Weight loss by low calorie diet versus gastric bypass surgery in people with diabetes results in divergent brain activation patterns – an fMRI study.

Running title: fMRI study of VLCD versus RYGB in diabetes

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ABSTRACT

Objective: Weight loss achieved with very low-calorie diets (VLCD) can produce remission of Type 2 diabetes (T2D), but weight regain very often occurs with reintroduction of higher calorie intakes. In contrast, bariatric surgery produces clinically significant and durable weight loss, with diabetes remission that translates into reductions in mortality. We hypothesised that in patients living with obesity and pre-diabetes/T2D, longitudinal changes in brain activity in response to food cues as measured using functional MRI would explain this difference. **Methods:** 16 participants underwent gastric bypass surgery and 19 matched participants undertook a very low calorie (meal replacement) diet for 4 weeks. Brain responses to food cues and resting state functional connectivity was assessed with functional MRI pre- and post-intervention and compared across groups. **Results:** We show that RYGB results in three divergent brain responses in comparison to VLCD-induced weight loss: (i) VLCD resulted in increased brain reward centre food cue responsiveness whereas in RYGB this was reduced; (ii) VLCD resulted in higher neural activation of cognitive control regions in response to food cues, associated with exercising increased cognitive restraint over eating, whereas RYGB did not; (iii) a homeostatic appetitive system (centred on the hypothalamus) is better engaged following RYGB-induced weight loss than VLCD. **Conclusion:** Taken together these findings point to divergent brain responses to different methods of weight loss in patients with diabetes, which may explain weight regain after a short-term VLCD in contrast with the enduring weight loss after RYGB.

INTRODUCTION

Obesity and Type 2 diabetes mellitus (T2D) are amongst the greatest global health challenges of our time (1,2). Population-based weight loss strategies are being heavily invested in by health systems, with ongoing debate about the most effective way to achieve long-term improvements in health. Weight loss achieved with very low calorie diets can produce remission of T2D, lasting up to 2 years in motivated participants (3). However, at population level, lifestyle interventions to achieve long term weight loss are not widely successful, with some suggestion that severe calorie restriction induces hormonal and metabolic responses that encourage the high rates of weight regain (4,5). Only bariatric surgery has been shown to produce enduring weight loss that translates into long-lasting amelioration and even remission of diabetes (6) and risk reduction for cardiovascular and cancer-related mortality (7). The mechanisms by which bariatric surgery produces its metabolic benefits remain incompletely understood (8), but changes in food preference and hunger after the surgery have been frequently reported and are likely to play a role (9). Functional Magnetic Resonance Imaging (fMRI) offers the opportunity to investigate the neural pathways that underpin different methods of weight loss.

A number of studies have reported changes in brain activation patterns before and after bariatric surgery (10–12), but few have focussed on participants with diabetes and prediabetes (13) and none have longitudinally investigated the evolution of these patterns in participants with obesity and diabetes that lose weight through diet versus surgery. Furthermore, reductions in insulin levels or hyperglycaemia may confound observed differences in fMRI signal in longitudinal studies of people with diabetes who have lost weight (15), highlighting the need for a study in which groups of people with diabetes, matched for improvements in glycaemia by different interventions, are compared and contrasted.

To address these important clinical questions, we have compared two groups of participants with pre-diabetes or non-insulin requiring T2D using fMRI before and after weight loss achieved by Roux-en-Y gastric bypass surgery (RYGB) or very low-calorie diet (VLCD). We hypothesised that there would be differences in brain activation patterns in patients undergoing RYGB versus VLCD. Drawing on previous literature, we identify divergent longitudinal changes in brain activity, that fall into three themes. Theme 1 centres on the hypothesis that RYGB induces a reduction in brain reward centre food cue responsiveness not seen with calorie restriction alone. Theme 2 is centred on whether neural activation in regions associated with cognitive control or “rational” decision-making differs with dieting compared with surgery in people with diabetes. Theme 3 focuses on whether a homeostatic system (centred on the hypothalamus) is differentially engaged following RYGB induced weight loss than VLCD.

METHODS

Recruitment. Participants were recruited (ClinicalTrials.gov NCT01945840) at the National Institute for Health Research (NIHR) Imperial Clinical Research Facility at Hammersmith Hospital, London, from July 2016 to October 2018 (14). All participants gave their written consent and the study was performed in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the UK National Health Service (NHS) Health Research Authority West London Research Ethics Committee (reference number 13/LO/1510). Volunteers for the VLCD group were recruited from clinics at the Imperial Weight Centre (IWC) or from newspaper advertising, whereas patients already listed for surgery at the IWC were recruited to the RYGB group. Key eligibility criteria were male or female participants aged between 18 and 70 years, meeting the NHS criteria for bariatric surgery, and with a diagnosis of prediabetes (impaired fasting glucose, impaired glucose tolerance, or HbA1c of 6.0–6.4% [42–47 mmol/mol]) or T2D according to World Health Organization criteria. Accepted participants had a stable HbA1c of <9.0% (75 mmol/mol) either on diet or a single oral hypoglycaemic agent. Key exclusion criteria were any comorbidities or medications that could compromise the validity and safety aspects of the study, a current history of smoking or substance misuse, pregnancy, and a history of eating disorders.

