GABA and opioid agonists and antagonists administered into the ventral tegmental area region of rats." Physiol Behav **76**(1): 107-116.

Egberts, K., W. A. Brown, et al. (2012). "Does exercise improve weight loss after bariatric surgery? A systematic review." Obes Surg **22**(2): 335-341.

Egecioglu, E., E. Jerlhag, et al. (2010). "Ghrelin increases intake of rewarding food in rodents." Addict Biol.

Egecioglu, E., K. P. Skibicka, et al. (2011). "Hedonic and incentive signals for body weight control." Rev Endocr Metab Disord **12**(3): 141-151.

Elder, K. A. and B. M. Wolfe (2007). "Bariatric surgery: a review of procedures and outcomes." Gastroenterology **132**(6): 2253-2271.

Epstein, L. H., J. L. Temple, et al. (2007). "Food reinforcement, the dopamine D2 receptor genotype, and energy intake in obese and nonobese humans." Behav Neurosci **121**(5): 877-886.

Epstein, L. H., R. Truesdale, et al. (2003). "Effects of deprivation on hedonics and reinforcing value of food." Physiol Behav **78**(2): 221-227.

Epstein, L. H., A. Valoski, et al. (1989). "Perception of eating and exercise in children as a function of child and parent weight status." Appetite **12**(2): 105-118.

Ernst, B., M. Thurnheer, et al. (2009). "Differential changes in dietary habits after gastric bypass versus gastric banding operations." Obes Surg **19**(3): 274-280.

Escareno, C. E., D. E. Azagury, et al. (2012). "Establishing a reproducible large animal survival model of laparoscopic Roux-en-Y gastric bypass." Surg Obes Relat Dis **8**(6): 764-769.

Eysenck, H., Eysenck MW (1985). Personality and Individual Differences:

A Natural Science Approach. Plenum. New York, Plenum.

Fairburn, C. G. and S. J. Beglin (1994). "Assessment of eating disorders: interview or self-report questionnaire?" Int J Eat Disord **16**(4): 363-370.

Fantino, M., J. Hosotte, et al. (1986). "An opioid antagonist, naltrexone, reduces preference for sucrose in humans." Am J Physiol **251**(1 Pt 2): R91-96.

Faraj, M., P. J. Havel, et al. (2003). "Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects." J Clin Endocrinol Metab **88**(4): 1594-1602.

Farooqi, I. S., E. Bullmore, et al. (2007). "Leptin regulates striatal regions and human eating behavior." Science **317**(5843): 1355.

Faulconbridge, L. F., T. A. Wadden, et al. (2009). "Changes in symptoms of depression with weight loss: results of a randomized trial." Obesity (Silver Spring) **17**(5): 1009-1016.

Felsted, J. A., X. Ren, et al. (2010). "Genetically determined differences in brain response to a primary food reward." J Neurosci **30**(7): 2428-2432.

Fenske, W. K., M. Bueter, et al. (2012). "Exogenous peptide YY3-36 and Exendin-4 further decrease food intake, whereas octreotide increases food intake in rats after Roux-en-Y gastric bypass." Int J Obes (Lond) **36**(3): 379-384.

Ferreira, L. K. and G. F. Busatto (2011). "Neuroimaging in Alzheimer's disease: current role in clinical practice and potential future applications." Clinics (Sao Paulo) **66 Suppl 1**: 19-24.

Ferreira, L. K., B. S. Diniz, et al. (2011). "Neurostructural predictors of Alzheimer's disease: a meta-analysis of VBM studies." Neurobiol Aging **32**(10): 1733-1741.

Ferriday, D. and J. M. Brunstrom (2011). "'I just can't help myself': effects of food-cue exposure in overweight and lean individuals." Int J Obes (Lond) **35**(1): 142-149.

Field, B. C., O. B. Chaudhri, et al. (2009). "Obesity treatment: novel peripheral targets." Br J Clin Pharmacol **68**(6): 830-843.

Field, B. C., A. M. Wren, et al. (2010). "PYY3-36 and oxyntomodulin can be additive in their effect on food intake in overweight and obese humans." Diabetes **59**(7): 1635-1639.

Figlewicz, D. P., J. L. Bennett, et al. (2008). "Insulin acts at different CNS sites to decrease acute sucrose intake and sucrose self-administration in rats." Am J Physiol Regul Integr Comp Physiol **295**(2): R388-394.

Figlewicz, D. P., J. L. Bennett, et al. (2006). "Intraventricular insulin and leptin decrease sucrose self-administration in rats." Physiol Behav **89**(4): 611-616.

Figlewicz, D. P. and S. C. Benoit (2009). "Insulin, leptin, and food reward: update 2008." Am J Physiol Regul Integr Comp Physiol **296**(1): R9-R19.

Figlewicz, D. P., M. S. Higgins, et al. (2001). "Leptin reverses sucrose-conditioned place preference in food-restricted rats." Physiol Behav **73**(1-2): 229-234.

Fink, H., A. Rex, et al. (1998). "Major biological actions of CCK--a critical evaluation of research findings." Exp Brain Res **123**(1-2): 77-83.

Finkelstein, E. A., B. T. Allaire, et al. (2011). "Financial implications of coverage for laparoscopic adjustable gastric banding." Surg Obes Relat Dis **7**(3): 295-303.

Finlayson, G., I. Bordes, et al. (2012). "Susceptibility to overeating affects the impact of savory or sweet drinks on satiation, reward, and food intake in nonobese women." J Nutr **142**(1): 125-130.

Finlayson, G., E. Bryant, et al. (2009). "Acute compensatory eating following exercise is associated with implicit hedonic wanting for food." Physiol Behav **97**(1): 62-67.

Finlayson, G. and M. Dalton (2012). "Current progress in the assessment of 'liking' vs. 'wanting' food in human appetite. Comment on '"You say it's liking, i say it's wanting...". On the difficulty of disentangling food reward in man'." Appetite **58**(1): 373-378; discussion 252-375.

Finlayson, G., N. King, et al. (2008). "The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control." Appetite **50**(1): 120-127.

Finlayson, G., N. King, et al. (2007). "Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure." Physiol Behav **90**(1): 36-42.

Fischer, L., C. Hildebrandt, et al. (2012). "Excessive weight loss after sleeve gastrectomy: a systematic review." Obes Surg **22**(5): 721-731.

Fitzgerald, P. B., T. J. Oxley, et al. (2006). "An analysis of functional neuroimaging studies of dorsolateral prefrontal cortical activity in depression." Psychiatry Res **148**(1): 33-45.

Flancbaum, L., P. S. Choban, et al. (1997). "Changes in measured resting energy expenditure after Roux-en-Y gastric bypass for clinically severe obesity." Surgery **122**(5): 943-949.

Fletcher, P. C., A. Napolitano, et al. (2010). "Distinct modulatory effects of satiety and sibutramine on brain responses to food images in humans: a double dissociation across hypothalamus, amygdala, and ventral striatum." J Neurosci **30**(43): 14346-14355.

Flier, J. S. (2004). "Obesity wars: molecular progress confronts an expanding epidemic." Cell **116**(2): 337-350.

Flint, A., A. Raben, et al. (2000). "Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies." Int J Obes Relat Metab Disord **24**(1): 38-48.

Flint, A., A. Raben, et al. (2001). "The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity." Int J Obes Relat Metab Disord **25**(6): 781-792.

Flum, D. R., S. Kwon, et al. (2011). "The use, safety and cost of bariatric surgery before and after Medicare's national coverage decision." Ann Surg **254**(6): 860-865.

Fotuhi, M., D. Do, et al. (2012). "Modifiable factors that alter the size of the hippocampus with ageing." Nat Rev Neurol **8**(4): 189-202.

Foxx-Orenstein, A., M. Camilleri, et al. (2003). "Effect of a somatostatin analogue on gastric motor and sensory functions in healthy humans." Gut **52**(11): 1555-1561.

Frank, T. C., G. L. Kim, et al. (2010). "Effect of menstrual cycle phase on corticolimbic brain activation by visual food cues." Brain Res **1363**: 81-92.

Franken, I. H. (2003). "Drug craving and addiction: integrating psychological and neuropsychopharmacological approaches." Prog Neuropsychopharmacol Biol Psychiatry **27**(4): 563-579.

Frankort, A., A. Roefs, et al. (2012). "Reward activity in satiated overweight women is decreased during unbiased viewing but increased when imagining taste: an event-related fMRI study." Int J Obes (Lond) **36**(5): 627-637.

Frederich, R. C., A. Hamann, et al. (1995). "Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action." Nat Med **1**(12): 1311-1314.

Fricker, G., S. Nobmann, et al. (2002). "Permeability of porcine blood brain barrier to somatostatin analogues." Br J Pharmacol **135**(5): 1308-1314.

Frieling, H., J. Fischer, et al. (2012). "Microstructural abnormalities of the posterior thalamic radiation and the mediodorsal thalamic nuclei in females with anorexia nervosa--a voxel based diffusion tensor imaging (DTI) study." J Psychiatr Res **46**(9): 1237-1242.

Fruhbeck, G., A. Diez Caballero, et al. (2004). "Fundus functionality and ghrelin concentrations after bariatric surgery." N Engl J Med **350**(3): 308-309.

Fruhbeck, G., F. Rotellar, et al. (2004). "Fasting plasma ghrelin concentrations 6 months after gastric bypass are not determined by weight loss or changes in insulinemia." Obes Surg **14**(9): 1208-1215.

Fung, A., M. Vizcaychipi, et al. (2012). "Central nervous system inflammation in disease related conditions: mechanistic prospects." Brain Res **1446**: 144-155.

Furet, J. P., L. C. Kong, et al. (2010). "Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers." Diabetes **59**(12): 3049-3057.

Furnes, M. W., B. Stenstrom, et al. (2008). "Feeding behavior in rats subjected to gastrectomy or gastric bypass surgery." Eur Surg Res **40**(3): 279-288.

Furnes, M. W., K. Tommeras, et al. (2008). "Gastric bypass surgery causes body weight loss without reducing food intake in rats." Obes Surg **18**(4): 415-422.

Furnes, M. W., C. M. Zhao, et al. (2009). "Feeding behavior and body weight development: lessons from rats subjected to gastric bypass surgery or high-fat diet." J Physiol Pharmacol **60 Suppl 7**: 25-31.

Fusar-Poli, P., S. Borgwardt, et al. (2011). "Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis." Neurosci Biobehav Rev **35**(5): 1175-1185.

Gadde, K. M., D. B. Allison, et al. (2011). "Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial." Lancet **377**(9774): 1341-1352.

Garber, A. K. and R. H. Lustig (2011). "Is fast food addictive?" Curr Drug Abuse Rev **4**(3): 146-162.

Garcia-Garcia, I., M. A. Jurado, et al. (2012). "Functional connectivity in obesity during reward processing." NeuroImage.

Garcia-Garcia, I., M. A. Jurado, et al. (2012). "Alterations of the salience network in obesity: A resting-state fMRI study." Hum Brain Mapp.

Garcia de la Torre, N., M. A. Rubio, et al. (2008). "Effects of weight loss after bariatric surgery for morbid obesity on vascular endothelial growth factor-A, adipocytokines, and insulin." J Clin Endocrinol Metab **93**(11): 4276-4281.

Gautier, J. F., K. Chen, et al. (2000). "Differential brain responses to satiation in obese and lean men." Diabetes **49**(5): 838-846.

Gautier, J. F., A. Del Parigi, et al. (2001). "Effect of satiation on brain activity in obese and lean women." Obes Res **9**(11): 676-684.

Gearhardt, A. N., W. R. Corbin, et al. (2009). "Preliminary validation of the Yale Food Addiction Scale." Appetite **52**(2): 430-436.

Gearhardt, A. N., S. Yokum, et al. (2011). "Neural correlates of food addiction." Arch Gen Psychiatry **68**(8): 808-816.

Gebhard, B., J. J. Holst, et al. (2001). "Postprandial GLP-1, norepinephrine, and reactive hypoglycemia in dumping syndrome." Dig Dis Sci **46**(9): 1915-1923.

Geliebter, A., T. Ladell, et al. (2006). "Responsivity to food stimuli in obese and lean binge eaters using functional MRI." Appetite **46**(1): 31-35.

Geloneze, B., M. A. Tambascia, et al. (2003). "Ghrelin: a gut-brain hormone: effect of gastric bypass surgery." Obes Surg **13**(1): 17-22.

Gibson, E. L. (2006). "Emotional influences on food choice: sensory, physiological and psychological pathways." Physiol Behav **89**(1): 53-61.

Gibson, E. L. (2012). "The psychobiology of comfort eating: implications for neuropharmacological interventions." Behav Pharmacol **23**(5-6): 442-460.

Gill, R. S., D. W. Birch, et al. (2010). "Sleeve gastrectomy and type 2 diabetes mellitus: a systematic review." Surg Obes Relat Dis **6**(6): 707-713.

Giuliano, C., T. W. Robbins, et al. (2012). "Inhibition of Opioid Transmission at the mu-Opioid Receptor Prevents Both Food Seeking and Binge-Like Eating." Neuropsychopharmacology.

Goldstein, R. Z. and N. D. Volkow (2002). "Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex." Am J Psychiatry **159**(10): 1642-1652.

Goldstone, A. P. (2006). "The hypothalamus, hormones, and hunger: alterations in human obesity and illness." Prog Brain Res **153**: 57-73.

Goldstone, A. P., C. G. de Hernandez, et al. (2009). "Fasting biases brain reward systems towards high-calorie foods." Eur J Neurosci **30**(8): 1625-1635.

Goldstone, A. P., M. Patterson, et al. (2005). "Fasting and postprandial hyperghrelinemia in Prader-Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma." J Clin Endocrinol Metab **90**(5): 2681-2690.

Goldstone, A. P., C. G. Prechtl de Hernandez, et al. (2009). "Fasting biases brain reward systems towards high-calorie foods." Eur J Neurosci **30**: 1625-1635.

Goldstone, A. P., C. G. Prechtl de Hernandez, et al. (2010). "Ghrelin mimics fasting in biasing food appeal towards high-calorie foods." Obesity Reviews **11 Suppl 1**(Suppl 1): 187.

Gray, L. J., N. Cooper, et al. (2012). "A systematic review and mixed treatment comparison of pharmacological interventions for the treatment of obesity." Obes Rev **13**(6): 483-498.

Grayson, B. E., M. F. Fitzgerald, et al. (2013). "Improvements in hippocampal-dependent memory and microglial infiltration with calorie restriction and gastric bypass surgery, but not with vertical sleeve gastrectomy." Int J Obes (Lond).

Greenway, F. L., K. Fujioka, et al. (2010). "Effect of naltrexone plus bupropion on weight loss in overweight and obese adults (COR-I): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial." Lancet **376**(9741): 595-605.

Griffioen-Roose, S., M. Mars, et al. (2012). "Protein status elicits compensatory changes in food intake and food preferences." Am J Clin Nutr **95**(1): 32-38.

Grosshans, M., C. Vollmert, et al. (2012). "Association of leptin with food cue-induced activation in human reward pathways." Arch Gen Psychiatry **69**(5): 529-537.

Gruber, S. A., M. M. Silveri, et al. (2011). "Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers." Exp Clin Psychopharmacol **19**(3): 231-242.

Guijarro, A., D. Osei-Hyiaman, et al. (2008). "Sustained weight loss after Roux-en-Y gastric bypass is characterized by down regulation of endocannabinoids and mitochondrial function." Ann Surg **247**(5): 779-790.

Gunstad, J., R. H. Paul, et al. (2008). "Relationship between body mass index and brain volume in healthy adults." Int J Neurosci **118**(11): 1582-1593.

Hajnal, A., P. Kovacs, et al. (2010). "Gastric bypass surgery alters behavioral and neural taste functions for sweet taste in obese rats." Am J Physiol Gastrointest Liver Physiol **299**(4): G967-979.

Haller, S., L. Curtis, et al. (2013). "Combined Grey Matter VBM and White Matter TBSS Analysis in Young First Episode Psychosis Patients With and Without Cannabis Consumption." Brain Topogr.

Hallschmid, M., C. Benedict, et al. (2008). "Obese men respond to cognitive but not to catabolic brain insulin signaling." Int J Obes (Lond) **32**(2): 275-282.

Halmi, K. A., E. Mason, et al. (1981). "Appetitive behavior after gastric bypass for obesity." Int J Obes **5**(5): 457-464.

Haltia, L. T., J. O. Rinne, et al. (2008). "Effects of intravenous placebo with glucose expectation on human basal ganglia dopaminergic function." Synapse **62**(9): 682-688.

Haltia, L. T., A. Viljanen, et al. (2007). "Brain white matter expansion in human obesity and the recovering effect of dieting." J Clin Endocrinol Metab **92**(8): 3278-3284.

Hankir, M. K., J. R. Parkinson, et al. (2011). "Peptide YY 3-36 and pancreatic polypeptide differentially regulate hypothalamic neuronal activity in mice in vivo as measured by manganese-enhanced magnetic resonance imaging." J Neuroendocrinol **23**(4): 371-380.

