

Elevated postoperative endogenous GLP-1 levels mediate effects of Roux-en-Y gastric bypass on neural responsivity to food cues.

Jennifer S. ten Kulve¹ M.D. Ph.D., Dick J. Veltman² M.D. Ph.D., Victor E.A. Gerdes³ M.D. Ph.D., Liselotte van Bloemendaal¹ M.D. Ph.D., Frederik Barkhof⁴ M.D. Ph.D., Carolyn F Deacon⁵ Ph.D., Jens J Holst⁵ M.D. Ph.D., Madeleine L. Drent⁶ M.D. Ph.D., Michaela Diamant^{1†} M.D. Ph.D., Richard G. IJzerman¹ M.D. Ph.D.

¹Diabetes Center / Department of Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands; ²Department of Psychiatry, VU University Medical Center, Amsterdam, the Netherlands; ³Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands; ⁴Department of Radiology & Nuclear Medicine, VU University Medical Center, Amsterdam, the Netherlands; ⁵The NNF Center for Basic Metabolic Research and Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Denmark; ⁶Department of Internal Medicine / Endocrine Section, VU University Medical Center, and the Department of Clinical Neuropsychology, VU University, Amsterdam, the Netherlands; † deceased April 2014.

Corresponding author: J.S. ten Kulve, Department of Internal Medicine, Diabetes Center VU University Medical Center, PO BOX 7057, 1007 MB Amsterdam.

Phone: +31 20 444 2974, Fax: +31 20 444 3349, E-mail: js.tenkulve@vumc.nl

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ABSTRACT

Objective: It has been suggested that weight reduction and improvements in satiety after Roux-en-Y gastric bypass (RYGB) are partly mediated via postoperative neuroendocrine changes. Glucagon-like peptide-1 (GLP-1) is a gut-hormone secreted after food ingestion and is associated with appetite and weight reduction, mediated via effects on the central nervous system (CNS). Secretion of GLP-1 is greatly enhanced after RYGB. We hypothesized that postoperative elevated GLP-1 levels contribute to the improved satiety regulation after RYGB via effects on the CNS.

Research Design and Methods: Effects of the GLP-1 receptor antagonist exendin9-39 (Ex9-39) and placebo were assessed in ten women before and after RYGB. We used functional magnetic resonance imaging (fMRI) to investigate CNS activation in response to visual food cues (pictures) and gustatory food cues (consumption chocolate milk), comparing results with Ex9-39 vs. placebo before and after RYGB.

Results: After RYGB, CNS activation was reduced in the rolandic operculum and caudate nucleus in response to viewing food pictures ($P=0.03$) and in the insula in response to consumption of palatable food ($P=0.003$). GLP-1 levels were significantly elevated postoperatively ($P<0.001$). After RYGB, GLP-1 receptor blockade resulted in a larger increase in activation in the caudate nucleus in response to food pictures ($P=0.02$) and in the insula in response to palatable food consumption ($P=0.002$).

Conclusions: We conclude that the effects of RYGB on CNS activation to visual and gustatory food cues may be mediated by central effects of GLP-1. Our findings provide further insights into the mechanisms underlying the weight lowering effects of RYGB.

Bariatric surgery is currently the most effective therapeutic modality for severe obesity in terms of substantial weight loss and long-term efficacy(1). The most commonly performed procedure is Roux-en-Y gastric bypass surgery (RYGB), which comprises the formation of a small gastric pouch, which is connected to the mid-jejunum, bypassing the duodenum and proximal jejunum. This may lead to reduced ingestive capacity and also some reduction in the absorption of calories. However, it has been suggested that the reduction in caloric intake after RYGB is not only explained by these restrictive and/or absorption-limiting mechanisms, but that RYGB has additional effects on caloric intake by diminishing appetite via changes in the central nervous system (CNS) and endocrine system(2).