Study visits. Pre-intervention (VISIT 1), the RYGB participants underwent an fMRI scan in the morning having fasted from 10pm the night before, and filled in the Dutch Eating Behaviour Questionnaire (15). RYGB surgery was performed laparoscopically according to standardized techniques by three designated surgeons at IWC. An identical fMRI scan and study visit was repeated at postoperative week 4 (VISIT 2). VLCD participants attended the research unit at VISIT 1, before starting a complete meal replacement VLCD of 800 kcal/day for 4 weeks (Cambridge Weight Plan, UK). An fMRI scan was performed at VISIT 1 and

VISIT 2 at week 4 (Supplementary Figure 1A). At arrival, before and after the MRI scan, then before and after lunch subjects completed a 100 mm visual analogue score (VAS) that rated nausea and pleasantness to eat. At each visit, fasting blood samples for gut hormones were collected in lithium heparin tubes containing Aprotinin (Nordic Pharma, UK) and a dipeptidyl peptidase 4 inhibitor Diprotin A (20 µg/mL blood; Enzo Life Sciences, UK). Samples were placed on ice, centrifuged at 4°C within 10 min of collection, and separated plasma stored at -80°C until analysis. Active GLP-1, Ghrelin and GIP levels were measured by a customised Milliplex magnetic bead-based multianalyte, metabolic panel immunoassay (Millipore, US). Total PYY was measured by an in-house radioimmunoassay utilizing a polyclonal antiserum recognizing both PYY₁₋₃₆ and PYY₃₋₃₆ (16). The intra- and inter-assay CV was <10% and <15% respectively for the Milliplex metabolic panel and <10% for the RIA. The lowest limit of detection was 0.8 pmol/L for GLP-1, 4.1 pmol/L for Ghrelin, 0.3 pmol/L for GIP and 8.7 pmol/L for PYY.

Functional MRI. Pre- and post-intervention MRI sessions lasted approximately 60 minutes and in addition to a localizer and a high-resolution T1-weighted anatomical image, included:

Food images task: This was a block-design task that was presented in two separate runs and consisted of food (appetising and bland) and object images derived from the study of Beaver et al (17). For the purposes of the present study the images were grouped into two categories: food images (divided into low and high calorie types) and non-food objects (see exemplar images in Supplementary Figure 1B). Each block included 5 images and each image was presented for approximately 3 seconds. The blocks were presented in a pseudo-random order and they were mixed with equally long resting blocks showing a blank screen. Each run consisted of 40 blocks (10 of each category) and the images in each run differed.

Resting state: During the resting state scan, participants were asked to keep their eyes open and fixate on a white cross in the middle of a grey screen.

MRI acquisition. Scanning was performed on a 3T Siemens Verio scanner with 32-channel phased-array head coil. Anatomical images were acquired at the beginning of each scan using a T1-weighted MPRAGE pulse sequence (1 mm isotropic voxels, TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, 160 axial slices). Functional images were acquired using a 3D Echo Planar Imaging (EPI) Sequence with the following parameters: TR = 2000 ms, TE = 30 ms, flip angle = 80°, 35 axial slices, voxel size = 3 mm isotropic. A different number of volumes was acquired for each task depending on the task duration: each food images task: ~10 mins and 305 volumes and resting state: ~8 mins and 240 volumes.

Whole-brain analysis. Imaging data was processed using FSL (www.fmrib.ox.ac.uk/fsl/) version 5.0.4 (Oxford Centre for Functional Resonance Imaging of the Brain [FMRIB]). Anatomical images were skull-stripped and segmented using the anatomical processing script (fsl_anat) in FSL. Functional image series were pre-processed using the following parameters: high-pass filter (100s), head motion correction, 6 mm (full width at half maximum [FWHM], Gaussian) spatial smoothing. The functional images of each individual were then co-registered to their T1 structural image and a standard anatomical template in the MNI152 space. For the analysis of the food images and fMRI battery task, a standard general linear model (GLM) was used, as implemented in the FEAT module in FSL (18) and incorporated FILM (FMRIB's Improved Linear Model) correction for auto-correlation. In the first-level models, regressors derived from the onset times of each stimulus condition were convolved with a gamma function to simulate the hemodynamic response function (HRF), and the first temporal derivatives of each stimuli time series were included in the model. Additionally, regressors derived from head-motion parameters were also included as regressors of no interest. First, a group-level analysis employed a mixed-effects (FMRIB's Local Analysis of Mixed Effects or FLAME-1) model and a statistical threshold of $Z = 2.3$ ($P < 0.05$ cluster-corrected for multiple

comparisons). Second, a follow-up group-level analyses was performed on a sub-group of tightly weight-matched participants ($n=7$ per intervention) using a fixed-effects model and a statistical threshold of $Z = 2.3$ ($P < 0.05$ cluster-corrected for multiple comparisons). Contrasts were computed to model the effect of each stimuli against baseline and the effects between the different stimuli in each task. For the food images task, the contrasts were: food or objects > baseline, and food > objects.