Hanusch-Enserer, U., G. Brabant, et al. (2003). "Ghrelin concentrations in morbidly obese patients after adjustable gastric banding." N Engl J Med **348**(21): 2159-2160.

Haqq, A. M., D. D. Stadler, et al. (2003). "Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi syndrome." J Clin Endocrinol Metab **88**(8): 3573-3576.

Hare, T. A., C. F. Camerer, et al. (2009). "Self-control in decision-making involves modulation of the vmPFC valuation system." Science **324**(5927): 646-648.

Harvey, J., R. R. Wing, et al. (1993). "Effects on food cravings of a very low calorie diet or a balanced, low calorie diet." Appetite **21**(2): 105-115.

Hasan, K. M., A. Iftikhar, et al. (2009). "Development and aging of the healthy human brain uncinate fasciculus across the lifespan using diffusion tensor tractography." Brain Res **1276**: 67-76.

Haslam, D. W. and W. P. James (2005). "Obesity." Lancet **366**(9492): 1197-1209.

Hawkins, M. A. and J. C. Stewart (2012). "Do negative emotional factors have independent associations with excess adiposity?" J Psychosom Res **73**(4): 243-250.

Hayes, M. R., B. C. De Jonghe, et al. (2010). "Role of the glucagon-like-peptide-1 receptor in the control of energy balance." Physiol Behav **100**(5): 503-510.

Haynes, C., M. D. Lee, et al. (2003). "Interactive effects of stress, dietary restraint, and disinhibition on appetite." Eat Behav **4**(4): 369-383.

Heatherton, T. F., C. P. Herman, et al. (1991). "Effects of physical threat and ego threat on eating behavior." J Pers Soc Psychol **60**(1): 138-143.

Hejazi, R. A., H. Patil, et al. (2010). "Dumping syndrome: establishing criteria for diagnosis and identifying new etiologies." Dig Dis Sci **55**(1): 117-123.

Hellyer, P. J., R. Leech, et al. (2012). "Individual prediction of white matter injury following traumatic brain injury." Ann Neurol.

Hempel, R., R. Onopa, et al. (2012). "Type 2 diabetes affects hippocampus volume differentially in men and women." Diabetes Metab Res Rev **28**(1): 76-83.

Herman, C. P. and J. Polivy (1984). "A boundary model for the regulation of eating." Res Publ Assoc Res Nerv Ment Dis **62**: 141-156.

Herman, C. P. and J. Polivy (2008). "External cues in the control of food intake in humans: the sensory-normative distinction." Physiol Behav **94**(5): 722-728.

Hernandez, L. and B. G. Hoebel (1988). "Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens." Physiol Behav **44**(4-5): 599-606.

Hernandez, L. and B. G. Hoebel (1988). "Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis." Life Sci **42**(18): 1705-1712.

Hess, D. S., D. W. Hess, et al. (2005). "The biliopancreatic diversion with the duodenal switch: results beyond 10 years." Obes Surg **15**(3): 408-416.

Hesslinger, B., L. Tebartz van Elst, et al. (2002). "Frontoorbital volume reductions in adult patients with attention deficit hyperactivity disorder." Neurosci Lett **328**(3): 319-321.

Hill, A. J. (2004). "Does dieting make you fat?" Br J Nutr **92 Suppl 1**: S15-18.

Himpens, J., G. Dapri, et al. (2006). "A prospective randomized study between laparoscopic gastric banding and laparoscopic isolated sleeve gastrectomy: results after 1 and 3 years." Obes Surg **16**(11): 1450-1456.

Ho, A., J. Kennedy, et al. (2012). "Neural correlates to food-related behavior in normal-weight and overweight/obese participants." PLoS One **7**(9): e45403.

Ho, A. J., C. A. Raji, et al. (2010). "Obesity is linked with lower brain volume in 700 AD and MCI patients." Neurobiol Aging **31**(8): 1326-1339.

Ho, A. J., J. L. Stein, et al. (2010). "A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly." Proc Natl Acad Sci U S A **107**(18): 8404-8409.

Holdstock, C., B. E. Engstrom, et al. (2003). "Ghrelin and adipose tissue regulatory peptides: effect of gastric bypass surgery in obese humans." J Clin Endocrinol Metab **88**(7): 3177-3183.

Hollmann, M., L. Hellrung, et al. (2012). "Neural correlates of the volitional regulation of the desire for food." Int J Obes (Lond) **36**(5): 648-655.

Hollmann, M., B. Pleger, et al. (2013). "Brain imaging in the context of food perception and eating." Curr Opin Lipidol **24**(1): 18-24.

Holsen, L. M., J. R. Zarcone, et al. (2006). "Neural mechanisms underlying hyperphagia in Prader-Willi syndrome." Obesity (Silver Spring) **14**(6): 1028-1037.

Holsen, L. M., J. R. Zarcone, et al. (2009). "Genetic subtype differences in neural circuitry of food motivation in Prader-Willi syndrome." Int J Obes (Lond) **33**(2): 273-283.

Holzer, P., F. Reichmann, et al. (2012). "Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis." Neuropeptides **46**(6): 261-274.

Horder, J., C. J. Harmer, et al. (2010). "Reduced neural response to reward following 7 days treatment with the cannabinoid CB1 antagonist rimonabant in healthy volunteers." Int J Neuropsychopharmacol **13**(8): 1103-1113.

Horowitz, M., P. J. Collins, et al. (1986). "Gastric emptying after gastric bypass." Int J Obes **10**(2): 117-121.

Horstmann, A., F. P. Busse, et al. (2011). "Obesity-Related Differences between Women and Men in Brain Structure and Goal-Directed Behavior." Front Hum Neurosci **5**: 58.

Hrabosky, J. I., R. M. Masheb, et al. (2006). "A prospective study of body dissatisfaction and concerns in extremely obese gastric bypass patients: 6- and 12-month postoperative outcomes." Obes Surg **16**(12): 1615-1621.

Hsu, J. L., Y. L. Chen, et al. (2012). "Microstructural white matter abnormalities in type 2 diabetes mellitus: a diffusion tensor imaging study." Neuroimage **59**(2): 1098-1105.

Huda, M. S., H. Mani, et al. (2010). "Ghrelin inhibits autonomic function in healthy controls, but has no effect on obese and vagotomized subjects." Clin Endocrinol (Oxf) **73**(5): 678-685.

Hudson, S. M., J. B. Dixon, et al. (2002). "Sweet eating is not a predictor of outcome after Lap-Band placement. Can we finally bury the myth?" Obes Surg **12**(6): 789-794.

Ifland, J. R., H. G. Preuss, et al. (2009). "Refined food addiction: a classic substance use disorder." Med Hypotheses **72**(5): 518-526.

Isbell, J. M., R. A. Tamboli, et al. (2010). "The importance of caloric restriction in the early improvements in insulin sensitivity after Roux-en-Y gastric bypass surgery." Diabetes Care **33**(7): 1438-1442.

Ivleva, E. I., A. S. Bidesi, et al. (2012). "Brain gray matter phenotypes across the psychosis dimension." Psychiatry Res **204**(1): 13-24.

Ivo, R., A. Nicklas, et al. (2013). "Brain structural and psychometric alterations in chronic low back pain." Eur Spine J.

Jacobsen, S. H., S. C. Olesen, et al. (2012). "Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects." Obes Surg **22**(7): 1084-1096.

James, W. P., I. D. Caterson, et al. (2010). "Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects." N Engl J Med **363**(10): 905-917.

Jansen, A., N. Theunissen, et al. (2003). "Overweight children overeat after exposure to food cues." Eat Behav **4**(2): 197-209.

Jansen, P. L., J. van Werven, et al. (2011). "Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery." Dig Dis **29**(1): 48-51.

Janssen, P., A. Rotondo, et al. (2013). "Review article: a comparison of glucagon-like peptides 1 and 2." Aliment Pharmacol Ther **37**(1): 18-36.

Jastreboff, A. M., R. Sinha, et al. (2013). "Neural correlates of stress- and food cue-induced food craving in obesity: association with insulin levels." Diabetes Care **36**(2): 394-402.

Jenkinson, M., P. Bannister, et al. (2002). "Improved optimization for the robust and accurate linear registration and motion correction of brain images." Neuroimage **17**(2): 825-841.

Jenkinson, M. and S. Smith (2001). "A global optimisation method for robust affine registration of brain images." Med Image Anal **5**(2): 143-156.

Jentsch, J. D. and J. R. Taylor (1999). "Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli." Psychopharmacology (Berl) **146**(4): 373-390.

Jones, R. B., S. McKie, et al. (2012). "Functional neuroimaging demonstrates that ghrelin inhibits the central nervous system response to ingested lipid." Gut **61**(11): 1543-1551.

Jonsson, B., H. Bjorvell, et al. (1986). "Personality traits predicting weight loss outcome in obese patients." Acta Psychiatr Scand **74**(4): 384-387.

Jonsson, E. G., M. M. Nothen, et al. (1999). "Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers." Mol Psychiatry **4**(3): 290-296.

Kalarchian, M. A., G. T. Wilson, et al. (1999). "Effects of bariatric surgery on binge eating and related psychopathology." Eat Weight Disord **4**(1): 1-5.

Kalarchian, M. A., G. T. Wilson, et al. (2000). "Assessment of eating disorders in bariatric surgery candidates: self-report questionnaire versus interview." Int J Eat Disord **28**(4): 465-469.

Karhunen, L. J., E. J. Vanninen, et al. (2000). "Regional cerebral blood flow during exposure to food in obese binge eating women." Psychiatry Res **99**(1): 29-42.

Karra, E., x, et al. (2013). "A link between FTO, ghrelin, and impaired brain food-cue responsivity." The Journal of Clinical Investigation **123**(8): 0-0.

Kashyap, S. R., D. L. Bhatt, et al. (2010). "Bariatric surgery vs. advanced practice medical management in the treatment of type 2 diabetes mellitus: rationale and design of the Surgical Therapy And Medications Potentially Eradicate Diabetes Efficiently trial (STAMPEDE)." Diabetes Obes Metab **12**(5): 452-454.

Kastin, A. J., V. Akerstrom, et al. (2002). "Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier." J Mol Neurosci **18**(1-2): 7-14.

Keitel, V., B. Gorg, et al. (2010). "The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain." Glia **58**(15): 1794-1805.

Kellogg, T. A., J. P. Bantle, et al. (2008). "Postgastric bypass hyperinsulinemic hypoglycemia syndrome: characterization and response to a modified diet." Surg Obes Relat Dis **4**(4): 492-499.

Kellum, J. M., J. F. Kuemmerle, et al. (1990). "Gastrointestinal hormone responses to meals before and after gastric bypass and vertical banded gastroplasty." Ann Surg **211**(6): 763-770; discussion 770-761.

Kenler, H. A., R. E. Brolin, et al. (1990). "Changes in eating behavior after horizontal gastroplasty and Roux-en-Y gastric bypass." Am J Clin Nutr **52**(1): 87-92.

Kennedy, S. H., M. Javanmard, et al. (1997). "A review of functional neuroimaging in mood disorders: positron emission tomography and depression." Can J Psychiatry **42**(5): 467-475.

Killgore, W. D., A. D. Young, et al. (2003). "Cortical and limbic activation during viewing of high- versus low-calorie foods." Neuroimage **19**(4): 1381-1394.

Killgore, W. D. and D. A. Yurgelun-Todd (2006). "Affect modulates appetite-related brain activity to images of food." Int J Eat Disord **39**(5): 357-363.

Kindel, T. L., S. M. Yoder, et al. (2009). "Duodenal-jejunal exclusion improves glucose tolerance in the diabetic, Goto-Kakizaki rat by a GLP-1 receptor-mediated mechanism." J Gastrointest Surg **13**(10): 1762-1772.

King, W. C., J. Y. Chen, et al. (2012). "Prevalence of alcohol use disorders before and after bariatric surgery." JAMA **307**(23): 2516-2525.

Kishi, T. and J. K. Elmquist (2005). "Body weight is regulated by the brain: a link between feeding and emotion." Mol Psychiatry **10**(2): 132-146.

Kleiner, K. D., M. S. Gold, et al. (2004). "Body mass index and alcohol use." J Addict Dis **23**(3): 105-118.

Koch, K., G. Wagner, et al. (2013). "Association between white matter fiber structure and reward-related reactivity of the ventral striatum." Hum Brain Mapp.

Kohli, R., M. Kirby, et al. (2010). "Intestinal adaptation after ileal interposition surgery increases bile acid recycling and protects against obesity-related comorbidities." Am J Physiol Gastrointest Liver Physiol **299**(3): G652-660.

Kohli, R., M. A. Stefater, et al. (2011). "Molecular insights from bariatric surgery." Rev Endocr Metab Disord **12**(3): 211-217.

Konrad, C., T. Ukas, et al. (2009). "Defining the human hippocampus in cerebral magnetic resonance images--an overview of current segmentation protocols." Neuroimage **47**(4): 1185-1195.

Kopelman, P. (2007). "Health risks associated with overweight and obesity." Obes Rev **8 Suppl 1**: 13-17.

Korbonits, M., A. P. Goldstone, et al. (2004). "Ghrelin--a hormone with multiple functions." Front Neuroendocrinol **25**(1): 27-68.

Korner, J., W. Inabnet, et al. (2006). "Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels." Obesity (Silver Spring) **14**(9): 1553-1561.

Korner, J., W. Inabnet, et al. (2009). "Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass." Int J Obes (Lond) **33**(7): 786-795.

Kramer, H., G. Cao, et al. (2010). "Increasing BMI and waist circumference and prevalence of obesity among adults with Type 2 diabetes: the National Health and Nutrition Examination Surveys." J Diabetes Complications **24**(6): 368-374.

Kraschnewski, J. L., J. Boan, et al. (2010). "Long-term weight loss maintenance in the United States." Int J Obes (Lond) **34**(11): 1644-1654.

Kreymann, B., G. Williams, et al. (1987). "Glucagon-like peptide-1 7-36: a physiological incretin in man." Lancet **2**(8571): 1300-1304.

Kristeller, J. L. and R. Q. Wolever (2011). "Mindfulness-based eating awareness training for treating binge eating disorder: the conceptual foundation." Eat Disord **19**(1): 49-61.

Kruseman, M., A. Leimgruber, et al. (2010). "Dietary, weight, and psychological changes among patients with obesity, 8 years after gastric bypass." J Am Diet Assoc **110**(4): 527-534.

Kuhn, S., F. Schubert, et al. (2011). "Structural correlates of trait anxiety: reduced thickness in medial orbitofrontal cortex accompanied by volume increase in nucleus accumbens." J Affect Disord **134**(1-3): 315-319.

Kullmann, S., M. Heni, et al. (2012). "The obese brain: association of body mass index and insulin sensitivity with resting state network functional connectivity." Hum Brain Mapp **33**(5): 1052-1061.

Kullmann, S., A. A. Pape, et al. (2012). "Functional Network Connectivity Underlying Food Processing: Disturbed Salience and Visual Processing in Overweight and Obese Adults." Cereb Cortex.

Kumar, R., J. C. Lieske, et al. (2011). "Fat malabsorption and increased intestinal oxalate absorption are common after Roux-en-Y gastric bypass surgery." Surgery **149**(5): 654-661.

Kuo, Y. T., J. R. Parkinson, et al. (2007). "The temporal sequence of gut peptide CNS interactions tracked in vivo by magnetic resonance imaging." J Neurosci **27**(45): 12341-12348.

Kurth, F., J. G. Levitt, et al. (2012). "Relationships between gray matter, body mass index, and waist circumference in healthy adults." Hum Brain Mapp.

LaBar, K. S., D. R. Gitelman, et al. (2001). "Hunger selectively modulates corticolimbic activation to food stimuli in humans." Behav Neurosci **115**(2): 493-500.

Laferrere, B. (2011). "Diabetes remission after bariatric surgery: is it just the incretins?" Int J Obes (Lond) **35 Suppl 3**: S22-25.

Laferrere, B. (2011). "Do we really know why diabetes remits after gastric bypass surgery?" Endocrine **40**(2): 162-167.

Laferrere, B., D. Reilly, et al. (2011). "Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss." Sci Transl Med **3**(80): 80re82.

Laferrere, B., J. Teixeira, et al. (2008). "Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes." J Clin Endocrinol Metab **93**(7): 2479-2485.

Lagerros, Y. T. and S. Rossner (2013). "Obesity management: what brings success?" Therap Adv Gastroenterol **6**(1): 77-88.

Lai, C. H. (2013). "Gray matter volume in major depressive disorder: a meta-analysis of voxel-based morphometry studies." Psychiatry Res **211**(1): 37-46.

Lang, T., R. Hauser, et al. (2002). "Impact of gastric banding on eating behavior and weight." Obes Surg **12**(1): 100-107.

Langer, F. B., M. A. Reza Hoda, et al. (2005). "Sleeve gastrectomy and gastric banding: effects on plasma ghrelin levels." Obes Surg **15**(7): 1024-1029.

Lappalainen, R., P. O. Sjoden, et al. (1990). "Hunger/craving responses and reactivity to food stimuli during fasting and dieting." Int J Obes **14**(8): 679-688.

Larsen, T. M., S. Dalskov, et al. (2010). "The Diet, Obesity and Genes (Diogenes) Dietary Study in eight European countries - a comprehensive design for long-term intervention." Obes Rev **11**(1): 76-91.