The CNS is important in the regulation of food intake, and it has been proposed that altered CNS responses may contribute to disturbances in this regulation. Altered responses to visual and gustatory food cues have indeed been described in obese individuals, using functional magnetic resonance imaging (fMRI)(3-5). Interestingly, weight loss after RYGB is paralleled by decreased responsivity of the CNS to high-calorie visual food cues, measured with fMRI(6;7), which may contribute to the reduced hedonic drive to consume high palatable food and therefore contribute to the substantial weight loss after RYGB. However, the mechanism explaining this altered CNS responsivity to food cues after RYGB is unknown.

Appetite and satiety are regulated by interaction of several neurological and hormonal signals. Gut hormones, as a part of the gut-brain axis, convey information about the nutritional status to the CNS and contribute to the central regulation of food intake(8). RYGB is consistently associated with increased postoperative levels of the gut hormone glucagon-like peptide-1 (GLP-1)(9;10), which is secreted after food ingestion from enteroendocrine L-cells. In addition to its glucose regulating effects, GLP-1 is associated with reduced appetite, food intake and body weight(11), which is at least partly mediated via effects in the CNS(12;13). Neuroendocrine changes after RYGB, such as the enhanced GLP-1 secretion,

are regarded as possible mechanisms to account for a part of appetite and weight reduction and the sustained efficacy of this procedure(14;15).

We have previously shown, by means of a GLP-1 receptor antagonists, that endogenous GLP-1 mediates satiating effects of meal intake on CNS responsivity to food cues in humans(13). We therefore hypothesized that the increased GLP-1 response after RYGB may enhance effects of GLP-1 on the satiety and reward pathways in the CNS, thereby contributing to the observed postoperative decreases in food intake and body weight. In the current fMRI study, we investigated the role of endogenous GLP-1 in the improved responsivity of the CNS to food cues after RYGB by comparing the effects of the selective GLP-1 receptor antagonist exendin 9-39 (Ex9-39) with placebo before and after RYGB.

RESEARCH DESIGN AND METHOTDS

Participants

The study (NCT01363609) was approved by the Medical Ethics Review Committee of the VU University Medical Center. Subjects were included after written informed consent was obtained. Ten female candidates for RYGB were recruited from the Center for Bariatric Surgery at the Slotervaartziekenhuis, Amsterdam, The Netherlands. Subjects were eligible if they were 40-65years, had a body mass index (BMI) $>35\text{kg/m}^2$, had a stable body weight during the previous one month (i.e. $<5\%$ reported change), and were right-handed. Subjects were not on a formal caloric restricted diet prior and/or during the study, but received general advice on healthy food choices. Exclusion criteria were a history of neurological disease, the use of any centrally acting agent, psychiatric disorders or current diabetes. Three patients used antihypertensive medication, one patient used a cholesterol lowering agent, and three patients used thyroxin for the treatment of hypothyroidism.

General experimental protocol

The study consisted of four separate test visits. The first two visits were scheduled eight weeks to two weeks before RYGB, the final two visits were scheduled four weeks after RYGB (Figure1A). All patients had laparoscopic RYGB procedures. Following an overnight fast, participants arrived at 8:30AM at the research unit. During each visit, two fMRI scans were performed; one while the participant was fasted and one 30minutes after intake of a standardized liquid meal. The liquid meal was consumed over a 25min. interval. The first four participants received 200mL, Nutridrink, Nutricia®, Zoetermeer, The Netherlands (300kcal, carbohydrate 37.5g, fat 11.6g and protein 12.0g.) at each visit (i.e. the two visits before and the two visits after RYGB). However, since these participants reported that this amount was very difficult to consume during the visits *after* RYGB, the protocol was adapted during the

study. The remaining six participants received 150mL during all test visits. At each visit, a catheter was inserted into a cubital vein for infusion (random order) of either placebo (0.9% sodium-chloride solution) or the GLP-1 receptor antagonist Ex9-39 (Bachem; Clinalfa, Bubendorf, Switzerland: used to block effects of endogenous GLP-1), using MRI-compatible-infusion pumps (MRIdium3850pump, Iradimed, WinterPark, USA). Ex9-39 was diluted in 0.9% sodium-chloride solution containing 0.5% human serum albumin and infused at a rate of 600pmol/kg/min. A test visit with Ex9-39 infusion was performed once *before* and once *after* RYGB. In addition, a test visit with placebo infusion was performed once *before* and once *after* RYGB. Each infusion started one hour before the start of the MR-imaging, and was continued during the whole MR-scanning period. The order of infusion was determined by block randomization and the participants were blinded for the type of infusion. Blood was drawn at fixed moments to measure GLP-1 and glucose levels. Body composition was measured using bioelectrical impedance analysis. A summary of the protocol is presented in Figure1B.