For the ROI analysis we specified a combination of functional and anatomical ROIs depending on the particular hypotheses tested and the most appropriate and practical methods for defining regions in different areas. Most of our ROIs were functionally defined since many areas that are described as relevant to food cue responsivity, such as the dlPFC, are not accurately defined as distinct anatomical areas in the brain, and may either encompass two or more anatomical regions, or be a sub-part of a larger anatomical region. In addition, functional definitions are often considered superior to anatomical with regards to inter-subject variability (19,20). To avoid biases, all the functional ROIs for the food task were independently defined based on the group mean effect of the task in all participants in the contrast “all stimuli > baseline”. This means that all scans from all participants were grouped to look for clusters of brain activity that were activated in response to seeing pictures (of any variety; food and non-food) compared with baseline (resting periods where no pictures were shown). These brain areas were visually compared with an established map (the Harvard-Oxford Atlas) of specified regions (21). This procedure is in line with the recommendations of Friston et al who suggest that this method of fROI definition is preferable to procedures using a separate localiser experiment (22). ROIs in this constrained functional space that corresponded to the five areas that made up our “Reward System” and the five areas that comprised the “Executive Control System” were created, and their coordinates (in MNI space) are listed in Supplementary Table 1.

For the hypothalamus, a functional definition was not possible due to its small size and location in an area with high magnetic susceptibility effects. Therefore an anatomical definition was

used following the localisation procedure described in Baroncini et al. (23). The same mask was used as a seed in the resting-state network analysis. The primary purpose for incorporating the hypothalamus in this analysis was to test the hypothesis that interconnectedness between homeostatic and hedonic networks in the brain might be modulated differentially by different means of weight loss.

Data from ROIs were extracted for each scan. Food cue induced signal (above baseline) was compared for each subject (post-intervention minus baseline) and each ROI. Differences between intervention groups was compared using two-tailed unpaired Student t-tests, after testing for Gaussian distribution using a D'Agostino-Pearson test.

Resting state analysis. A seed-based connectivity analysis was performed on the resting state data using the anatomical hypothalamus ROI as a mask, as previously applied (24–26). Time-series were extracted from the hypothalamus seed for each participant and included as regressors in the first-level model. Additionally, each participant's anatomical images were segmented into white matter and CSF masks and time-series were extracted for each (using FSL's `fsl_anat` and `FAST` modules). These time-series were included in the models as regressors of no interest along with standard head-motion regressors, and the model also incorporated FILM correction for autocorrelation. Subsequently, a higher-level analysis compared baseline and post-intervention scans using FSL's FEAT using a mixed-effects cluster-corrected ($Z > 2.3$, $P < 0.05$) FLAME-1 model.

Statistical Analyses. A power calculation, using the standard deviation of the reward-system ROI dataset from previous fMRI studies in our unit comparing reward network activation in the fed versus the fasted state, estimated that 16 participants per group were required to establish a VISIT 2 – VISIT 1 mean % BOLD signal difference between RYGB versus VLCD of 0.1 with a power of 0.8 and an alpha of 0.05 (18). Continuous data for other measurements

between VLCD and RYGB groups are presented as mean $\pm$ standard error of the mean (SEM) following normality testing with the D'Agostino-Pearson test. Fisher's exact tests were used to compare categorical variables and differences between proportions. Paired *t*-tests were used to determine changes in clinical measurements between baseline and post intervention unpaired between groups. VAS scores over the course of the study visits were adjusted for baseline were compared by repeated-measures nonparametric Friedman's test with Dunn's multiple comparison *post hoc* test. Changes in DEBQ scores pre- and post-intervention in each group were compared using Mann-Whitney tests. Linear regression was performed to assess the relationship between longitudinal changes in MRI signal per ROI and changes in gut hormone levels or questionnaire score. A threshold of $P < 0.05$ was used for statistical significance. Statistical analysis was performed using Prism version 8 (GraphPad Software, US).