Laurenius, A., I. Larsson, et al. (2012). "Changes in eating behaviour and meal pattern following Roux-en-Y gastric bypass." Int J Obes (Lond) **36**(3): 348-355.

Lavin, J. H., G. Wittert, et al. (1996). "Appetite regulation by carbohydrate: role of blood glucose and gastrointestinal hormones." Am J Physiol **271**(2 Pt 1): E209-214.

Le, D. S., N. Pannacciulli, et al. (2006). "Less activation of the left dorsolateral prefrontal cortex in response to a meal: a feature of obesity." Am J Clin Nutr **84**(4): 725-731.

Le, D. S., N. Pannacciulli, et al. (2007). "Less activation in the left dorsolateral prefrontal cortex in the reanalysis of the response to a meal in obese than in lean women and its association with successful weight loss." Am J Clin Nutr **86**(3): 573-579.

le Roux, C. W., S. J. Aylwin, et al. (2006). "Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters." Ann Surg **243**(1): 108-114.

le Roux, C. W., R. L. Batterham, et al. (2006). "Attenuated peptide YY release in obese subjects is associated with reduced satiety." Endocrinology **147**(1): 3-8.

le Roux, C. W. and S. R. Bloom (2005). "Peptide YY, appetite and food intake." Proc Nutr Soc **64**(2): 213-216.

le Roux, C. W. and S. R. Bloom (2005). "Why do patients lose weight after Roux-en-Y gastric bypass?" J Clin Endocrinol Metab **90**(1): 591-592.

le Roux, C. W., C. Borg, et al. (2010). "Gut hypertrophy after gastric bypass is associated with increased glucagon-like peptide 2 and intestinal crypt cell proliferation." Ann Surg **252**(1): 50-56.

le Roux, C. W., M. Bueter, et al. (2011). "Gastric bypass reduces fat intake and preference." Am J Physiol Regul Integr Comp Physiol **301**(4): R1057-1066.

le Roux, C. W., M. Patterson, et al. (2005). "Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects." J Clin Endocrinol Metab **90**(2): 1068-1071.

le Roux, C. W., R. Welbourn, et al. (2007). "Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass." Ann Surg **246**(5): 780-785.

Lee, T. Y., S. N. Kim, et al. (2013). "Neural correlate of impulsivity in subjects at ultra-high risk for psychosis." Prog Neuropsychopharmacol Biol Psychiatry **45**: 165-169.

Lee, W. J., C. Y. Chen, et al. (2011). "Changes in postprandial gut hormones after metabolic surgery: a comparison of gastric bypass and sleeve gastrectomy." Surg Obes Relat Dis **7**(6): 683-690.

Leonetti, F., G. Silecchia, et al. (2003). "Different plasma ghrelin levels after laparoscopic gastric bypass and adjustable gastric banding in morbid obese subjects." J Clin Endocrinol Metab **88**(9): 4227-4231.

Ley, R. E., P. J. Turnbaugh, et al. (2006). "Microbial ecology: human gut microbes associated with obesity." Nature **444**(7122): 1022-1023.

Li, J. V., H. Ashrafian, et al. (2011). "Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk." Gut **60**(9): 1214-1223.

Liddle, P. F., K. A. Kiehl, et al. (2001). "Event-related fMRI study of response inhibition." Hum Brain Mapp **12**(2): 100-109.

Lieverse, R. J., J. B. Jansen, et al. (1995). "Effects of somatostatin on human satiety." Neuroendocrinology **61**(2): 112-116.

Lim, E. L., K. G. Hollingsworth, et al. (2011). "Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol." Diabetologia **54**(10): 2506-2514.

Lin, E., N. Gletsu, et al. (2004). "The effects of gastric surgery on systemic ghrelin levels in the morbidly obese." Arch Surg **139**(7): 780-784.

Liou, A. P., M. Paziuk, et al. (2013). "Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity." Sci Transl Med **5**(178): 178ra141.

Lissner, L. (2002). "Measuring food intake in studies of obesity." Public Health Nutr **5**(6A): 889-892.

Liu, J., C. E. Prudom, et al. (2008). "Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men." J Clin Endocrinol Metab **93**(5): 1980-1987.

Liu, W. Y., B. Weber, et al. (2013). "The Big Five of Personality and structural imaging revisited: a VBM - DARTEL study." Neuroreport **24**(7): 375-380.

Liu, X., A. Lagoy, et al. (2012). "Metabolic and Neuroendocrine Responses to Roux-en-Y Gastric Bypass. I: Energy Balance, Metabolic Changes, and Fat Loss." J Clin Endocrinol Metab **97**(8): E1440-1450.

Livhits, M., C. Mercado, et al. (2012). "Preoperative predictors of weight loss following bariatric surgery: systematic review." Obes Surg **22**(1): 70-89.

Lofgren, P., I. Andersson, et al. (2005). "Long-term prospective and controlled studies demonstrate adipose tissue hypercellularity and relative leptin deficiency in the postobese state." J Clin Endocrinol Metab **90**(11): 6207-6213.

Lopresti, A. L. and P. D. Drummond (2013). "Obesity and psychiatric disorders: Commonalities in dysregulated biological pathways and their implications for treatment." Prog Neuropsychopharmacol Biol Psychiatry **45C**: 92-99.

Lowe, M. R., M. L. Butryn, et al. (2009). "The Power of Food Scale. A new measure of the psychological influence of the food environment." Appetite **53**(1): 114-118.

Lowe, M. R., J. van Steenburgh, et al. (2009). "Neural correlates of individual differences related to appetite." Physiol Behav **97**(5): 561-571.

Luchtman, D. W. and C. Song (2013). "Cognitive enhancement by omega-3 fatty acids from child-hood to old age: findings from animal and clinical studies." Neuropharmacology **64**: 550-565.

Luppino, F. S., L. M. de Wit, et al. (2010). "Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies." Arch Gen Psychiatry **67**(3): 220-229.

Maayan, L., C. Hoogendoorn, et al. (2011). "Disinhibited eating in obese adolescents is associated with orbitofrontal volume reductions and executive dysfunction." Obesity (Silver Spring) **19**(7): 1382-1387.

MacDonald, A. F., C. J. Billington, et al. (2003). "Effects of the opioid antagonist naltrexone on feeding induced by DAMGO in the ventral tegmental area and in the nucleus accumbens shell region in the rat." Am J Physiol Regul Integr Comp Physiol **285**(5): R999-R1004.

MacDonald, A. F., C. J. Billington, et al. (2004). "Alterations in food intake by opioid and dopamine signaling pathways between the ventral tegmental area and the shell of the nucleus accumbens." Brain Res **1018**(1): 78-85.

MacLean, L. D., B. M. Rhode, et al. (2001). "Long- or short-limb gastric bypass?" J Gastrointest Surg **5**(5): 525-530.

Malik, S., F. McGlone, et al. (2008). "Ghrelin modulates brain activity in areas that control appetitive behavior." Cell Metab **7**(5): 400-409.

Maller, J. J., R. H. Thomson, et al. (2010). "Traumatic brain injury, major depression, and diffusion tensor imaging: making connections." Brain Res Rev **64**(1): 213-240.

Mallory, G. N., A. M. Macgregor, et al. (1996). "The Influence of Dumping on Weight Loss After Gastric Restrictive Surgery for Morbid Obesity." Obes Surg **6**(6): 474-478.

Marinella, M. A. (2008). "Anemia following Roux-en-Y surgery for morbid obesity: a review." South Med J **101**(10): 1024-1031.

Marrazzi, M. A., J. P. Bacon, et al. (1995). "Naltrexone use in the treatment of anorexia nervosa and bulimia nervosa." Int Clin Psychopharmacol **10**(3): 163-172.

Marrazzi, M. A., J. Kinzie, et al. (1995). "A detailed longitudinal analysis on the use of naltrexone in the treatment of bulimia." Int Clin Psychopharmacol **10**(3): 173-176.

Martel, P. and M. Fantino (1996). "Mesolimbic dopaminergic system activity as a function of food reward: a microdialysis study." Pharmacol Biochem Behav **53**(1): 221-226.

Martin, B., C. D. Dotson, et al. (2009). "Modulation of taste sensitivity by GLP-1 signaling in taste buds." Ann N Y Acad Sci **1170**: 98-101.

Martin, C. K., P. M. O'Neil, et al. (2006). "Changes in food cravings during low-calorie and very-low-calorie diets." Obesity (Silver Spring) **14**(1): 115-121.

Martin, L. E., L. M. Holsen, et al. (2010). "Neural mechanisms associated with food motivation in obese and healthy weight adults." Obesity (Silver Spring) **18**(2): 254-260.

Mason, E. E. and C. Ito (1969). "Gastric bypass." Ann Surg **170**(3): 329-339.

Mathes, C. M., M. Bueter, et al. (2012). "Roux-en-Y gastric bypass in rats increases sucrose taste-related motivated behavior independent of pharmacological GLP-1-receptor modulation." Am J Physiol Regul Integr Comp Physiol **302**(6): R751-767.

Mathes, C. M. and A. C. Spector (2012). "Food selection and taste changes in humans after Roux-en-Y gastric bypass surgery: a direct-measures approach." Physiol Behav **107**(4): 476-483.

Mathes, W. F., K. A. Brownley, et al. (2009). "The biology of binge eating." Appetite **52**(3): 545-553.

Matochik, J. A., E. D. London, et al. (2005). "Effect of leptin replacement on brain structure in genetically leptin-deficient adults." J Clin Endocrinol Metab **90**(5): 2851-2854.

Matsuo, K., M. Nicoletti, et al. (2009). "A voxel-based morphometry study of frontal gray matter correlates of impulsivity." Hum Brain Mapp **30**(4): 1188-1195.

McCaffery, J. M., A. P. Haley, et al. (2009). "Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls." Am J Clin Nutr **90**(4): 928-934.

McGuire, M. T., R. R. Wing, et al. (1999). "Behavioral strategies of individuals who have maintained long-term weight losses." Obes Res **7**(4): 334-341.

McIntyre, R. S., H. A. Kenna, et al. (2010). "Brain volume abnormalities and neurocognitive deficits in diabetes mellitus: points of pathophysiological commonality with mood disorders?" Adv Ther **27**(2): 63-80.

McKinnon, M. C., K. Yucel, et al. (2009). "A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder." J Psychiatry Neurosci **34**(1): 41-54.

McLaughlin, T., M. Peck, et al. (2010). "Reversible hyperinsulinemic hypoglycemia after gastric bypass: a consequence of altered nutrient delivery." J Clin Endocrinol Metab **95**(4): 1851-1855.

McPherson, K., T. Marsh, et al. (2007). "Foresight report on obesity." Lancet **370**(9601): 1755; author reply 1755.

Mehta, M. A., N. I. Golembo, et al. (2009). "Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot." J Child Psychol Psychiatry **50**(8): 943-951.

Mela, D. J. (2001). "Determinants of food choice: relationships with obesity and weight control." Obes Res **9 Suppl 4**: 249S-255S.

Melka, M. G., J. Gillis, et al. (2013). "FTO, obesity and the adolescent brain." Hum Mol Genet **22**(5): 1050-1058.

Meule, A., C. Vogele, et al. (2012). "Restrained eating is related to accelerated reaction to high caloric foods and cardiac autonomic dysregulation." Appetite **58**(2): 638-644.

Middleton, K. M., S. M. Patidar, et al. (2012). "The impact of extended care on the long-term maintenance of weight loss: a systematic review and meta-analysis." Obes Rev **13**(6): 509-517.

Miller, J. L., J. Couch, et al. (2009). "Early childhood obesity is associated with compromised cerebellar development." Dev Neuropsychol **34**(3): 272-283.

Miller, J. L., J. A. Couch, et al. (2007). "Sylvian fissure morphology in Prader-Willi syndrome and early-onset morbid obesity." Genet Med **9**(8): 536-543.

Miller, J. L., A. P. Goldstone, et al. (2008). "Pituitary abnormalities in Prader-Willi syndrome and early onset morbid obesity." Am J Med Genet A **146A**(5): 570-577.

Miller, J. L., G. A. James, et al. (2007). "Enhanced activation of reward mediating prefrontal regions in response to food stimuli in Prader-Willi syndrome." J Neurol Neurosurg Psychiatry **78**(6): 615-619.

Miras, A. D., R. N. Jackson, et al. (2012). "Gastric bypass surgery for obesity decreases the reward value of a sweet-fat stimulus as assessed in a progressive ratio task." Am J Clin Nutr **96**(3): 467-473.

Miras, A. D. and C. W. le Roux (2010). "Bariatric surgery and taste: novel mechanisms of weight loss." Curr Opin Gastroenterol **26**(2): 140-145.

Miras, A. D. and C. W. le Roux (2013). "Mechanisms underlying weight loss after bariatric surgery." Nat Rev Gastroenterol Hepatol.

Mistiaen, W., W. Vaneerdeweg, et al. (2000). "Gastric emptying rate measurement after vertical banded gastroplasty." Obes Surg **10**(3): 245-249.

Mitchell, J. E., G. Christenson, et al. (1989). "A placebo-controlled, double-blind crossover study of naltrexone hydrochloride in outpatients with normal weight bulimia." J Clin Psychopharmacol **9**(2): 94-97.

Mitchell, J. E., J. E. Morley, et al. (1987). "High-dose naltrexone therapy and dietary counseling for obesity." Biol Psychiatry **22**(1): 35-42.

Mitterschiffthaler, M. T., U. Ettinger, et al. (2006). "Applications of functional magnetic resonance imaging in psychiatry." J Magn Reson Imaging **23**(6): 851-861.

Modlin, I. M., M. Pavel, et al. (2010). "Review article: somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours." Aliment Pharmacol Ther **31**(2): 169-188.

Molteni, R., R. J. Barnard, et al. (2002). "A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning." Neuroscience **112**(4): 803-814.

Monteiro, M., J. Monteiro, et al. (2006). "A Rat Model of Restrictive Bariatric Surgery with Gastric Banding." Obesity Surgery **16**(1): 48-51.

Monteiro, M., J. Monteiro, et al. (2006). "Rats Submitted to Gastric Banding are Leaner and Show Distinctive Feeding Patterns." Obesity Surgery **16**(5): 597-602.

Moreira, R. O., R. B. Moreira, et al. (2008). "Post-prandial hypoglycemia after bariatric surgery: pharmacological treatment with verapamil and acarbose." Obes Surg **18**(12): 1618-1621.

Moreno-Lopez, L., C. Soriano-Mas, et al. (2012). "Brain structural correlates of reward sensitivity and impulsivity in adolescents with normal and excess weight." PLoS One **7**(11): e49185.

Morinigo, R., R. Casamitjana, et al. (2004). "Short-term effects of gastric bypass surgery on circulating ghrelin levels." Obes Res **12**(7): 1108-1116.

Morinigo, R., V. Moize, et al. (2006). "Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects." J Clin Endocrinol Metab **91**(5): 1735-1740.

Moriya, R., S. Mashiko, et al. (2009). "Comparison of independent and combined chronic anti-obese effects of NPY Y2 receptor agonist, PYY(3-36), and NPY Y5 receptor antagonist in diet-induced obese mice." Peptides **30**(7): 1318-1322.

Mostofsky, S. H., J. G. Schafer, et al. (2003). "fMRI evidence that the neural basis of response inhibition is task-dependent." Brain Res Cogn Brain Res **17**(2): 419-430.

Mueller, K., A. Anwander, et al. (2011). "Sex-dependent influences of obesity on cerebral white matter investigated by diffusion-tensor imaging." PLoS One **6**(4): e18544.

Murdaugh, D. L., J. E. Cox, et al. (2012). "fMRI reactivity to high-calorie food pictures predicts short- and long-term outcome in a weight-loss program." NeuroImage **59**(3): 2709-2721.

Murray, E. A. (2007). "The amygdala, reward and emotion." Trends Cogn Sci **11**(11): 489-497.

Myint, A. M. (2012). "Kynurenines: from the perspective of major psychiatric disorders." FEBS J **279**(8): 1375-1385.

Myint, K. S., J. R. Greenfield, et al. (2012). "Prolonged successful therapy for hyperinsulinaemic hypoglycaemia after gastric bypass: the pathophysiological role of GLP1 and its response to a somatostatin analogue." Eur J Endocrinol **166**(5): 951-955.

Myslobodsky, M. (2003). "Gourmand savants and environmental determinants of obesity." Obes Rev **4**(2): 121-128.

Nakatani, H., K. Kasama, et al. (2009). "Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery." Metabolism **58**(10): 1400-1407.

Nakazato, M., N. Murakami, et al. (2001). "A role for ghrelin in the central regulation of feeding." Nature **409**(6817): 194-198.

Nannipieri, M., A. Mari, et al. (2011). "The role of beta-cell function and insulin sensitivity in the remission of type 2 diabetes after gastric bypass surgery." J Clin Endocrinol Metab **96**(9): E1372-1379.

Narayanaswamy, J. C., D. Jose, et al. (2013). "Clinical correlates of nucleus accumbens volume in drug-naive, adult patients with obsessive-compulsive disorder." Aust N Z J Psychiatry.

Narita, K., H. Kosaka, et al. (2009). "Relationship between plasma leptin level and brain structure in elderly: a voxel-based morphometric study." Biol Psychiatry **65**(11): 992-994.

Naslund, E., B. Barkeling, et al. (1999). "Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men." Int J Obes Relat Metab Disord **23**(3): 304-311.

Naslund, I. (1987). "Gastric bypass versus gastroplasty. A prospective study of differences in two surgical procedures for morbid obesity." Acta Chir Scand Suppl **536**: 1-60.

Nasser, J. A., S. M. Evans, et al. (2008). "Use of an operant task to estimate food reinforcement in adult humans with and without BED." Obesity (Silver Spring) **16**(8): 1816-1820.

Neary, M. T. and R. L. Batterham (2010). "Gaining new insights into food reward with functional neuroimaging." Forum Nutr **63**: 152-163.

Neary, N. M., M. R. Druce, et al. (2006). "Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect." Gut **55**(1): 135.

Neary, N. M., C. J. Small, et al. (2004). "Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial." J Clin Endocrinol Metab **89**(6): 2832-2836.

Nederkoorn, C., C. Braet, et al. (2006). "Why obese children cannot resist food: the role of impulsivity." Eat Behav **7**(4): 315-322.

Ng, J., E. Stice, et al. (2011). "An fMRI study of obesity, food reward, and perceived caloric density. Does a low-fat label make food less appealing?" Appetite **57**(1): 65-72.

Nichols, T. E. and A. P. Holmes (2002). "Nonparametric permutation tests for functional neuroimaging: a primer with examples." Hum Brain Mapp **15**(1): 1-25.

Niego, S. H., M. D. Kofman, et al. (2007). "Binge eating in the bariatric surgery population: a review of the literature." Int J Eat Disord **40**(4): 349-359.

Nijs, I. M., I. H. Franken, et al. (2010). "Food-related Stroop interference in obese and normal-weight individuals: behavioral and electrophysiological indices." Eat Behav **11**(4): 258-265.

Nisbett, R. E. (1968). "Determinants of food intake in obesity." Science **159**(3820): 1254-1255.

Noble, E. P. (2000). "Addiction and its reward process through polymorphisms of the D2 dopamine receptor gene: a review." Eur Psychiatry **15**(2): 79-89.

Noble, E. P., L. A. Gottschalk, et al. (1997). "D2 dopamine receptor polymorphism and brain regional glucose metabolism." Am J Med Genet **74**(2): 162-166.

Nummenmaa, L., J. Hirvonen, et al. (2012). "Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity." PLoS One **7**(2): e31089.

O'Brien, P. E. (2010). "Bariatric surgery: mechanisms, indications and outcomes." J Gastroenterol Hepatol **25**(8): 1358-1365.

Obermann, M., R. Rodriguez-Raecke, et al. (2013). "Gray matter volume reduction reflects chronic pain in trigeminal neuralgia." Neuroimage **74**: 352-358.

Ochner, C. N., Y. Kwok, et al. (2010). "Selective Reduction in Neural Responses to High Calorie Foods Following Gastric Bypass Surgery." Ann Surg.

Ochner, C. N., Y. Kwok, et al. (2011). "Selective reduction in neural responses to high calorie foods following gastric bypass surgery." Ann Surg **253**(3): 502-507.

Ochner, C. N., B. Laferrere, et al. (2012). "Neural responsivity to food cues in fasted and fed states pre and post gastric bypass surgery." Neurosci Res **74**(2): 138-143.

Ochner, C. N., E. Stice, et al. (2012). "Relation between changes in neural responsivity and reductions in desire to eat high-calorie foods following gastric bypass surgery." Neuroscience **209**: 128-135.

Odstrcil, E. A., J. G. Martinez, et al. (2010). "The contribution of malabsorption to the reduction in net energy absorption after long-limb Roux-en-Y gastric bypass." Am J Clin Nutr **92**(4): 704-713.

Ogundare, M., S. Theofilopoulos, et al. (2010). "Cerebrospinal fluid steroidomics: are bioactive bile acids present in brain?" J Biol Chem **285**(7): 4666-4679.

Ogura, K., T. Fujii, et al. (2011). "Small gray matter volume in orbitofrontal cortex in Prader-Willi syndrome: a voxel-based MRI study." Hum Brain Mapp **32**(7): 1059-1066.

Olbers, T., S. Bjorkman, et al. (2006). "Body composition, dietary intake, and energy expenditure after laparoscopic Roux-en-Y gastric bypass and laparoscopic vertical banded gastroplasty: a randomized clinical trial." Ann Surg **244**(5): 715-722.

Olbers, T., H. Lonroth, et al. (2003). "Laparoscopic gastric bypass: development of technique, respiratory function, and long-term outcome." Obes Surg **13**(3): 364-370.

Oliver, G., J. Wardle, et al. (2000). "Stress and food choice: a laboratory study." Psychosom Med **62**(6): 853-865.

Olson, E. A., P. F. Collins, et al. (2009). "White matter integrity predicts delay discounting behavior in 9- to 23-year-olds: a diffusion tensor imaging study." J Cogn Neurosci **21**(7): 1406-1421.

Olszewski, P. K. and A. S. Levine (2007). "Central opioids and consumption of sweet tastants: when reward outweighs homeostasis." Physiol Behav **91**(5): 506-512.

Orsi, G., G. Perlaki, et al. (2011). "Body weight and the reward system: the volume of the right amygdala may be associated with body mass index in young overweight men." Brain Imaging Behav **5**(2): 149-157.

Ostlund, M. P., O. Backman, et al. (2013). "Increased admission for alcohol dependence after gastric bypass surgery compared with restrictive bariatric surgery." JAMA Surg **148**(4): 374-377.

Osto, M., K. Abegg, et al. (2013). "Roux-en-y gastric bypass surgery in rats alters gut microbiota profile along the intestine." Physiol Behav.

Padoin, A. V., M. Galvao Neto, et al. (2009). "Obese patients with type 2 diabetes submitted to banded gastric bypass: greater incidence of dumping syndrome." Obes Surg **19**(11): 1481-1484.

Padwal, R., S. Klarenbach, et al. (2011). "Bariatric surgery: a systematic review of the clinical and economic evidence." J Gen Intern Med **26**(10): 1183-1194.

Padwal, R., S. K. Li, et al. (2004). "Long-term pharmacotherapy for obesity and overweight." Cochrane Database Syst Rev(3): CD004094.

Palacios, E. M., R. Sala-Llonch, et al. (2013). "Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury." JAMA Neurol: 1-7.

Pan, P., H. Shi, et al. (2012). "Chronic smoking and brain gray matter changes: evidence from meta-analysis of voxel-based morphometry studies." Neurol Sci.

Pan, P. L., H. C. Shi, et al. (2012). "Gray matter atrophy in Parkinson's disease with dementia: evidence from meta-analysis of voxel-based morphometry studies." Neurol Sci.

Pannacciulli, N., A. Del Parigi, et al. (2006). "Brain abnormalities in human obesity: a voxel-based morphometric study." Neuroimage **31**(4): 1419-1425.

Pannacciulli, N., D. S. Le, et al. (2007). "Relationships between plasma leptin concentrations and human brain structure: a voxel-based morphometric study." Neurosci Lett **412**(3): 248-253.

Park, S. Q., T. Kahnt, et al. (2010). "Prefrontal cortex fails to learn from reward prediction errors in alcohol dependence." J Neurosci **30**(22): 7749-7753.

Parkinson, C., W. M. Drake, et al. (2002). "A comparison of the effects of pegvisomant and octreotide on glucose, insulin, gastrin, cholecystokinin, and pancreatic polypeptide responses to oral glucose and a standard mixed meal." J Clin Endocrinol Metab **87**(4): 1797-1804.

Parkinson, J. R., O. B. Chaudhri, et al. (2009). "Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxyntomodulin and lithium chloride in mice detected by manganese-enhanced magnetic resonance imaging (MEMRI)." Neuroimage **44**(3): 1022-1031.

Pasco, J. A., L. J. Williams, et al. (2013). "Obesity and the relationship with positive and negative affect." Aust N Z J Psychiatry.

Passamonti, L., J. B. Rowe, et al. (2009). "Personality predicts the brain's response to viewing appetizing foods: the neural basis of a risk factor for overeating." J Neurosci **29**(1): 43-51.

Patenaude, B., S. M. Smith, et al. (2011). "A Bayesian model of shape and appearance for subcortical brain segmentation." Neuroimage **56**(3): 907-922.

Paton, J. J., M. A. Belova, et al. (2006). "The primate amygdala represents the positive and negative value of visual stimuli during learning." Nature **439**(7078): 865-870.

Patti, M. E., S. M. Houten, et al. (2009). "Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism." Obesity (Silver Spring) **17**(9): 1671-1677.

Patton, J. H., M. S. Stanford, et al. (1995). "Factor structure of the Barratt impulsiveness scale." J Clin Psychol **51**(6): 768-774.

Pecina, S. and K. C. Berridge (2000). "Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: map based on microinjection Fos plumes." Brain Res **863**(1-2): 71-86.

Pecina, S. and K. C. Berridge (2005). "Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness?" J Neurosci **25**(50): 11777-11786.

Peng, Z., S. S. Lui, et al. (2012). "Brain structural abnormalities in obsessive-compulsive disorder: converging evidence from white matter and grey matter." Asian J Psychiatr **5**(4): 290-296.

Peper, J. S., R. C. Mandl, et al. (2013). "Delay discounting and frontostriatal fiber tracts: a combined DTI and MTR study on impulsive choices in healthy young adults." Cereb Cortex **23**(7): 1695-1702.

Perello, M., I. Sakata, et al. (2010). "Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner." Biol Psychiatry **67**(9): 880-886.

Peters, A., A. Bosy-Westphal, et al. (2011). "Why doesn't the brain lose weight, when obese people diet?" Obes Facts **4**(2): 151-157.

Picot, J., J. Jones, et al. (2009). "The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation." Health Technol Assess **13**(41): 1-190, 215-357, iii-iv.

Pike, G. B. (2012). "Quantitative functional MRI: concepts, issues and future challenges." Neuroimage **62**(2): 1234-1240.

Piras, F., F. Piras, et al. (2013). "Widespread structural brain changes in OCD: A systematic review of voxel-based morphometry studies." Cortex.

Pliner, P. L. (1973). "Effect of external cues on the thinking behavior of obese and normal subjects." J Abnorm Psychol **82**(2): 233-238.

Poldrack, R. A. (2006). "Can cognitive processes be inferred from neuroimaging data?" Trends Cogn Sci **10**(2): 59-63.

Polivy, J. and C. P. Herman (1999). "The effects of resolving to diet on restrained and unrestrained eaters: the "false hope syndrome"." Int J Eat Disord **26**(4): 434-447.

Polivy, J., C. P. Herman, et al. (2008). "Caloric restriction in the presence of attractive food cues: external cues, eating, and weight." Physiol Behav **94**(5): 729-733.

Pontiroli, A. E., F. Frige, et al. (2009). "In morbid obesity, metabolic abnormalities and adhesion molecules correlate with visceral fat, not with subcutaneous fat: effect of weight loss through surgery." Obes Surg **19**(6): 745-750.

Pontiroli, A. E. and A. Morabito (2011). "Long-term prevention of mortality in morbid obesity through bariatric surgery. a systematic review and meta-analysis of trials performed with gastric banding and gastric bypass." Ann Surg **253**(3): 484-487.

Pories, W. J., E. G. Flickinger, et al. (1982). "The effectiveness of gastric bypass over gastric partition in morbid obesity: consequence of distal gastric and duodenal exclusion." Ann Surg **196**(4): 389-399.

Porubska, K., R. Veit, et al. (2006). "Subjective feeling of appetite modulates brain activity: an fMRI study." Neuroimage **32**(3): 1273-1280.

Potter, G. M., A. Moshirfar, et al. (1999). "Insulin affects dopamine overflow in the nucleus accumbens and the striatum." Physiol Behav **65**(4-5): 811-816.

Pournaras, D. J., E. T. Aasheim, et al. (2012). "Effect of bypassing the proximal gut on gut hormones involved with glycemic control and weight loss." Surg Obes Relat Dis **8**(4): 371-374.

Pournaras, D. J., C. Glicksman, et al. (2012). "The Role of Bile After Roux-en-Y Gastric Bypass in Promoting Weight Loss and Improving Glycaemic Control." Endocrinology **153**(8): 3613-3619.

Pournaras, D. J. and C. W. le Roux (2009). "Obesity, gut hormones, and bariatric surgery." World J Surg **33**(10): 1983-1988.

Pournaras, D. J., A. Osborne, et al. (2010). "The gut hormone response following Roux-en-Y gastric bypass: cross-sectional and prospective study." Obes Surg **20**(1): 56-60.

Pournaras, D. J., A. Osborne, et al. (2010). "Remission of type 2 diabetes after gastric bypass and banding: mechanisms and 2 year outcomes." Ann Surg **252**(6): 966-971.

Provencher, V., V. Drapeau, et al. (2003). "Eating behaviors and indexes of body composition in men and women from the Quebec family study." Obes Res **11**(6): 783-792.

Punjabi, M., M. Arnold, et al. (2011). "Peripheral glucagon-like peptide-1 (GLP-1) and satiation." Physiol Behav **105**(1): 71-76.

Raji, C. A., A. J. Ho, et al. (2010). "Brain structure and obesity." Hum Brain Mapp **31**(3): 353-364.

Rao, R. S. and S. Kini (2012). "Diabetic and bariatric surgery: a review of the recent trends." Surg Endosc **26**(4): 893-903.

Rao, R. S., V. Rao, et al. (2010). "Animal models in bariatric surgery--a review of the surgical techniques and postsurgical physiology." Obes Surg **20**(9): 1293-1305.

Raynor, H. A. and L. H. Epstein (2003). "The relative-reinforcing value of food under differing levels of food deprivation and restriction." Appetite **40**(1): 15-24.

Reidelberger, R. D., A. C. Haver, et al. (2013). "Role of Peptide YY(3-36) in the Satiety Produced by Gastric Delivery of Macronutrients in Rats." Am J Physiol Endocrinol Metab.

Ridgway, G. R., S. M. Henley, et al. (2008). "Ten simple rules for reporting voxel-based morphometry studies." Neuroimage **40**(4): 1429-1435.

Riediger, T., C. Bothe, et al. (2004). "Peptide YY directly inhibits ghrelin-activated neurons of the arcuate nucleus and reverses fasting-induced c-Fos expression." Neuroendocrinology **79**(6): 317-326.

Rissanen, A., P. Hakala, et al. (2002). "Acquired preference especially for dietary fat and obesity: a study of weight-discordant monozygotic twin pairs." Int J Obes Relat Metab Disord **26**(7): 973-977.

Ritz, P. and H. Hanaire (2011). "Post-bypass hypoglycaemia: a review of current findings." Diabetes Metab **37**(4): 274-281.

Rodgers, R. J., M. H. Tschop, et al. (2012). "Anti-obesity drugs: past, present and future." Dis Model Mech **5**(5): 621-626.

Rodin, J., D. Schank, et al. (1989). "Psychological features of obesity." Med Clin North Am **73**(1): 47-66.

Roefs, A. and A. Jansen (2002). "Implicit and explicit attitudes toward high-fat foods in obesity." J Abnorm Psychol **111**(3): 517-521.

Roemer, L. and S. M. Orsillo (2007). "An open trial of an acceptance-based behavior therapy for generalized anxiety disorder." Behav Ther **38**(1): 72-85.

Rogers, P. J. and H. J. Smit (2000). "Food craving and food "addiction": a critical review of the evidence from a biopsychosocial perspective." Pharmacol Biochem Behav **66**(1): 3-14.

Rolls, B. J., I. C. Fedoroff, et al. (1991). "Gender differences in eating behavior and body weight regulation." Health Psychol **10**(2): 133-142.

Rosenbaum, M., M. Sy, et al. (2008). "Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli." J Clin Invest **118**(7): 2583-2591.

Rosenthal, R. J., A. A. Diaz, et al. (2012). "International Sleeve Gastrectomy Expert Panel Consensus Statement: best practice guidelines based on experience of >12,000 cases." Surg Obes Relat Dis **8**(1): 8-19.

Rothemund, Y., C. Preuschhof, et al. (2007). "Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals." Neuroimage **37**(2): 410-421.

Rubino, F., M. Gagner, et al. (2004). "The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism." Ann Surg **240**(2): 236-242.

Rubino, F., P. R. Schauer, et al. (2010). "Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action." Annu Rev Med **61**: 393-411.

Ruderman, A. J. (1985). "Dysphoric mood and overeating: a test of restraint theory's disinhibition hypothesis." J Abnorm Psychol **94**(1): 78-85.

Ryan, K. K., R. Kohli, et al. (2013). "Fibroblast growth factor-19 action in the brain reduces food intake and body weight and improves glucose tolerance in male rats." Endocrinology **154**(1): 9-15.

Ryden, A., M. Sullivan, et al. (2003). "Severe obesity and personality: a comparative controlled study of personality traits." Int J Obes Relat Metab Disord **27**(12): 1534-1540.