RESULTS

A total of 16 participants in the RYGB group and 19 in the VLCD group completed pre- and post- intervention study visits and were included in the analysis. Pre-intervention, subjects were matched for age and gender, with similar spread of diabetes diagnoses and use of oral antidiabetic drugs (Table 1). The RYGB group at baseline weighed on average 119.9 ± 6.1 kg and lost 12.3 ± 0.89 kg (-10.4%) whereas the VLCD group started on average at 109.2 ± 4.98 kg and lost 8.4 ± 0.56 kg (-8.4%) by VISIT 2 at 4 weeks ($P=0.002$ for absolute weight loss between groups at 4 weeks). At 12 months, the VLCD group regained all the weight previously lost while the RYGB group continued to lose weight. HbA1c values between VISIT 1 and VISIT 2 fell to a similar degree in each group (53.9 ± 2.65 mmol/mol to 46.0 ± 1.97 for the RYGB group and 53.1 ± 2.52 to 46.6 ± 2.1 for the VLCD group). Importantly, there was also a comparable reduction at 4 weeks in both fasting glucose (-2.48 ± 0.44 for RYGB vs -1.84 ± 0.50 mmol/L for VLCD, $P=0.35$) and fasting insulin (-7.13 ± 1.82 for RYGB vs -6.81 ± 1.15 mIU/L for VLCD, $P=0.88$) following the interventions. A subgroup of very closely matched patients for weight loss ($n=7$ weight-matched from each intervention, weight loss 8.2 ± 0.68 kg for the RYGB subgroup and 8.2 ± 0.67 kg for the VLCD subgroup) was also identified for subgroup analyses that were free of the potential confounding effects of unmatched weight loss (Supplementary Table 2).

There was a significant increase in fMRI activity in both groups when subjects looked at images of food compared to non-food objects, confirming the validity of the task design. Group-level (FLAME-1) analysis did not reveal a between group and between treatment difference. Fixed-effects group-level analysis on the weight-matched subgroups ($n=7$) confirmed brain areas involved in both hedonic responses and executive control were significantly deactivated in the RYGB group post-intervention compared with the VLCD group (Supplementary Figure 2D).

These concepts are expanded in the *a priori* ROI analysis below. Of note, comparison of VISIT 1 food cue responses showed no differences between the groups (Supplementary Figure 2A). VAS scores revealed no differences between the VLCD group and the RYGB group both pre- and post-intervention in terms of nausea ratings or prospective scores for pleasantness to eat on the study visit days (Supplementary Figure 2B and 2C).

Functional ROIs related to the Reward System

Functional ROIs pertaining to the hedonic reward/salience network in feeding studies (amygdala, caudate, insula, nucleus accumbens, OFC, putamen) were analysed, chosen because of their functional implications in other similar studies (10,27). All of the reward system regions of interest tended towards a relative hypo-responsiveness to food cues post-intervention in the RYGB group compared with the VLCD group (Figure 1A: *P*-values for each comparison are given in the figure legend, none reached <0.05). In the subgroup analysis of $n=7$ highly weight-loss matched participants, the divergent pattern of activation in the VLCD group versus deactivation in the RYGB group held true significantly for the nucleus accumbens ($P=0.001$) and the putamen ($P=0.004$) (Supplementary Figure 2E). Overall, the complete VLCD group demonstrated a significant augmentation in responsivity to food cues within the combined “Reward System” ROIs post intervention ($P=0.03$, Figure 1B). This finding was in keeping with the whole brain analysis of that group and was also evident in the weight-matched subgroup comparison ($P=0.01$, Supplementary Figure 2F). A correlation analysis of changes in gut hormone levels between visits and changes in reward ROI activity did not reveal any strong associations (Supplementary Table 3).

The DEBQ questionnaire looks at three elements of disordered eating: restraint, emotional eating and susceptibility to external food cues. 8 people in the VLCD group and 5 in the RYGB

group did not fill in or return their second questionnaire and so baseline datasets for these patients were also excluded. The reduction in external cue score (which best reflects how a person is enticed by food cues), from baseline to post intervention, was significantly greater for the RYGB group compared with the VLCD group ($P=0.01$, Figure 1C). Taken together, these findings suggest that RYGB-mediated weight loss results in a brain activation pattern that underpins a reduced reward drive to food cues.

Functional ROIs related to the Executive Control System

When we compared post-intervention to pre-intervention (VISIT 2 – VISIT 1) in each group, we saw no significant difference in whole brain activation in response to food cues in the RYGB group, whereas in the VLCD group we saw significantly increased brain activation in the posterior cingulate gyrus, inferior frontal gyrus and middle temporal gyrus. These are all areas with a role in executive control that have previously been shown to be modulated with weight loss (28,29) and suggest that weight loss with VLCD requires a stronger executive (inhibitory/restraint) response to food images compared with RYGB, implying that subjects may need to engage a greater restraint or effort to suppress food cravings following VLCD than RYGB. Using functional ROIs based on the Neurosynth guide to the regions involved in executive control/decision making (30), we defined functional ROIs that grouped into our “Executive Control System”. As for the “Reward System” analysis, no single ROI was significantly more engaged in one treatment group compared with the other post intervention (Figure 1D), however taken as a whole, the Executive Control System was significantly deactivated post intervention in the RYGB group compared with the VLCD group ($P=0.017$ Figure 1E).

The Restraint subsection of the DEBQ questionnaire is most likely to reflect the participant's ability to exert executive control on their eating. Interestingly, both the RYGB and the VLCD groups reported higher restraint scores after their weight loss intervention, although this only reached significance compared to baseline in the RYGB group (Figure 1C). A correlation matrix of DEBQ score changes and ROI signal changes by group revealed that, allowing for multiple comparisons, longitudinal changes in food cue responsivity in the paracingulate gyrus correlated with DEBQ-R score increases in the VLCD group but not the RYGB group (Figures 2A and 2B). The signal change in the paracingulate gyrus ROI did diverge in the two groups, but not significantly so (mean VISIT 2-VISIT 1 signal change in the paracingulate gyrus in the RYGB group was -12.2 vs +0.3 in the VLCD group, $P=0.08$). Caudate signal similarly diverged between the two groups (Figure 1A) but again only in the VLCD group did this longitudinal signal change correlate with changes in DEBQ-R scores ($R^2=0.78$, $P=0.007$ for VLCD and $R^2=-0.41$, $P=0.21$ for RYGB group) (Figures 2C and 2D). Taken together these results suggest that there appears to be a reduced requirement to activate the executive control system following RYGB in comparison to VLCD. Whilst both groups report greater restraint scores on questionnaires, the neural substrates underpinning this may be different.

Homeostatic versus Non-homeostatic systems

An anatomically defined ROI for the hypothalamus also revealed a divergent pattern in hypothalamic signal change pre- and post-treatment between the two intervention groups. The task-based signal change in the hypothalamus between VISIT 1 and VISIT 2 reduced in the RYGB group whereas it increased in the VLCD group ($P=0.046$). Hypothalamic signal change pre-and post-intervention was significantly correlated with the achieved weight loss for the RYGB group but not the VLCD group (Figure 2E and 2F). When the hypothalamus was used as a seed region in resting state analysis, we found an increased post-surgical resting state

connectivity between the hypothalamus and numerous brain areas including the dPFC, putamen, globus pallidus and the cingulate gyrus, whereas this connectivity increase was absent in the VLCD group after their weight loss (Figure 2G). Taken together, these findings suggest that RYGB engages a stronger homeostatic regulation of food intake than VLCD.

DISCUSSION

This study is the first to report on these three interconnecting phenomena occurring in people with diabetes in a longitudinal comparison between weight loss groups. The importance of such longitudinal trial designs in weight loss settings has been highlighted by others (31). The complex appetitive brain processes that control food intake have been described as three interconnecting networks: cognitive, hedonic and homeostatic (32). We show that RYGB results in three divergent brain responses in comparison to VLCD-induced weight loss: first, RYGB induces a relative reduction in food cue responsiveness in areas of the brain known to be associated with food reward processing. Second, weight loss with RYGB is facilitated by reduced requirement for higher neural activation of cognitive control regions, which is linked to cognitive restraint over eating as measured by the DEBQ. Third, a homeostatic appetitive system centred on the hypothalamus is better engaged following RYGB induced weight loss than VLCD, with reduced activation to food cues in the hypothalamus and increased connectivity to reward areas in the RYGB group at rest, suggesting that reduced hunger may mediate the observed changes in food reward after RYGB. The findings suggest that RYGB induces multiple brain activation patterns that may guard against weight regain, whereas VLCD induces changes which may make continued weight loss more difficult to sustain. As we studied our participants at a relatively early stage post-operatively, we cannot rule out that other changes in brain activity may become apparent at later stages. It should also be noted that whilst the groups were not randomised to their intervention, there was no obvious difference in their baseline characteristics. We are not able to provide data for brain responses in response to a return to an eucaloric diet in patients who had lost weight. Both groups lost over 10% of their body weight by the second scan, however the RYGB group lost significantly more than the VLCD cohort, although there is no evidence to suggest that the results presented here are not due to the different mechanisms of weight loss as opposed to any baseline or total body weight

loss differences between the comparator groups. We have also presented subjective scores filled in by participants during the scan visits to exclude the possibility that the surgical group were experiencing more nausea or other noxious sensations (due to their recent surgery) than the VLCD group.

The first theme in our study is that RYGB induces a reduction in brain reward centre food cue responsiveness compared with calorie restriction alone. The phenomenon of reward hypo-reactivity after surgical weight loss is well established in the fMRI literature, although these studies did not include people with diabetes. Ochner et al. reported a reduction in post-RYGB activation to high fat food cues in a range of corticolimbic areas within the mesolimbic reward pathway, including the ventral striatum and putamen (12), and Faulconbridge et al. reported a significant decline in ventral tegmental area response to food images after RYGB but not weight stable controls (11). We have previously shown that within a hedonic reward system (OFC, amygdala, anterior insula, nucleus accumbens and caudate) the fMRI signal in response to high calorie food cues was lower in people who had undergone RYGB than weight matched controls and also in patients who have undergone RYGB compared with gastric band surgery (10). Of particular note in the present study was the finding that caudate activity correlated strongly with restraint scores in the VLCD group, who also exhibited significantly less improvement in external cue DEBQ scores post intervention compared with RYGB patients. These findings point to the possibility that patients in the VLCD group were more susceptible to food cravings. Indeed, Volkow et al. demonstrated increased dopamine levels in the caudate nucleus of hungry subjects exposed to food cues (33), and Small et al. conversely report a decrease in dopaminergic binding potential in the dorsal striatum of fasted subjects who have been allowed to eat to satiety (34). An fMRI study by Pelchat et al. designed to elicit food cravings in hungry subjects confirmed significant caudate activation in this setting (35).