Sabatinelli, D., M. M. Bradley, et al. (2007). "Pleasure rather than salience activates human nucleus accumbens and medial prefrontal cortex." J Neurophysiol **98**(3): 1374-1379.

Sadry, S. A. and D. J. Drucker (2013). "Emerging combinatorial hormone therapies for the treatment of obesity and T2DM." Nat Rev Endocrinol **9**(7): 425-433.

Saeidi, N., L. Meoli, et al. (2013). "Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass." Science **341**(6144): 406-410.

Sahin, M., A. Kartal, et al. (1999). "Effect of octreotide (Sandostatin 201-995) on bile flow and bile components." Dig Dis Sci **44**(1): 181-185.

Salbe, A. D., A. DelParigi, et al. (2004). "Taste preferences and body weight changes in an obesity-prone population." Am J Clin Nutr **79**(3): 372-378.

Salehi, M., R. L. Prigeon, et al. (2011). "Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans." Diabetes **60**(9): 2308-2314.

Sam, A. H., R. C. Troke, et al. (2012). "The role of the gut/brain axis in modulating food intake." Neuropharmacology **63**(1): 46-56.

Santiago, J. V. (1986). "Overview of the complications of diabetes." Clin Chem **32**(10 Suppl): B48-53.

Saper, C. B., T. C. Chou, et al. (2002). "The need to feed: homeostatic and hedonic control of eating." Neuron **36**(2): 199-211.

Sarwer, D. B., N. I. Cohn, et al. (2004). "Psychiatric diagnoses and psychiatric treatment among bariatric surgery candidates." Obes Surg **14**(9): 1148-1156.

Sarwer, D. B., T. A. Wadden, et al. (2010). "Changes in quality of life and body image after gastric bypass surgery." Surg Obes Relat Dis **6**(6): 608-614.

Sato, Y., K. Ito, et al. (2013). "Postoperative Increase in Cerebral White Matter Fractional Anisotropy on Diffusion Tensor Magnetic Resonance Imaging is Associated With Cognitive Improvement After Uncomplicated Carotid Endarterectomy: Tract-Based Spatial Statistics Analysis." Neurosurgery.

Schachter, S. (1968). "Obesity and eating. Internal and external cues differentially affect the eating behavior of obese and normal subjects." Science **161**(3843): 751-756.

Schafer, A., D. Vaitl, et al. (2010). "Regional grey matter volume abnormalities in bulimia nervosa and binge-eating disorder." Neuroimage **50**(2): 639-643.

Scharmuller, W., S. Ubel, et al. (2012). "Appetite regulation during food cue exposure: A comparison of normal-weight and obese women." Neurosci Lett **518**(2): 106-110.

Schienle, A., A. Schafer, et al. (2009). "Binge-eating disorder: reward sensitivity and brain activation to images of food." Biol Psychiatry **65**(8): 654-661.

Schindler, K., G. Prager, et al. (2004). "Impact of laparoscopic adjustable gastric banding on plasma ghrelin, eating behaviour and body weight." Eur J Clin Invest **34**(8): 549-554.

Scholtz, S., L. Bidlake, et al. (2007). "Long-term outcomes following laparoscopic adjustable gastric banding: postoperative psychological sequelae predict outcome at 5-year follow-up." Obes Surg **17**(9): 1220-1225.

Schultz, W. (2001). "Reward signaling by dopamine neurons." Neuroscientist **7**(4): 293-302.

Schur, E. A., N. M. Kleinhans, et al. (2009). "Activation in brain energy regulation and reward centers by food cues varies with choice of visual stimulus." Int J Obes (Lond) **33**(6): 653-661.

Schur, E. A., N. M. Kleinhans, et al. (2012). "Acquired differences in brain responses among monozygotic twins discordant for restrained eating." Physiol Behav **105**(2): 560-567.

Schwartz, M. W. and G. J. Morton (2002). "Obesity: keeping hunger at bay." Nature **418**(6898): 595-597.

Schwartz, M. W., S. C. Woods, et al. (2000). "Central nervous system control of food intake." Nature **404**(6778): 661-671.

Scruggs, D. M., C. Buffington, et al. (1994). "Taste Acuity of the Morbidly Obese before and after Gastric Bypass Surgery." Obes Surg **4**(1): 24-28.

See, R. E. (2002). "Neural substrates of conditioned-cued relapse to drug-seeking behavior." Pharmacol Biochem Behav **71**(3): 517-529.

Seeley, R. J., G. van Dijk, et al. (1996). "Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats." Horm Metab Res **28**(12): 664-668.

Segura, B., M. A. Jurado, et al. (2009). "Microstructural white matter changes in metabolic syndrome: a diffusion tensor imaging study." Neurology **73**(6): 438-444.

Service, G. J., G. B. Thompson, et al. (2005). "Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery." N Engl J Med **353**(3): 249-254.

Shapira, N. A., M. C. Lessig, et al. (2005). "Satiety dysfunction in Prader-Willi syndrome demonstrated by fMRI." J Neurol Neurosurg Psychiatry **76**(2): 260-262.

Shaw, K., P. O'Rourke, et al. (2005). "Psychological interventions for overweight or obesity." Cochrane Database Syst Rev(2): CD003818.

Shi, H. and D. J. Clegg (2009). "Sex differences in the regulation of body weight." Physiol Behav **97**(2): 199-204.

Shin, A. C. and H. R. Berthoud (2011). "Food reward functions as affected by obesity and bariatric surgery." Int J Obes (Lond) **35 Suppl 3**: S40-44.

Shin, A. C., R. L. Townsend, et al. (2011). ""Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition." Am J Physiol Regul Integr Comp Physiol **301**(5): R1267-1280.

Shin, A. C., H. Zheng, et al. (2011). "Roux-en-Y gastric bypass surgery changes food reward in rats." Int J Obes (Lond) **35**(5): 642-651.

Shin, A. C., H. Zheng, et al. (2010). "Meal-induced hormone responses in a rat model of Roux-en-Y gastric bypass surgery." Endocrinology **151**(4): 1588-1597.

Shin, Y. K., B. Martin, et al. (2008). "Modulation of taste sensitivity by GLP-1 signaling." J Neurochem **106**(1): 455-463.

Shriner, R. L. (2011). "Food as a bariatric drug." Curr Pharm Des **17**(12): 1198-1208.

Sigstad, H. (1970). "A clinical diagnostic index in the diagnosis of the dumping syndrome. Changes in plasma volume and blood sugar after a test meal." Acta Med Scand **188**(6): 479-486.

Simmonds, D. J., J. J. Pekar, et al. (2008). "Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent." Neuropsychologia **46**(1): 224-232.

Simmons, W. K., A. Martin, et al. (2005). "Pictures of appetizing foods activate gustatory cortices for taste and reward." Cereb Cortex **15**(10): 1602-1608.

Sjostrom, L. (2008). "Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study." Int J Obes (Lond) **32 Suppl 7**: S93-97.

Sjostrom, L. (2013). "Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery." J Intern Med **273**(3): 219-234.

Sjostrom, L., A. K. Lindroos, et al. (2004). "Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery." N Engl J Med **351**(26): 2683-2693.

Sjostrom, L., K. Narbro, et al. (2007). "Effects of bariatric surgery on mortality in Swedish obese subjects." N Engl J Med **357**(8): 741-752.

Sjostrom, L., M. Peltonen, et al. (2012). "Bariatric surgery and long-term cardiovascular events." JAMA **307**(1): 56-65.

Skibicka, K. P. and S. L. Dickson (2011). "Ghrelin and food reward: the story of potential underlying substrates." Peptides **32**(11): 2265-2273.

Skibicka, K. P., R. H. Shirazi, et al. (2012). "Ghrelin interacts with neuropeptide Y Y1 and opioid receptors to increase food reward." Endocrinology **153**(3): 1194-1205.

Sloth, B., J. J. Holst, et al. (2007). "Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects." Am J Physiol Endocrinol Metab **292**(4): E1062-1068.

Small, D. M. (2009). "Individual differences in the neurophysiology of reward and the obesity epidemic." Int J Obes (Lond) **33 Suppl 2**: S44-48.

Small, D. M., G. Bender, et al. (2007). "The role of the human orbitofrontal cortex in taste and flavor processing." Ann N Y Acad Sci **1121**: 136-151.

Small, D. M., R. J. Zatorre, et al. (2001). "Changes in brain activity related to eating chocolate: from pleasure to aversion." Brain **124**(Pt 9): 1720-1733.

Smeets, P. A., L. Charbonnier, et al. (2012). "Food-induced brain responses and eating behaviour." Proc Nutr Soc **71**(4): 511-520.

Smeets, P. A., C. de Graaf, et al. (2006). "Effect of satiety on brain activation during chocolate tasting in men and women." Am J Clin Nutr **83**(6): 1297-1305.

Smith, K. S. and K. C. Berridge (2007). "Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum." J Neurosci **27**(7): 1594-1605.

Smith, S. M. (2002). "Fast robust automated brain extraction." Hum Brain Mapp **17**(3): 143-155.

Smith, S. M., N. De Stefano, et al. (2001). "Normalized accurate measurement of longitudinal brain change." J Comput Assist Tomogr **25**(3): 466-475.

Smith, S. M., M. Jenkinson, et al. (2006). "Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data." Neuroimage **31**(4): 1487-1505.

Smith, S. M., Y. Zhang, et al. (2002). "Accurate, robust, and automated longitudinal and cross-sectional brain change analysis." Neuroimage **17**(1): 479-489.

Smucny, J., M. A. Cornier, et al. (2012). "Brain structure predicts risk for obesity." Appetite **59**(3): 859-865.

Soetens, B. and C. Braet (2007). "Information processing of food cues in overweight and normal weight adolescents." Br J Health Psychol **12**(Pt 2): 285-304.

Soussignan, R., B. Schaal, et al. (2012). "Orofacial reactivity to the sight and smell of food stimuli. Evidence for anticipatory liking related to food reward cues in overweight children." Appetite **58**(2): 508-516.

St-Onge, M. P., A. McReynolds, et al. (2012). "Sleep restriction leads to increased activation of brain regions sensitive to food stimuli." Am J Clin Nutr **95**(4): 818-824.

St-Onge, M. P., M. Sy, et al. (2005). "Human cortical specialization for food: a functional magnetic resonance imaging investigation." J Nutr **135**(5): 1014-1018.

Stanek, K. M., S. M. Grieve, et al. (2011). "Obesity is associated with reduced white matter integrity in otherwise healthy adults." Obesity (Silver Spring) **19**(3): 500-504.

Starke JA, A. M., Prechtl CG, Scholtz S, Miras AD, Chhina N, Sleeth M, Frost GS, Bell JD, Goldstone AP (2013). Salience resting state network integrity is increased in obesity and predicts orbitofrontal cortex activation to high-calorie food cues. 1st Annual Meeting of Society for the Study of Ingestive Behavior.

Stebbins, G. T. and C. M. Murphy (2009). "Diffusion tensor imaging in Alzheimer's disease and mild cognitive impairment." Behav Neurol **21**(1): 39-49.

Steele, K. E., G. P. Prokopowicz, et al. (2010). "Alterations of central dopamine receptors before and after gastric bypass surgery." Obes Surg **20**(3): 369-374.

Stefanidis, D., T. S. Kuwada, et al. (2011). "The importance of the length of the limbs for gastric bypass patients--an evidence-based review." Obes Surg **21**(1): 119-124.

Stefater, M. A., H. E. Wilson-Perez, et al. (2012). "All bariatric surgeries are not created equal: insights from mechanistic comparisons." Endocr Rev **33**(4): 595-622.

Steiner, G. (1981). "Diabetes and atherosclerosis: an overview." Diabetes **30**(Suppl 2): 1-7.

Stengel, A., M. Goebel, et al. (2010). "Activation of brain somatostatin 2 receptors stimulates feeding in mice: analysis of food intake microstructure." Physiol Behav **101**(5): 614-622.

Sterpenich, V., G. Albouy, et al. (2007). "Sleep-related hippocampo-cortical interplay during emotional memory recollection." PLoS Biol **5**(11): e282.

Stice, E., K. Burger, et al. (2013). "Caloric deprivation increases responsivity of attention and reward brain regions to intake, anticipated intake, and images of palatable foods." Neuroimage **67**: 322-330.

Stice, E., M. Fisher, et al. (2004). "Are dietary restraint scales valid measures of acute dietary restriction? Unobtrusive observational data suggest not." Psychol Assess **16**(1): 51-59.

Stice, E., S. Spoor, et al. (2008). "Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele." Science **322**(5900): 449-452.

Stice, E., S. Spoor, et al. (2008). "Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study." J Abnorm Psychol **117**(4): 924-935.

Stice, E., S. Spoor, et al. (2009). "Relation of obesity to consummatory and anticipatory food reward." Physiol Behav **97**(5): 551-560.

Stice, E., S. Yokum, et al. (2010). "Weight gain is associated with reduced striatal response to palatable food." J Neurosci **30**(39): 13105-13109.

Stice, E., S. Yokum, et al. (2010). "Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of DRD2 and DRD4." NeuroImage **50**(4): 1618-1625.

Stice, E., S. Yokum, et al. (2012). "Multilocus genetic composite reflecting dopamine signaling capacity predicts reward circuitry responsivity." J Neurosci **32**(29): 10093-10100.

Stice, E., S. Yokum, et al. (2011). "Youth at risk for obesity show greater activation of striatal and somatosensory regions to food." J Neurosci **31**(12): 4360-4366.

Stoeckel, L. E., J. Kim, et al. (2009). "Effective connectivity of a reward network in obese women." Brain Res Bull **79**(6): 388-395.

Stoeckel, L. E., R. E. Weller, et al. (2008). "Widespread reward-system activation in obese women in response to pictures of high-calorie foods." Neuroimage **41**(2): 636-647.

Stoeckli, R., R. Chanda, et al. (2004). "Changes of body weight and plasma ghrelin levels after gastric banding and gastric bypass." Obes Res **12**(2): 346-350.

Stubbs, R. J., D. A. Hughes, et al. (2000). "The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings." Br J Nutr **84**(4): 405-415.

Stunkard, A. J. and S. Messick (1985). "The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger." J Psychosom Res **29**(1): 71-83.

Stylopoulos, N., A. G. Hoppin, et al. (2009). "Roux-en-Y gastric bypass enhances energy expenditure and extends lifespan in diet-induced obese rats." Obesity (Silver Spring) **17**(10): 1839-1847.

Sugerman, H. J., J. V. Starkey, et al. (1987). "A randomized prospective trial of gastric bypass versus vertical banded gastroplasty for morbid obesity and their effects on sweets versus non-sweets eaters." Ann Surg **205**(6): 613-624.

Sumithran, P., L. A. Prendergast, et al. (2011). "Long-term persistence of hormonal adaptations to weight loss." N Engl J Med **365**(17): 1597-1604.

Sundbom, M., C. Holdstock, et al. (2007). "Early changes in ghrelin following Roux-en-Y gastric bypass: influence of vagal nerve functionality?" Obes Surg **17**(3): 304-310.

Suzuki, S., E. J. Ramos, et al. (2005). "Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model." Surgery **138**(2): 283-290.

Swarbrick, M. M., K. L. Stanhope, et al. (2008). "Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery." Diabetologia **51**(10): 1901-1911.

Sweeney, T. E. and J. M. Morton (2013). "The Human Gut Microbiome: A Review of the Effect of Obesity and Surgically Induced Weight Loss." JAMA Surg: 1-7.

Szczypka, M. S., K. Kwok, et al. (2001). "Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice." Neuron **30**(3): 819-828.

Taber, M. T., G. Zernig, et al. (1998). "Opioid receptor modulation of feeding-evoked dopamine release in the rat nucleus accumbens." Brain Res **785**(1): 24-30.

Tachibana, T., M. A. Cline, et al. (2011). "Feeding responses to central administration of several somatostatin analogs in chicks." Comp Biochem Physiol A Mol Integr Physiol **158**(1): 47-51.

Tadross, J. A. and C. W. le Roux (2009). "The mechanisms of weight loss after bariatric surgery." Int J Obes (Lond) **33 Suppl 1**: S28-32.

Tagliacozzi, D., A. F. Mozzi, et al. (2003). "Quantitative analysis of bile acids in human plasma by liquid chromatography-electrospray tandem mass spectrometry: a simple and rapid one-step method." Clin Chem Lab Med **41**(12): 1633-1641.

Taki, Y., S. Kinomura, et al. (2008). "Relationship between body mass index and gray matter volume in 1,428 healthy individuals." Obesity (Silver Spring) **16**(1): 119-124.

Taki, Y., B. Thyreau, et al. (2012). "A longitudinal study of age- and gender-related annual rate of volume changes in regional gray matter in healthy adults." Hum Brain Mapp.

Tam, C. S., V. Lecoultre, et al. (2011). "Novel strategy for the use of leptin for obesity therapy." Expert Opin Biol Ther **11**(12): 1677-1685.

Tan, T. M., M. Vanderpump, et al. (2004). "Somatostatin infusion lowers plasma ghrelin without reducing appetite in adults with Prader-Willi syndrome." J Clin Endocrinol Metab **89**(8): 4162-4165.