The concept that weight loss might be related to activity in regions implicated in cognitive control seems intuitive to anyone who has tried, and failed, to diet. In 2008 Rosenbaum et al. (28) reported that maintenance of a reduced body weight was associated with increased neural activity in systems mediating aspects of executive and decision-making functions (middle temporal gyrus, inferior frontal gyrus and lingual gyrus). In line with this, Kahathuduwa et al. investigated the effects of a 3-week hypocaloric total meal replacement diet compared with an iso-caloric typical diet (portion control), and reported that meal-replacement increased activation in the anterior cingulate, primary motor and left insular cortices, areas involved in executive inhibitory control over ingestion and food reward (36). Recently, Neseliler et al. tested the hypothesis that weight loss would be related to activity in regions implicated in cognitive control. They showed increased fMRI signal in the same regions associated with cognitive control as depicted in our results, which correlated with weight loss at one month (37). Conversely, Zoon et al. reported that the post RYGB shift in food preferences away from high-calorie foods correlates with decreased superior parietal activation to food cues (38). However, in the closest comparable study to ours, Baboumian et al. compared whole brain activation in response to food cues after RYGB with a control group that lost weight with diet alone, and reported that the surgery group showed *increased* dorsolateral prefrontal cortex (dlPFC) activation in response to appetising food cues, suggesting *greater* cognitive dietary inhibition (39). This differs from our findings but it is important to note that the dietary weight loss group in this study was no longer on caloric restriction at the time of the second scan, which may have removed the metabolic drive for enhanced cognitive restraint. dlPFC activation is believed to be instrumental in weight loss (40) and it may seem counterintuitive that a reduced activity in areas of cognitive control would protect against weight regain. However, our data suggests that there is a reduction in requirement for cognitive control after RYGB which may be driven by the reduction in reward appeal to food cues and an improved

connectivity with the homeostatic system. In our surgical group this seemed to be driven largely by changes in the paracingulate gyrus, an area well described to be involved in cognitive response inhibition (41).

The homeostatic regulation of body weight control is centred on the hypothalamus, which is able to assimilate hormonal cues from adipose tissue and the gut to modulate appetite (42). Here, we propose that this homeostatic system is better engaged following RYGB induced weight loss than VLCD. It has been previously reported that hypothalamic activation in response to food cues is more pronounced after surgery, as a marker of enhanced satiation (38). It has been reported that in healthy normal weight volunteers hypothalamic fMRI signal positively co-varies with circulating PYY levels (43). Both PYY and GLP-1 levels are elevated in the post-prandial state following bariatric surgery (44) while fasting ghrelin levels have been shown to decline post-operatively (45). Tenkolve et al. have reported that the GLP-1 analogue exenatide promotes hypothalamic activity in humans and hypothalamic connectivity with the rest of the brain (46) and Meyer-Gerspach et al. extended this to the action of endogenous GLP-1, revealing that administration of the GLP-1 receptor antagonist exendin 9-39 disrupted functional connectivity between homeostatic (hypothalamus) and reward-related (orbitofrontal cortex) brain regions (47). More recently another study by Tenkolve et al. showed that in ten obese females undergoing RYGB, activation was reduced in the caudate nucleus to response to food and that this was interrupted after RYGB with GLP-1R blockade (48). Circulating levels of ghrelin have also been implicated in modulating activation of the reward system. Changes in fasting ghrelin levels post RYGB have been positively linked with changes in BOLD activation in the Ventral Tegmental Area (VTA), a key area for reward processing (11) while similar associations were also found with other reward-related areas (dorsolateral prefrontal cortices) in obesity (49). We did not see any correlation between this increase in

hypothalamic connectivity or reward hypo-activity with prevailing gut hormone levels in our patient groups, suggesting that the changes in brain activity we have detected are not simply explained by acute changes in gut hormone secretion but may represent a longer-term response. We find that the hypothalamus becomes significantly more connected to higher brain centres following bariatric surgery, suggesting the engagement of a homeostatic appetitive system that feasibly promotes maintenance of satiety. The hedonic drive may therefore be reduced by the hypothalamic drive in a bottom-up fashion, since a reduced subconscious hunger drive feeds into reduced cravings and a drive to eat high calorie foods (50).

In summary this is the first longitudinal fMRI study comparing groups of people with diabetes who have lost weight through either RYGB or VLCD. We provide evidence that those who have undergone bariatric surgery experience three complementary changes in appetitive brain processing compared with those who have lost weight through calorie restriction. These divergent brain responses to different methods of weight loss may explain why there is a tendency to regain weight after completion of a short-term VLCD. Any effective and durable weight loss programme aimed at inducing remission of T2D through weight loss should perhaps focus on interventions that induce changes in brain activity similar to those induced by RYGB, and our findings point the way towards fMRI-based methods for assessing the likely long-term efficacy of such interventions.