Tanabe, J., J. R. Tregellas, et al. (2009). "Medial orbitofrontal cortex gray matter is reduced in abstinent substance-dependent individuals." Biol Psychiatry **65**(2): 160-164.

Taqi, E., L. E. Wallace, et al. (2010). "The influence of nutrients, biliary-pancreatic secretions, and systemic trophic hormones on intestinal adaptation in a Roux-en-Y bypass model." J Pediatr Surg **45**(5): 987-995.

Taren, A. A., J. D. Creswell, et al. (2013). "Dispositional mindfulness co-varies with smaller amygdala and caudate volumes in community adults." PLoS One **8**(5): e64574.

Tataranni, P. A., J. F. Gautier, et al. (1999). "Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography." Proc Natl Acad Sci U S A **96**(8): 4569-4574.

Tetley, A., J. Brunstrom, et al. (2009). "Individual differences in food-cue reactivity. The role of BMI and everyday portion-size selections." Appetite **52**(3): 614-620.

Thirlby, R. C., F. Bahiraei, et al. (2006). "Effect of Roux-en-Y gastric bypass on satiety and food likes: the role of genetics." J Gastrointest Surg **10**(2): 270-277.

Thirumangalakudi, L., A. Prakasam, et al. (2008). "High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice." J Neurochem **106**(1): 475-485.

Thomas, J. R., F. Gizis, et al. (2010). "Food selections of Roux-en-Y gastric bypass patients up to 2.5 years postsurgery." J Am Diet Assoc **110**(4): 608-612.

Tice, J. A., L. Karliner, et al. (2008). "Gastric banding or bypass? A systematic review comparing the two most popular bariatric procedures." Am J Med **121**(10): 885-893.

Tichansky, D. S., J. D. Boughter, Jr., et al. (2006). "Taste change after laparoscopic Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding." Surg Obes Relat Dis **2**(4): 440-444.

Tichansky, D. S., A. R. Glatt, et al. (2011). "Decrease in sweet taste in rats after gastric bypass surgery." Surg Endosc **25**(4): 1176-1181.

Titomanlio, L., D. De Brasi, et al. (2006). "Partial cerebellar hypoplasia in a patient with Prader-Willi syndrome." Acta Paediatr **95**(7): 861-863.

Titova, O. E., O. C. Hjorth, et al. (2013). "Anorexia nervosa is linked to reduced brain structure in reward and somatosensory regions: a meta-analysis of VBM studies." BMC Psychiatry **13**(1): 110.

Trakhtenbroit, M. A., J. G. Leichman, et al. (2009). "Body weight, insulin resistance, and serum adipokine levels 2 years after 2 types of bariatric surgery." Am J Med **122**(5): 435-442.

Travers, B. G., N. Adluru, et al. (2012). "Diffusion tensor imaging in autism spectrum disorder: a review." Autism Res **5**(5): 289-313.

Trostler, N., A. Mann, et al. (1995). "Weight Loss and Food Intake 18 Months following Vertical Banded Gastroplasty or Gastric Bypass for Severe Obesity." Obes Surg **5**(1): 39-51.

Trostler, N., A. Mann, et al. (1995). "Nutrient Intake following Vertical Banded Gastroplasty or Gastric Bypass." Obes Surg **5**(4): 403-410.

Tschop, M., C. Weyer, et al. (2001). "Circulating ghrelin levels are decreased in human obesity." Diabetes **50**(4): 707-709.

Tschritter, O., H. Preissl, et al. (2006). "The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study." Proc Natl Acad Sci U S A **103**(32): 12103-12108.

Turton, M. D., D. O'Shea, et al. (1996). "A role for glucagon-like peptide-1 in the central regulation of feeding." Nature **379**(6560): 69-72.

Tuulari, J. J., H. K. Karlsson, et al. (2013). "Weight loss after bariatric surgery reverses insulin-induced increases in brain glucose metabolism of the morbidly obese." Diabetes.

Tymitz, K., A. Engel, et al. (2011). "Changes in ghrelin levels following bariatric surgery: review of the literature." Obes Surg **21**(1): 125-130.

Uher, R., J. Treasure, et al. (2006). "Cerebral processing of food-related stimuli: effects of fasting and gender." Behav Brain Res **169**(1): 111-119.

Ukleja, A. (2005). "Dumping syndrome: pathophysiology and treatment." Nutr Clin Pract **20**(5): 517-525.

Unwin, N., D. Gan, et al. (2010). "The IDF Diabetes Atlas: providing evidence, raising awareness and promoting action." Diabetes Res Clin Pract **87**(1): 2-3.

Urosevic, S., P. Collins, et al. (2012). "Longitudinal changes in behavioral approach system sensitivity and brain structures involved in reward processing during adolescence." Dev Psychol **48**(5): 1488-1500.

Vainik, U., A. Dagher, et al. (2013). "Neurobehavioural correlates of body mass index and eating behaviours in adults: A systematic review." Neurosci Biobehav Rev **37**(3): 279-299.

Van der Schueren, B. J., P. Homel, et al. (2012). "Magnitude and variability of the glucagon-like peptide-1 response in patients with type 2 diabetes up to 2 years following gastric bypass surgery." Diabetes Care **35**(1): 42-46.

van Hout, G. C., I. van Oudheusden, et al. (2004). "Psychological profile of the morbidly obese." Obes Surg **14**(5): 579-588.

van Jaarsveld, C. H., C. H. Llewellyn, et al. (2011). "Prospective associations between appetitive traits and weight gain in infancy." Am J Clin Nutr **94**(6): 1562-1567.

Van Oudenhove, L., S. McKie, et al. (2011). "Fatty acid-induced gut-brain signaling attenuates neural and behavioral effects of sad emotion in humans." J Clin Invest **121**(8): 3094-3099.

van Strien, T., Frijters, J. E. R., Bergers, G. P. A., Defares, P. B. (1986). "The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior." Int. J. Eat. Disord **5**(2): 295–315.

van Strien, T., H. M. Snoek, et al. (2010). "Parental control and the dopamine D2 receptor gene (DRD2) interaction on emotional eating in adolescence." Appetite **54**(2): 255-261.

Vanderschuren, L. J., P. Di Ciano, et al. (2005). "Involvement of the dorsal striatum in cue-controlled cocaine seeking." J Neurosci **25**(38): 8665-8670.

Vecht, J., C. B. Lamers, et al. (1999). "Long-term results of octreotide-therapy in severe dumping syndrome." Clin Endocrinol (Oxf) **51**(5): 619-624.

Vederine, F. E., M. Wessa, et al. (2011). "A meta-analysis of whole-brain diffusion tensor imaging studies in bipolar disorder." Prog Neuropsychopharmacol Biol Psychiatry **35**(8): 1820-1826.

Velasquez-Mieyer, P. A., P. A. Cowan, et al. (2003). "Suppression of insulin secretion is associated with weight loss and altered macronutrient intake and preference in a subset of obese adults." Int J Obes Relat Metab Disord **27**(2): 219-226.

Verstynen, T. D., A. M. Weinstein, et al. (2012). "Increased body mass index is associated with a global and distributed decrease in white matter microstructural integrity." Psychosom Med **74**(7): 682-690.

Vidarsdottir, S., P. A. Smeets, et al. (2007). "Glucose ingestion fails to inhibit hypothalamic neuronal activity in patients with type 2 diabetes." Diabetes **56**(10): 2547-2550.

Vilarrasa, N., J. Vendrell, et al. (2007). "Effect of weight loss induced by gastric bypass on proinflammatory interleukin-18, soluble tumour necrosis factor-alpha receptors, C-reactive protein and adiponectin in morbidly obese patients." Clin Endocrinol (Oxf) **67**(5): 679-686.

Viollet, C., G. Lepousez, et al. (2008). "Somatostatinergic systems in brain: networks and functions." Mol Cell Endocrinol **286**(1-2): 75-87.

Volkow, N. D., G.-J. Wang, et al. (2008). "Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: Possible contributing factors." NeuroImage **42**(4): 1537-1543.

Volkow, N. D., G. J. Wang, et al. (2011). "Reward, dopamine and the control of food intake: implications for obesity." Trends Cogn Sci **15**(1): 37-46.

Volkow, N. D., G. J. Wang, et al. (2008). "Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology." Philos Trans R Soc Lond B Biol Sci **363**(1507): 3191-3200.

Wadden, T. A., M. L. Butryn, et al. (2007). "Lifestyle modification for the management of obesity." Gastroenterology **132**(6): 2226-2238.

Wadden, T. A., G. D. Foster, et al. (1994). "One-year behavioral treatment of obesity: comparison of moderate and severe caloric restriction and the effects of weight maintenance therapy." J Consult Clin Psychol **62**(1): 165-171.

Wadden, T. A., V. L. Webb, et al. (2012). "Lifestyle modification for obesity: new developments in diet, physical activity, and behavior therapy." Circulation **125**(9): 1157-1170.

Wajchenberg, B. L. (2007). "beta-cell failure in diabetes and preservation by clinical treatment." Endocr Rev **28**(2): 187-218.

Wallis, D. J. and M. M. Hetherington (2004). "Stress and eating: the effects of ego-threat and cognitive demand on food intake in restrained and emotional eaters." Appetite **43**(1): 39-46.

Wallner-Liebmann, S., K. Koschutnig, et al. "Insulin and Hippocampus Activation in Response to Images of High-Calorie Food in Normal Weight and Obese Adolescents." Obesity (Silver Spring).

Wallner-Liebmann, S., K. Koschutnig, et al. (2010). "Insulin and hippocampus activation in response to images of high-calorie food in normal weight and obese adolescents." Obesity (Silver Spring) **18**(8): 1552-1557.

Walther, K., A. C. Birdsill, et al. (2010). "Structural brain differences and cognitive functioning related to body mass index in older females." Hum Brain Mapp **31**(7): 1052-1064.

Wang, G., K. Agenor, et al. (2012). "Accelerated Gastric Emptying but No Carbohydrate Malabsorption 1 Year After Gastric Bypass Surgery (GBP)." Obes Surg **22**(8): 1263-1267.

Wang, G. J., A. Geliebter, et al. (2011). "Enhanced striatal dopamine release during food stimulation in binge eating disorder." Obesity (Silver Spring) **19**(8): 1601-1608.

Wang, G. J., N. D. Volkow, et al. (2001). "Brain dopamine and obesity." Lancet **357**(9253): 354-357.

Wang, G. J., N. D. Volkow, et al. (2009). "Evidence of gender differences in the ability to inhibit brain activation elicited by food stimulation." Proc Natl Acad Sci U S A **106**(4): 1249-1254.

Wang, L., G. Gourcerol, et al. (2010). "Peripheral peptide YY inhibits propulsive colonic motor function through Y2 receptor in conscious mice." Am J Physiol Gastrointest Liver Physiol **298**(1): G45-56.

Ward, M. A., C. M. Carlsson, et al. (2005). "The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study." BMC Neurol **5**: 23.

Warde-Kamar, J., M. Rogers, et al. (2004). "Calorie intake and meal patterns up to 4 years after Roux-en-Y gastric bypass surgery." Obes Surg **14**(8): 1070-1079.

Wardle, J. (1987). "Eating style: a validation study of the Dutch Eating Behaviour Questionnaire in normal subjects and women with eating disorders." J Psychosom Res **31**(2): 161-169.

Watson, D., L. A. Clark, et al. (1988). "Development and validation of brief measures of positive and negative affect: the PANAS scales." J Pers Soc Psychol **54**(6): 1063-1070.

Weiland, B. J., R. C. Welsh, et al. (2013). "Accumbens functional connectivity during reward mediates sensation-seeking and alcohol use in high-risk youth." Drug Alcohol Depend **128**(1-2): 130-139.

Weir, G. C., L. Marselli, et al. (2009). "Towards better understanding of the contributions of overwork and glucotoxicity to the beta-cell inadequacy of type 2 diabetes." Diabetes Obes Metab **11 Suppl 4**: 82-90.

Weise, C. M., P. Thiyyagura, et al. (2012). "Postprandial plasma PYY concentrations are associated with increased regional gray matter volume and rCBF declines in caudate nuclei--a combined MRI and H2(15)O PET study." Neuroimage **60**(1): 592-600.

Weiss, E. C., D. A. Galuska, et al. (2007). "Weight regain in U.S. adults who experienced substantial weight loss, 1999-2002." Am J Prev Med **33**(1): 34-40.

Welch, C. C., E. M. Kim, et al. (1996). "Palatability-induced hyperphagia increases hypothalamic Dynorphin peptide and mRNA levels." Brain Res **721**(1-2): 126-131.

Weng, C. B., R. B. Qian, et al. (2013). "Gray matter and white matter abnormalities in online game addiction." Eur J Radiol **82**(8): 1308-1312.

Werthmann, J., A. Roefs, et al. (2011). "Can(not) take my eyes off it: attention bias for food in overweight participants." Health Psychol **30**(5): 561-569.

Westenhoefer, J., D. Engel, et al. (2013). "Cognitive and weight-related correlates of flexible and rigid restrained eating behaviour." Eat Behav **14**(1): 69-72.

Weygandt, M., K. Mai, et al. (2013). "The role of neural impulse control mechanisms for dietary success in obesity." Neuroimage.

White, M. J., C. P. Morris, et al. (2008). "Behavioral phenotypes of impulsivity related to the ANKK1 gene are independent of an acute stressor." Behav Brain Funct **4**: 54.

White, T., M. Nelson, et al. (2008). "Diffusion tensor imaging in psychiatric disorders." Top Magn Reson Imaging **19**(2): 97-109.

Whitson, B. A., D. B. Leslie, et al. (2007). "Adipokine response in diabetics and nondiabetics following the Roux-en-Y gastric bypass: a preliminary study." J Surg Res **142**(2): 295-300.

WHO (2005). The SuRF Report 2. Surveillance of chronic disease Risk Factors: Country-level data and comparable estimates. Geneva, World Health Organisation.

Will, M. J., E. B. Franzblau, et al. (2003). "Nucleus accumbens mu-opioids regulate intake of a high-fat diet via activation of a distributed brain network." J Neurosci **23**(7): 2882-2888.

Wilson-Perez, H. E., A. P. Chambers, et al. (2013). "Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency." Diabetes **62**(7): 2380-2385.

Wilson, S. J., M. A. Sayette, et al. (2004). "Prefrontal responses to drug cues: a neurocognitive analysis." Nat Neurosci **7**(3): 211-214.

Wing, R. R. and S. Phelan (2005). "Long-term weight loss maintenance." Am J Clin Nutr **82**(1 Suppl): 222S-225S.

Woelnerhanssen, B., R. Peterli, et al. (2011). "Effects of postbariatric surgery weight loss on adipokines and metabolic parameters: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy--a prospective randomized trial." Surg Obes Relat Dis **7**(5): 561-568.

Woods, S. C., K. Gotoh, et al. (2003). "Gender differences in the control of energy homeostasis." Exp Biol Med (Maywood) **228**(10): 1175-1180.

Woolrich, M. W., T. E. Behrens, et al. (2004). "Multilevel linear modelling for FMRI group analysis using Bayesian inference." NeuroImage **21**(4): 1732-1747.

Wyllys E, A. E., Mix CL. "‘‘Dumping stomach’’ and other results of gastrojejunostomy: operative cure by disconnecting old stoma." Surg Clin Chic **4**: 879–892.

Wynne, K. and S. R. Bloom (2006). "The role of oxyntomodulin and peptide tyrosine-tyrosine (PYY) in appetite control." Nat Clin Pract Endocrinol Metab **2**(11): 612-620.

Xu, J., H. Kober, et al. (2012). "White matter integrity and behavioral activation in healthy subjects." Hum Brain Mapp **33**(4): 994-1002.

Yamamoto, T. (1993). "Neural mechanisms of taste aversion learning." Neurosci Res **16**(3): 181-185.

Yates, K. F., V. Sweat, et al. (2012). "Impact of metabolic syndrome on cognition and brain: a selected review of the literature." Arterioscler Thromb Vasc Biol **32**(9): 2060-2067.

Yau, P. L., M. G. Castro, et al. (2012). "Obesity and metabolic syndrome and functional and structural brain impairments in adolescence." Pediatrics **130**(4): e856-864.

Yau, P. L., D. C. Javier, et al. (2010). "Preliminary evidence for brain complications in obese adolescents with type 2 diabetes mellitus." Diabetologia **53**(11): 2298-2306.

Yeomans, J. S., A. Mathur, et al. (1993). "Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons." Behav Neurosci **107**(6): 1077-1087.

Yeomans, M. R. and E. Coughlan (2009). "Mood-induced eating. Interactive effects of restraint and tendency to overeat." Appetite **52**(2): 290-298.

Yeomans, M. R. and R. W. Gray (1996). "Selective effects of naltrexone on food pleasantness and intake." Physiol Behav **60**(2): 439-446.

Yeomans, M. R. and R. W. Gray (1997). "Effects of naltrexone on food intake and changes in subjective appetite during eating: evidence for opioid involvement in the appetizer effect." Physiol Behav **62**(1): 15-21.