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Author contributions statement

P.B., K.A., G.T. contributed to the execution of the clinical study. T.M-M.T, S.R.B, W.S.D, V.S, S.S, M.W. designed the study. A.R.A., S.P. performed the surgery in the prospective RYGB cohort. V.S., P.B., L.D., M.W prepared the statistical analysis. V.S., P.B., T.M-M.T., K.A. wrote the manuscript and all authors contributed to the editing of the manuscript. T.M-M.T. is the guarantor of this work, had full access to the data, and accepts full responsibility for integrity of the data and the accuracy of the data analysis.

Conflict of Interest Statement

All authors report no potential conflicts of interest.

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TABLE 1

	Roux-en-Y Gastric Bypass (RYGB)	Very Low Calorie Diet (VLCD)	P-value between treatment groups
n	16	19	
Female: Male	13: 3	11: 8	0.17
Age (years)	48.6 ± 14.4	46.2 ± 10.8	0.57
Weight at baseline (VISIT 1) (kg)	119.9 ± 6.1	109.2 ± 4.98	0.18
Weight at with intervention at VISIT 2 (kg)	107.7 ± 5.98	100.8 ± 4.54	0.35
Weight loss between fMRI scans (VISIT 2-VISIT 1) (kg)	-12.3 ± 0.89	-8.42 ± 0.66	0.002
Percentage Weight loss between fMRI scans (%)	-10.42 ± 0.86	-7.66 ± 0.39	0.004
Follow up weight one year after study entry (kg)	83.80 ± 4.43	109.06 ± 7.15	0.0064
HbA1c at VISIT 1 (mmol/mol)	53.94 ± 2.65	53.11 ± 2.52	0.82
HbA1c at VISIT 2 (mmol/mol)	46.00 ± 1.97	46.63 ± 2.1	0.83
Fasting glucose at VISIT 1 (mmol/L)	8.37 ± 0.46	7.64 ± 0.60	0.37
Fasting glucose at VISIT 2 (mmol/L)	5.89 ± 0.25	5.83 ± 0.25	0.85
Δ Fasting glucose (mmol/L)	-2.48 ± 0.44	-1.84 ± 0.50	0.35
Fasting insulin at VISIT 1 (mIU/L)	19.52 ± 1.73	16.23 ± 1.38	0.14
Fasting insulin at VISIT 2 (mIU/L)	12.38 ± 1.40	9.43 ± 0.84	0.07
Δ Fasting insulin (mIU/L)	-7.13 ± 1.82	-6.81 ± 1.15	0.88
Treatment for diabetes during the study	Diet:8 Metformin:7 DPP4 inhibitor:1	Diet:10 Metformin:9 DPP4 inhibitor:0	>0.99

Table 1. Baseline clinical and biochemical characteristics of patients in the study. 16 patients with impaired glucose tolerance or non-insulin requiring Type 2 diabetes were treated with RYGB and 19 patients with VLCD for 4 weeks. An fMRI scan was performed at baseline (VISIT 1) and again after the intervention (VISIT 2). For most patients VISIT 2 occurred at 4 weeks after study entry, except for three patients in the RYGB group who had a scan at 12 weeks post operatively due to medical or logistic reasons. Demographic and clinical characteristics for both study groups at baseline (VISIT 1) and at the end of the intervention (VISIT 2) are shown. Continuous variables (checked for normality of distribution) are shown as means ± SEM. P-values represent the results of unpaired t-tests between the RYGB and VLCD treated groups.

FIGURE LEGENDS

Figure 1. Functional ROI analysis of food task.

(A) Mean % BOLD signal change in a priori (functional) reward-based ROIs in response to food images against objects in the VLCD (in black) and RYGB (in red) groups. Mean % BOLD signal change (post intervention vs baseline) across all six ROIs (amygdala, caudate, insula, nucleus accumbens, OFC, putamen – the Reward System) in response to food images in the VLCD (in black) and RYGB (in red) groups. Data are shown as mean $\pm$ SEM, VLCD: $n=19$, RYGB: $n=16$. p -values for the comparisons are: amygdala $p=0.23$, caudate $p=0.31$, insula $p=0.61$, nucleus accumbens $p=0.18$, OFC $p=0.76$, putamen $p=0.32$ (B) Taking the “Reward System” as a whole (summed ROIs described in 1A), there was a significant down-activation in the RYGB group in response to food cue after surgery compared with the pre- to post- change in the VLCD. (C) Changes in the restraint subsections of the DEBQ scores pre- and post-weight loss in either group are plotted. Using a one sample Wilcoxon test, only the scores for DEBQ-Restraint and DEBQ-External in the RYGB group (not the VLCD group) changed significantly post vs pre intervention ($p=0.008$ and $p=0.0008$ respectively). Between group comparison (VISIT 2 – VISIT 1 for RYGB vs VLCD) showed a significant divergence only on the DEBQ-Restraint scores, $p=0.01$ as highlighted on the graph. (D) Mean % BOLD signal change in a priori (functional) executive-control ROIs in response to food images against objects in the VLCD (in black) and RYGB (in red) groups. Mean % BOLD signal change (post intervention vs baseline) across all six ROIs (ventromedial prefrontal cortex (vmPFC), hippocampus, insular cortex, paracingulate gyrus, middle frontal gyrus, parietal lobule – the Executive Control System) in response to food images in the VLCD (in black) and RYGB (in red) groups. Data are shown as mean $\pm$ SEM, VLCD: $n=19$, RYGB: $n=16$. P -values for the comparisons are: vmPFC $p=0.80$, hippocampus $p=0.07$, insular cortex $p=0.99$, paracingulate gyrus $p=0.08$, middle frontal gyrus $p=0.18$, parietal lobule $p=0.39$. (E) Taking the “Executive Control System” as a whole (summed ROIs described in 1D), there was a significant down-activation in the RYGB group in response to food cue after surgery compared with the pre- to post- change in the VLCD.

Figure 2. Brain region correlates with DEBQ and weight loss and resting state data

(A-D) Paracingulate gyrus and caudate signal change from pre- to post-intervention strongly correlated with DEBQ-Restraint scores in the VLCD group but not the RYGB group (matched fMRI and DEBQ datasets were available for $n=11$ per group). (E-F) Hypothalamic signal (anatomical ROI) reduced in response to food cues in the RYGB group whereas it increased in the VLCD group ($P=0.05$). Of note, body weight loss correlated with signal change in the hypothalamus only in the RYGB group. (G) On resting state analysis, the hypothalamus was used as a seed region. Seed based connectivity analysis revealed a significantly augmented connectivity between the hypothalamus and numerous areas including the putamen, globus pallidus and the cingulate gyrus post-surgery, whereas this connectivity increase was absent in the VLCD group after their weight loss.

Supplementary Figure 1. Study Design

(A) Flow diagram illustrating the study visits for both groups. 16 patients with impaired glucose tolerance or non-insulin requiring Type 2 diabetes were treated with RYGB and 19 matched patients with VLCD for 4 weeks. An fMRI scan was performed at baseline (VISIT 1) and again after the intervention (VISIT 2). For the majority of patients VISIT 2 occurred at 4 weeks after study entry, except for three patients in the RYGB who had a scan at 12 weeks post operatively due to medical or logistic reasons. Importantly, all patients in the VLCD group were still on the diet at the time of the second scan (VISIT 2). (B) The BOLD task design consisted of a

block-design task that was presented in two separate runs and consisted of food (appetising and bland) and object images, examples of which are displayed here.

Supplementary Figure 2. Baseline characteristics, VAS scores and sub-group analyses

(A) VISIT 1 comparison of food cue induced brain activity patterns in the Reward System ROIs (see main Figure 1A). There were no differences in baseline ROI responses to visual food cues between the groups that went on to undergo RYGB or VLCD indicating no inherent differences in brain activity between the two groups. (B and C) Visual Analogue Scores (VAS) for nausea and pleasantness, as rated by participants at the start of their study visit and all the way through to after lunch at the end of their fMRI scan. There were no differences in any rating between the two groups or pre- to post- intervention. Black squares= VLCD group at baseline. White squares= VLCD group at 1 month post-intervention. Red circles= RYGB group at baseline. White circles= RYGB group 1-3 months post-intervention. (D) Sub-group whole brain between group analysis for $n=7$ per group of tightly weight-matched participants (see Table S2). Fixed effects analysis for the comparison VISIT 1>VISIT 2 AND RYGB > VLCD revealed several brain regions (subsequently labelled with reference to the Harvard-Oxford atlas) that were significantly deactivated in the RYGB group compared to the VLCD group in response to weight loss. All statistical maps thresholded at $z=2.3$, $P<0.05$ (cluster corrected for multiple comparisons), (VLCD: $n=19$, RYGB: $n=16$). (E) The weight-matched subgroup (see Table S2) were subjected to analysis of mean % BOLD signal change in a priori (functional) reward-based ROIs. Mean % BOLD signal change (post intervention vs baseline) across all six ROIs (amygdala, caudate, insula, nucleus accumbens, OFC, putamen – the Reward System) in response to food images in the VLCD (in black) and RYGB (in red) groups. Data are shown as mean $\pm$ SEM, VLCD: $n=7$, RYGB: $n=7$. P -values for the comparisons are: amygdala $p=0.32$, caudate $p=0.17$, insula $p=0.26$, nucleus accumbens $p=0.0095$, OFC $p=0.65$, putamen $p=0.043$ (F) Taking the “Reward System” as a whole (summed ROIs described in 1A), there was a highly significant ($p<0.01$) down-activation in the RYGB group in response to food cue after surgery compared with the pre- to post- change in the VLCD.