Yeomans, M. R. and R. W. Gray (2002). "Opioid peptides and the control of human ingestive behaviour." Neurosci Biobehav Rev **26**(6): 713-728.

Yeomans, M. R., R. W. Gray, et al. (1997). "Independent effects of palatability and within-meal pauses on intake and appetite ratings in human volunteers." Appetite **29**(1): 61-76.

Yeomans, M. R., M. Leitch, et al. (2008). "Impulsivity is associated with the disinhibition but not restraint factor from the Three Factor Eating Questionnaire." Appetite **50**(2-3): 469-476.

Yeomans, M. R. and P. Wright (1991). "Lower pleasantness of palatable foods in nalmefene-treated human volunteers." Appetite **16**(3): 249-259.

Yokum, S., J. Ng, et al. (2011). "Attentional bias to food images associated with elevated weight and future weight gain: an fMRI study." Obesity (Silver Spring) **19**(9): 1775-1783.

Yokum, S., J. Ng, et al. (2012). "Relation of regional gray and white matter volumes to current BMI and future increases in BMI: a prospective MRI study." Int J Obes (Lond) **36**(5): 656-664.

Yoshida, R., M. Niki, et al. (2012). "Modulation of sweet responses of taste receptor cells." Semin Cell Dev Biol.

Yu, R. (2012). "Regional white matter volumes correlate with delay discounting." PLoS One **7**(2): e32595.

Yuan, K., W. Qin, et al. (2011). "Microstructure abnormalities in adolescents with internet addiction disorder." PLoS One **6**(6): e20708.

Zagury, L., R. O. Moreira, et al. (2004). "Insulinoma misdiagnosed as dumping syndrome after bariatric surgery." Obes Surg **14**(1): 120-123.

Zhang, Y., M. Brady, et al. (2001). "Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm." IEEE Trans Med Imaging **20**(1): 45-57.

Zheng, H., A. C. Shin, et al. (2009). "Meal patterns, satiety, and food choice in a rat model of Roux-en-Y gastric bypass surgery." Am J Physiol Regul Integr Comp Physiol **297**(5): R1273-1282.

Zhou, Y. H., X. Q. Ma, et al. (2012). "Effect of anti-obesity drug on cardiovascular risk factors: a systematic review and meta-analysis of randomized controlled trials." PLoS One **7**(6): e39062.

Ziauddeen, H., I. S. Farooqi, et al. (2012). "Obesity and the brain: how convincing is the addiction model?" Nat Rev Neurosci **13**(4): 279-286.

APPENDICES

1. Information sheet for research participants
2. Participant consent form
3. Dutch Eating Behaviour Questionnaire
4. Eating Disorder Examination Questionnaire
5. Positive and Negative Affect Schedule
6. Beck Depression Inventory
7. Barratt Impulsivity Scale
8. Eysenck Personality Questionnaire
9. Behavioural Activation / Behavioural Inhibition Scales
10. Visual analogue scales
11. Dietary food record
12. Dumping syndrome questionnaire
13. Subject characteristics of whole cohort who attended screening visit
14. VBM whole brain results of obese and normal weight subjects
15. 15 VBM region of interest analysis of obese, overweight and normal weight subjects
16. Subcortical volumetric analysis of nucleus accumbens in obese, overweight and normal weight subjects

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THIS INFORMATION SHEET IS VALID FOR USE UNTIL: 1 October 2011

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

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

STUDY TITLE: OBESITY SURGERY AND FUNCTIONAL MAGNETIC RESONANCE IMAGING OF APPETITE.

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Thank you for reading this.

WHAT IS THE PURPOSE OF THE STUDY?

We do not understand fully why people with obesity overeat. Obesity surgery reduces appetite, which leads to weight loss. How this happens is not fully understood.

We do know that restriction of stomach size after surgery may play some part, but it is likely that changes in hormones released from the gut play a role by acting on the appetite and reward centers in the brain.

This study aims to see how obesity alters the brain's response to food and how obesity surgery changes this by comparing people who have undergone obesity surgery and those that have not. Participants will undergo brain scans while looking at pictures of food, after fasting overnight or after having eaten. They will also be injected under the skin either with Octreotide, a hormone that temporarily reduces the release of hormones from your gut, or with water as a dummy injection for comparison.

This study is an important step towards finding out how appetite is altered in obesity and how obesity surgery works. This will help with the development of new treatments for obesity, which are as successful as obesity surgery.

WHY HAVE I BEEN CHOSEN?

We are recruiting the following groups of people:

1. Healthy individuals who are not obese
2. People who are obese but have not had surgery.
3. People who have had obesity surgery, either gastric banding or gastric bypass surgery.

You should **not** take part in this study if you:

1) Have any illnesses which we feel make you unsuitable
2) If you take any medication which we feel make you unsuitable
3) If you are pregnant or breast feeding
4) If you have donated blood in the last three months

It is important that you **should not become pregnant** during the course of the study or for one month after. It is therefore important that you have adequate and reliable contraception during this period.

DO I HAVE TO TAKE PART?

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

WHAT WILL HAPPEN TO ME IF I TAKE PART?

Screening visit

If you agree to take part in this study, you will first be examined by one of the research doctors and have a number of blood tests after an overnight fast (no more than 30ml blood equivalent to around 2 tablespoons), a pregnancy test (if female) and a heart recording (ECG) to ensure you are fit and healthy enough to take part.

You will be asked to complete some questionnaires about your eating habits, personality and mood. This information will be related to the results from your brain scans. Individual differences in these factors between people have been shown to influence appetite and how the brain responds to looking at pictures, such as food. You will also be asked to look at some pictures of food and say which ones you prefer to eat. It will take about 30-40 minutes to complete these questionnaires. A researcher will be available to assist you with this if necessary.

You will also be asked to taste the meal that will be used later on in the study. We will also ask you to keep a record of all food and drink consumed for three days.

You will also have your height and weight taken and your body fat content measured using a 'bio-electrical impedance' machine. This is a painless safe method that involves measuring the electrical current from your body and takes only about 5 minutes. As long as these medical checks are satisfactory and you are still happy to participate, you will then be asked to attend for the first of up to 4 study visits.

With your permission, we will also take a sample of DNA from blood or saliva to look for changes in your genes that may be involved in the how the body controls appetite and body weight and responds to gut hormones. This will enable us to see what effect such changes may have on your brain scans.

Number of visits

You will be asked to attend the Clinical Investigation Ward at Hammersmith Hospital as an outpatient on up to another 4 occasions, following your screening visit, each separated by at least 3 days. These will be completed on dates convenient to you and the investigators, but should usually be completed within a maximum of 3 months.

Study design

On each study visit you will be asked to have nothing to eat and only water to drink from 8 pm on the evening before the study. You will be asked to attend in the morning and each visit will last around 5 hours. You will be asked to abstain from alcohol and strenuous exercise for 24 hours before the visit. We will also ask you to keep a record of all food and drink consumed for one day before the visit, the day of the visit and for one day afterwards. You will also have a pregnancy test on each visit (if female), and have your height, weight and body fat content measured. You will also complete questionnaires about your mood on each study visit. These questionnaires should take about 5 minutes to complete.

On each study day you will have a small plastic cannula tube inserted into a vein in one arm. A vein is the type of blood vessel commonly used for taking blood samples. You may feel some discomfort whilst the cannula is being inserted. After the cannula tube has been inserted this will be used to take blood samples.

On each of the visits, you will then receive either a saline (salt water) or Octreotide injection under the skin on the thigh or tummy. You may fell some slight discomfort at the time of the injection. You will also receive at the same time another injection under the skin on the thigh or tummy of either saline (salt water) or a small dose of insulin. The insulin injection is to prevent your sugar level going too high after the Octreotide injection.

What is Octreotide?

Octreotide is a man-made hormone that is very similar to a substance that occurs naturally in the body, called somatostatin. It temporarily reduces the release of some hormones in the body including those from the gut that reduce appetite. We have used this substance in other studies to investigate appetite without problems. Only very mild side effects are occasionally seen, such as abdominal discomfort and bloating, loose stools or nausea, increase in blood sugar level after eating. These effects of Octreotide are not expected to last longer than 6 hours. Octreotide is widely prescribed as a medicine for certain intestinal conditions.

What is saline?

The saline is a placebo or dummy treatment that is commonly used in studies of this nature. It contains no active ingredient and is not expected to alter your appetite. The saline treatment will serve as a baseline measurement to which all active treatments are compared. This is a randomised, double-blind trial. This means that neither you, nor your research doctors, will know what substance you are being given on some visits (although, if your doctor needs to find out, he/she can do so). Throughout the study, we will monitor your heart rate and blood pressure.

Breakfast

You will either be given a moderate size breakfast to eat over 20 minutes, or continue fasting.

Paracetamol

You will be given a solution of paracetamol in water with breakfast. The levels of paracetamol in your blood will then be measured. This will allow us to have an accurate measure of the rate at which your stomach is emptying. We do not expect that you will suffer any side effects from this. You should not take any further paracetamol at home for at least 12 hours after the study visit is over.

Blood tests

Blood samples will be taken from the cannula in your arm. The total amount of blood taken on each study visit will not be more than 150 ml (about 10 tablespoonfuls). The total amount of blood taken over all your visits will not be more than 630 ml (a little less than a pint and the same amount taken when making a single donation of blood for blood transfusion). During blood testing, you will be seated or lying on a couch, and can read or watch television.

Visual analogue scales

Over the morning, we will regularly ask you to score how you are feeling (e.g. rating your hunger) by placing a mark on a line called a visual analogue scale.

Scanning

You will have magnetic resonance imaging (MRI) brain scans by lying in an MRI scanner for up to 1 hour. This will take place in the Robert Steiner Magnetic Resonance Imaging Unit, nearby the Clinical Investigation Ward. This will enable us to look at the structure and activity of your brain.

During the functional brain scans we look at the activity of the brain at rest, while you look at a variety of pictures on a screen, and perform simple tasks like viewing a flashing light, pressing a button, reading, listening, speaking, recalling, thinking about words or numbers. You will be asked to make responses to the pictures while in the scanner using a keypad. You will have the opportunity to practice lying in the scanner while looking at various pictures on the screen. This will enable us to ensure that you can follow the instructions and lie still while in the scanner. While in the scanner your heart and breathing rate and finger skin sweat production may be monitored.

Meal

At the end of the brain scan, you will be taken back to the Clinical Investigation Ward. At this stage, you will be presented with a meal and you will be asked to eat as much as you want of the meal until you feel comfortably full.

We will continue to monitor you for another 2 hour after the scan, after which you are free to go. At the end of the final visit you will also be asked to score how much you usually like to eat the foodstuffs shown in the food pictures using a visual analogue scale.

WHAT DO I HAVE TO DO?

The only restrictions on your lifestyle are that you will be asked to have nothing to eat and only water to drink from 8pm on the night before the meal days. You will need to keep a record of all food and drinks consumed in the day before every study and for a day afterwards. For twenty-four hours before each study meal you will be asked to refrain from taking strenuous exercise and drinking alcohol.

Female volunteers should have adequate contraception for the period of the study and for one month afterwards. Pregnancy tests will be carried out to confirm that women of child bearing age are not pregnant on the morning before each study day.

WHAT IS THE DRUG THAT IS BEING USED?

Octreotide is a synthetic copy of the naturally occurring hormone found in the blood, called somatostatin. Octreotide has been used acutely in several other research studies in our department and worldwide, in men and women, without harmful effects. It is also widely prescribed to patients

with intestinal problems for long term use. The dose of Octreotide that you will be given is small and will decrease the levels of gut hormones in your body.

Insulin is a naturally occurring hormone found in the blood that regulates your blood sugar and is used to treat people with sugar diabetes. When we give you an injection of Octreotide you will also have a single injection of insulin since the Octreotide may temporarily suppress you body's own production of insulin which may cause your sugar to rise for a few hours. The injection of insulin will prevent the sugar rising too much. Your sugar levels will be monitored.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?

Some people experience mild abdominal discomfort, loose stools, nausea and flatulence after having Octreotide, but these symptoms are usually mild and settle within a few hours. If your sugar levels rise after the Octreotide injection you may feel thirsty, pass more urine than usual, or feel a little sleepy, but this will settle within a few hours and you will be able to drink water if you wish.

From our previous studies we do not expect any significant side effects, but the unexpected can occur. During the study, at least one experienced doctor will monitor you closely. If you suffer from any ill effects during the study you should report them to the doctors monitoring you immediately. If you suffer from any ill effects afterwards you should report them to one of the research doctors at the contact number below or when you next see them. You may ask for the study to stop at any time without giving a reason. If any unexpected side effects occur, the study will be stopped.

MRI is a powerful, diagnostic body scanning technique, which is used in hospitals worldwide to create images of the inside of the body. MRI has been used safely for several decades and has no known side-effects. Each scan is directed to the specific requirements of your referring doctor or to research study in which you are taking part.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

Octreotide has been administered in several studies by our laboratory and therefore we do not anticipate any problems with the injection. Insertion of the cannula (drip) into your arm on each of the study days may cause minor discomfort or superficial bruising, as may the injection of saline or Octreotide under the skin.

Magnetic resonance imaging (MRI) is a procedure that allows doctors to look inside the body by using a scanner that sends out a strong magnetic field and radio waves. MRI does not use X-rays. This procedure is used routinely for medical care and is very safe for most people, but you will be monitored during the entire MRI scan in case any problems occur. The risks of having an MRI scan are:

- The MRI scanner contains a very strong magnet. Therefore, you will not be able to have the MRI if you have any type of metal implanted in your body, for example, any pacing device (such as a heart pacer), any metal in your eyes, or certain types of heart valves or brain aneurysm clips. Someone will ask you questions about this before you have the MRI. If you have previously undergone gastric bypass or banding surgery, then the devices used in your surgery will have been approved as safe for MRI.

- There is not much room inside the MRI scanner. You may be uncomfortable if you do not like to be in close spaces ("claustrophobia"). During the procedure, you will be able to talk to and hear the MRI staff through a speaker and earphone system, and, in the event of an emergency, you

can tell them to stop the scan. You will be closely monitored and repeatedly checked on to make sure you are as comfortable as possible. While your head is in the scanner, we will support it, so you can't move it. If this upsets you, you will be able to signal and speak to the investigator and stop the scan through the use of a radio system and a signaling button. You will have the opportunity during the first MRI scan to ensure that you can tolerate having the scan before the next scans are done.

- The MRI produces a "hammering noise". You will wear earplugs and headphones to prevent discomfort or damage to hearing. The headphones will also allow you to be able to hear us talk to you.

- You will be fully awake during the MRI scan and will not be sedated at any time. We will make every effort to ensure your comfort during this experiment.

It should be noted that the MRI brain scan cannot be viewed as a comprehensive health screening procedure. However, vary rarely, unexpected information can be detected which may warrant further investigation. In this event, you will be informed and a report will be sent to your GP, who will arrange further tests and coordinate your further care. In the rare event that we find a significant abnormality on your structural brain scan on the first visit this may exclude you from continuing with the rest of the study.

It is possible that if the treatment is given to a pregnant woman it will harm the unborn child. Pregnant women must therefore not take part in this study; neither should women who plan to become pregnant during the study. Women who are at risk of pregnancy will be asked to have a pregnancy test before taking part, to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

The information that we get from this study will help us to better understand appetite regulation and may help us to better treat future patients who suffer from being overweight or obese.

If any of the screening questionnaires or blood tests reveal any medical problems (e.g. depression, diabetes, high cholesterol, thyroid, kidney or liver problems), your GP will be informed so that they can coordinate your further care, arrange any further tests, and refer you on to Hospital Doctors if necessary**.**

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

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

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

Once the study has finished, the results of the study can be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should

contact one of the research doctors on the numbers provided below.

WHAT IF SOMETHING GOES WRONG?

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

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator Dr. Goldstone (Tel: 020 8383 1029).

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

It is a requirement that your GP is informed, with your consent, of your participation in this study, at the start of the study.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

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

WHO IS ORGANISING AND FUNDING THE RESEARCH?

This study is being organised and funded by the MRC Clinical Sciences Centre and the Department of Investigative Medicine, Imperial College London.

PAYMENT

You will receive a fixed payment to cover expenses including travel costs. This sum of £20 for the screening visit and £50 for each study visit (total £220) will be paid when you have completed your visits.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed by the Hammersmith Hospitals Research Ethics Committee (Ref 08/H0707/139).

CONTACT FOR FURTHER INFORMATION

If you experience any problems during the study, you may withdraw at any stage. The doctors involved in the study, Dr Scholtz and Dr. Goldstone, will be available by telephone during working hours (020 8383 1029 or via the paging system). The hospital switchboard (020 8383 1000) has home and mobile phone numbers for all the doctors involved in the study and can contact them at any time outside normal working hours.

If you agree to take part in the trial, you will also be given the mobile phone numbers of the doctors.

Imperial College London
MRC Clinical Sciences Centre

Ref: 08/H0707/139 Version 2, 02/10/2008. Valid until 1st October 2011

Robert Steiner MRI Unit
MRC Clinical Sciences Centre, Imperial College London
Hammersmith Hospital Campus, Du Cane Road, London W12 0NN

Participant Consent Form

Title of project: Obesity surgery and functional magnetic resonance imaging of appetite.

Name of Principal Investigator: Dr. A.P.Goldstone. Please tick and initial each statement:

1. I confirm that I have read and understand the subject information sheet Protocol Version3…… dated …..03/08/2009…. for the above study. ☐

2. I have had the opportunity to ask questions and discuss this study. ☐

3. All my questions have been answered fully. ☐

4. I have received enough information about the study. ☐

5. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

6. I understand that my images and sections of any of my medical notes may be looked at by responsible individuals from Imperial College London or from regulatory authorities where it is relevant to my taking part in this research. ☐

7. I give permission for these individuals to access my records that are relevant to this research. ☐

8. I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my visits and brain scans. ☐

9. I give permission for my images to be used for research by responsible individuals from Imperial College London and Imperial College Healthcare NHS Trust so long as they do not contain identifying personal information. ☐

10. I agree for a DNA sample to be taken and stored to look for changes that may be involved in obesity and the control of appetite. ☐

11. The compensation arrangements have been discussed with me. ☐

12. I agree to take part in the above study. ☐

_____	_____	_____
Name of Subject (block capitals)	Signature	Date
_____	_____	_____
Principal Investigator	Signature	Date
_____		_____
Name of Person taking consent (if different from Principal Investigator)	Signature	Date

DUTCH EATING QUESTIONNAIRE

Volunteer Initials: _____ **Date:** _____

Volunteer No. _____

Please place an (✓) in the box which applies best to each of the numbered statements. All of the results will be *strictly* confidential. Most of the questions directly relate to food or eating, although other types of questions have been included. Please answer each question carefully. Thank you.

1. If you have put on weight, do you eat less than you usually do?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

2. Do you try to eat less at mealtimes than you would like to eat?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

3. How often do you refuse food or drink offered because you are concerned about your weight?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

4. Do you watch exactly what you eat?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

5. Do you deliberately eat foods that are slimming?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

6. When you have eaten too much, do you eat less than usual the following days?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

7. Do you deliberately eat less in order not to become heavier?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

8. How often do you try not to eat between meals because you are watching your weight?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

9. How often in the evening do you try not to eat because you are watching your weight?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

10. Do you take into account your weight with what you eat?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

11. Do you have the desire to eat when you are irritated?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

12. Do you have a desire to eat when you have nothing to do?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

13. Do you have a desire to eat when you are depressed or discouraged?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

14. Do you have a desire to eat when you are feeling lonely?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

15. Do you have a desire to eat when somebody lets you down?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

16. Do you have a desire to eat when you are cross?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

17. Do you have a desire to eat when you are approaching something unpleasant to happen?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

18. Do you get the desire to eat when you are anxious, worried or tense?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

19. Do you have a desire to eat when things are going against you or when things have gone wrong?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

20. Do you have a desire to eat when you are frightened?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

21. Do you have a desire to eat when you are disappointed?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

22. Do you have a desire to eat when you are bore or restless?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

23. Do you have a desire to eat when you are emotionally upset?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often ‰ Not Relevant

24. If food tastes good to you, do you eat more than usual?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

25. If food smells and looks good do you eat more than usual?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

26. If you see or smell something delicious, do you have the desire to eat it?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

27. If you have something delicious to eat, do you eat it straight away?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

28. If you walk past the baker do you have the desire to buy something delicious?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

29. If you walk past a snackbar or a café, do you have the desire to buy something delicious?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

30. If you see others eating, do you also have the desire to eat?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

31. Can you resist eating delicious foods?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

32. Do you eat more than usual, when you see others eating?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

33. When preparing a meal are you inclined to eat something?

‰ Never ‰ Seldom ‰ Sometimes ‰ Often ‰ Very Often

EATING QUESTIONNAIRE

Instructions: The following questions are concerned with the past four weeks (28 days) only. Please read each question carefully. Please answer all of the questions. Thank you.

Questions 1 to 12: Please circle the appropriate number on the right. Remember that the questions only refer to the past four weeks (28 days) only.

.

	On how many of the past 28 days	No days	1-5 days	6-12 days	13-15 days	16-22 days	23-27 days	Every day
1	Have you been deliberately trying to limit the amount of food you eat to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
2	Have you gone for long periods of time (8 waking hours or more) without eating anything at all in order to influence your shape or weight?	0	1	2	3	4	5	6
3	Have you tried to exclude from your diet any foods that you like in order to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
4	Have you tried to follow definite rules regarding your eating (for example, a calorie limit) in order to influence your shape or weight (whether or not you have succeeded)?	0	1	2	3	4	5	6
5	Have you had a definite desire to have an empty stomach with the aim of influencing your shape or weight?	0	1	2	3	4	5	6
6	Have you had a definite desire to have a totally flat stomach?	0	1	2	3	4	5	6
7	Has thinking about food, eating or calories made it very difficult to concentrate on things you are interested in (for example, working, following a conversation, or reading)?	0	1	2	3	4	5	6
8	Has thinking about shape or weight made it very difficult to concentrate on things you are interested in (for example, working, following a conversation, or reading)?	0	1	2	3	4	5	6
9	Have you had a definite fear of losing control over eating?	0	1	2	3	4	5	6
10	Have you had a definite fear that you might gain weight?	0	1	2	3	4	5	6
11	Have you felt fat?	0	1	2	3	4	5	6
12	Have you had a strong desire to lose weight?	0	1	2	3	4	5	6

Questions 22 to 28: Please circle the appropriate number on the right. Remember that the questions only refer to the past four weeks (28 days).

	Over the past 28 days	**Not at all**		**Slightly**		**Moderate -ly**		**Markedly**
22	Has your weight influenced how you think about (judge) yourself as a person?	0	1	2	3	4	5	6
23	Has your shape influenced how you think about (judge) yourself as a person?	0	1	2	3	4	5	6
24	How much would it have upset you if you had been asked to weigh yourself once a week (no more, or less, often) for the next four weeks?	0	1	2	3	4	5	6
25	How dissatisfied have you been with your weight?	0	1	2	3	4	5	6
26	How dissatisfied have you been with your shape?	0	1	2	3	4	5	6
27	How uncomfortable have you felt seeing your body (for example, seeing your shape in the mirror, in a shop window reflection, while undressing or taking a bath or shower)?	0	1	2	3	4	5	6
28	How uncomfortable have you felt about others seeing your shape or figure (for example, in communal changing rooms, when swimming, or wearing tight clothes)?	0	1	2	3	4	5	6

What is your weight at present? (Please give your best estimate.) .....

What is your height? (Please give your best estimate.) .....

If female: Over the past three-to-four months have you missed any menstrual periods? .....

If so, how many? .....
Have you been taking the "pill"? .....

THANK YOU

BDI-II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

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11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.
2b I sleep a lot less than usual.

3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.
2b My appetite is much greater than usual.

3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

_____ Subtotal Page 2
_____ Subtotal Page 1
_____ Total Score

NR15645

PANAS Scale

Initials: ID: Date:

This scale consists of a number of words that describe different feelings and emotions.

Read each item and then mark [x] the appropriate answer in the space next to the word.

Indicate to what extent you have felt this way **on average during the past week**.

Use the following scale to record your answer:

No.	Feeling	very slightly or not at all	a little	moderately	quite a bit	extremely
1	interested					
2	distressed					
3	excited					
4	upset					
5	strong					
6	guilty					
7	scared					
8	hostile					
9	enthusiastic					
10	proud					
11	irritable					
12	alert					
13	ashamed					
14	inspired					
15	nervous					
16	determined					
17	attentive					
18	jittery					
19	active					
20	afraid					

DIRECTIONS: People differ in the ways they act and think in different situations. This is a test to measure some of the ways in which you act and think. Read each statement and put an X on the appropriate circle on the right side of this page. Do not spend too much time on any statement. Answer quickly and honestly.

	O Rarely/Never	O Occasionally	O Often	O Almost Always/Always			
1	I plan tasks carefully.			O	O	O	O
2	I do things without thinking.			O	O	O	O
3	I make-up my mind quickly.			O	O	O	O
4	I am happy-go-lucky.			O	O	O	O
5	I don't "pay attention."			O	O	O	O
6	I have "racing" thoughts.			O	O	O	O
7	I plan trips well ahead of time.			O	O	O	O
8	I am self controlled.			O	O	O	O
9	I concentrate easily.			O	O	O	O
10	I save regularly.			O	O	O	O
11	I "squirm" at plays or lectures.			O	O	O	O
12	I am a careful thinker.			O	O	O	O
13	I plan for job security.			O	O	O	O
14	I say things without thinking.			O	O	O	O
15	I like to think about complex problems.			O	O	O	O
16	I change jobs.			O	O	O	O
17	I act "on impulse."			O	O	O	O
18	I get easily bored when solving thought problems.			O	O	O	O
19	I act on the spur of the moment.			O	O	O	O
20	I am a steady thinker.			O	O	O	O
21	I change residences.			O	O	O	O
22	I buy things on impulse.			O	O	O	O
23	I can only think about one thing at a time.			O	O	O	O
24	I change hobbies.			O	O	O	O
25	I spend or charge more than I earn.			O	O	O	O
26	I often have extraneous thoughts when thinking.			O	O	O	O
27	I am more interested in the present than the future.			O	O	O	O
28	I am restless at the theater or lectures.			O	O	O	O
29	I like puzzles.			O	O	O	O
30	I am future oriented.			O	O	O	O

EPQ-R

Instructions:

Please answer each question by putting a circle around the 'YES' or the 'NO' following the question.

There are no right or wrong answers, and no trick questions.

Work quickly and do not think too long about the exact meaning of the questions.

PLEASE REMEMBER TO ANSWER EACH QUESTION

1. Do you have many different hobbies? ..... YES NO
2. Do you stop to think things over before doing anything? ..... YES NO
3. Does your mood often go up and down? ..... YES NO
4. Have you ever taken the praise for something you knew someone else had really done?. YES NO
5. Do you take much notice of what people think? ..... YES NO
6. Are you a talkative person? ..... YES NO
7. Would being in debt worry you? ..... YES NO
8. Do you ever feel 'just miserable' for no reason? ..... YES NO
9. Do you give money to charities? ..... YES NO
10. Were you ever greedy by helping yourself to more than your share of anything? ..... YES NO
11. Are you rather lively? ..... YES NO
12. Would it upset you a lot to see a child or animal suffer? ..... YES NO
13. Do you often worry about things you should not have done or said? ..... YES NO
14. Do you dislike people who don't know how to behave themselves? ..... YES NO
15. If you say you will do something , do you always keep your promise no matter how inconvenient it might be? ..... YES NO
16. Can you usually let yourself go and enjoy yourself at a lively party? ..... YES NO
17. Are you an irritable person? ..... YES NO
18. Should people always respect the law? ..... YES NO
19. Have you ever blamed someone for doing something you knew was really your fault? ..... YES NO
20. Do you enjoy meeting new people? ..... YES NO
21. Are good manners very important? ..... YES NO
22. Are your feelings easily hurt? ..... YES NO
23. Are all your habits good and desirable ones? ..... YES NO
24. Do you tend to keep in the background on social occasions? ..... YES NO
25. Would you take drugs which may have strange or dangerous effects? ..... YES NO
26. Do you often feel 'fed-up'? ..... YES NO
27. Have you ever taken anything (even a pin or button) that belonged to someone else? .... YES NO
28. Do you like going out a lot? ..... YES NO
29. Do you prefer to go your own way rather than act by the rules? ..... YES NO

30. Do you enjoy hurting people you love? ..... YES NO
31. Are you often troubled about feelings of guilt? ..... YES NO
32. Do you sometimes talk about things you know nothing about? ..... YES NO
33. Do you prefer reading to meeting people? ..... YES NO
34. Do you have enemies who want to harm you? ..... YES NO
35. Would you call yourself a nervous person? ..... YES NO
36. Do you have many friends? ..... YES NO
37. Do you enjoy practical jokes that can sometimes really hurt people? ..... YES NO
38. Are you a worrier? ..... YES NO
39. As a child did you do us you were told immediately and without grumbling? ..... YES NO
40. Would you call yourself happy-go-lucky? ..... YES NO
41. Do good manners and cleanliness matter much to you? ..... YES NO
42. Have you often gone against your parents' wishes? ..... YES NO
43. Do you worry about awful things that might happen? ..... YES NO
44. Have you ever broken or lost something belonging to someone else? ..... YES NO
45. Do you usually take the initiative in making new friends? ..... YES NO
46. Would you call yourself tense or 'highly-strung'? ..... YES NO
47. Are you mostly quiet when you are with other people? ..... YES NO
48. Do you think marriage is old-fashioned and should be done away with? ..... YES NO
49. Do you sometimes boast a little? ..... YES NO
50. Are you more easy-going about right and wrong than most people? ..... YES NO
51. Can you easily get some life into a rather dull party? ..... YES NO
52. Do you worry about your health? ..... YES NO
53. Have you ever said anything bad or nasty about anyone? ..... YES NO
54. Do you enjoy co-operating with others? ..... YES NO
55. Do you like telling jokes and funny stories to your friends? ..... YES NO
56. Do most things taste the same to you? ..... YES NO
57. As a child were you ever cheeky to your parents? ..... YES NO
58. Do you like mixing with people? ..... YES NO
59. Does it worry you if you know there are mistakes in your work? ..... YES NO
60. Do you suffer from sleeplessness? ..... YES NO
61. Have people said that you sometimes act too rashly? ..... YES NO
62. Do you always wash before a meal? ..... YES NO
63. Do you nearly always have a 'ready answer' when people talk to you? ..... YES NO
64. Do you like to arrive at appointments in plenty of time? ..... YES NO
65. Have you often felt listless and tired for no reason? ..... YES NO
66. Have you ever cheated at a game? ..... YES NO
67. Do you like doing things in which you have to act quickly? ..... YES NO
68. Is (or was) your mother a good woman? ..... YES NO

Initials: ID #: Date:

69. Do you often make decisions on the spur of the moment? ..... YES NO
70. Do you often feel life is very dull? ..... YES NO
71. Have you ever taken advantage of someone? ..... YES NO
72. Do you often take on more activities than you have time for? ..... YES NO
73. Are there several people who keep trying to avoid you? ..... YES NO
74. Do you worry a lot about your looks? ..... YES NO
75. Do you think people spend too much time safeguarding their future with savings and insurance? ..... YES NO
76. Have you ever wished that you were dead? ..... YES NO
77. Would you dodge paying taxes if you were sure you could never be found out? ..... YES NO
78. Clan you get a party going? ..... YES NO
79. Do you try not to be rude to people? ..... YES NO
80. Do you worry too long after an embarrassing experience? ..... YES NO
81. Do you generally 'look before you leap'? ..... YES NO
82. Have you ever insisted on having your own way? ..... YES NO
83. Do you suffer from 'nerves'? ..... YES NO
84. Do you often feel lonely? ..... YES NO
85. Can you on the whole trust people to tell the truth? ..... YES NO
86. Do you always practice what you preach? ..... YES NO
87. Are you easily hurt when people find fault with you or the work you do? ..... YES NO
88. Is it better to follow society's rules than go your own way? ..... YES NO
89. Have you ever been late for an appointment or work? ..... YES NO
90. Do you like plenty of bustle and excitement around you? ..... YES NO
91. Would you like other people to be afraid of you? ..... YES NO
92. Are you sometimes bubbling over with energy and sometimes very sluggish? ..... YES NO
93. Do you sometimes put off until tomorrow what you ought to do today? ..... YES NO
94. Do other people think of you as being very lively? ..... YES NO
95. Do people tell you a lot of lies? ..... YES NO
96. Do you believe one has special duties to one's family? ..... YES NO
97. Are you touchy about some things? ..... YES NO
98. Are you always wiling to admit it when you have made a mistake? ..... YES NO
99. Would you feel very sorry for an animal caught in a trap? ..... YES NO
100. When your temper rises, do you find it difficult to control? ..... YES NO

PLEASE CHECK THAT YOU HAVE ANSWERED ALL THE QUESTIONS

FMRI Study visual analogue scales

Time -30

HOW HUNGRY DO YOU FEEL RIGHT NOW?

NOT AT ALL EXTREMELY

HOW SICK DO YOU FEEL RIGHT NOW?

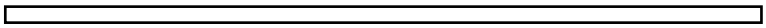
NOT AT ALL EXTREMELY

HOW PLEASANT WOULD IT BE TO EAT RIGHT NOW?

NOT AT ALL EXTREMELY

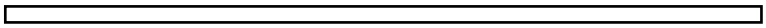
Time -30

HOW MUCH DO YOU THINK YOU COULD EAT RIGHT NOW?



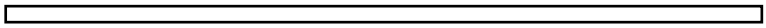
NOTHING A LARGE AMOUNT

HOW FULL DO YOU FEEL RIGHT NOW?



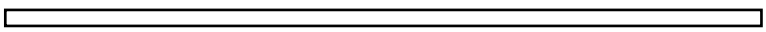
NOT AT ALL EXTREMELY

HOW STRESSED DO YOU FEEL RIGHT NOW?



NOT AT ALL EXTREMELY

HOW SLEEPY DO YOU FEEL RIGHT NOW?



NOT AT ALL EXTREMELY