fMRI tasks

At each visit, a visual food-cue task and a gustatory food-cue task were performed. The visual food-cue task was performed both in fasted condition and in postprandial condition. The gustatory food-cue task was performed only in postprandial condition (i.e. when endogenous GLP-1 levels would be at their highest). All the fMRI-tasks were created and presented via the software Eprime1.2 (Psychology-SoftwareTools, Pittsburg, PA).

Visual-food cues: Details of this fMRI-task have been described previously(5;13;16). Briefly, the fMRI-task consisted of pictures selected from three different categories; 1) high-calorie-food, 2) low-calorie-food and 3) non-food items. Pictures were presented in a block design. In total, 42pictures per category were presented divided in six blocks of 21sec. (Supplemental-

figure 1A). Given that each participant was scanned eight times, eight versions were created of this paradigm with different pictures, with the images being matched between the versions and between the categories for type and color.

Gustatory-food cues: Details of this fMRI-task have been described previously(17). Chocolate milk was used as a palatable food stimulus. As a neutral stimulus, a tasteless solution was used, designed to mimic the natural taste of saliva (consisting of 2.5mM NaHCO_3 and 25mM KCl (4)). This solution should provide a better neutral stimulus than water, which has previously been shown to be able to activate the gustatory cortex(18;19). Participants received 0.4ml of the chocolate milk or tasteless solution per 'trial'. In each trial, participants were presented a picture of an orange triangle (coupled to chocolate milk) or a blue star (coupled to tasteless solution), which was followed by the consumption of the coupled solution.

Participants were instructed to keep the solution within their mouth for 6sec. and to refrain from swallowing until the sign 'swallow' was presented afterwards (Supplemental-figure 1B).

The taste solutions were delivered with two programmable infusion pumps (Braun, InfusomatP, Melsungen, Germany) to ensure consistent volume and timing of the solution delivery.

MRI acquisition and analyzes

MRI-acquisition and analyzes have been described previously(5;13;16;17). MRI-data were acquired on a 3.0Tesla GE-SignaHDxt-scanner (GeneralElectric, Milwaukee, Wisconsin, USA). Functional images were analyzed with SPM8software (Wellcome-Trust-Centre-for-Neuroimaging, London, UK).

Functional scans were analyzed in the context of the general linear model. For the visual food-cue task, the high-calorie, low-calorie and non-food block were defined in the model. Next, to assess CNS activation related to food cues and, more specifically, their hedonic

quality, we computed two contrasts of interest: food>non-food and high-calorie>non-food, which refer to the activity during viewing food or high-calorie-food that is greater compared to during viewing non-food pictures. These contrast images were entered into three-way-ANOVA(5;13;16) with factors surgery (preRYGB, postRYGB), infusion (placebo, Ex9-39) and state of feeding (fasted, postprandial) to assess effects of surgery and to compare the effect of Ex9-39 vs. placebo infusion before and after RYGB in both meal states. For the gustatory food-cue task, the events of the consumption of solution were modelled and the contrast of chocolate milk greater than tasteless solution consumption (chocolate>tasteless) was computed. These contrast images were entered into a separate two-way-ANOVA, comparable to the visual food-cue task but without the factor meal, since the gustatory task was only performed in postprandial state.

First we explored, using whole brain analyses, if differences in activation in *a priori* regions of interest (ROIs) were present at an uncorrected $P < 0.001$. *A priori* ROIs were determined based on previous studies (i.e. insula (including adjacent opercular cortices), striatum (i.e. putamen and caudate nucleus), amygdala and orbitofrontal cortex (OFC)), as these regions are consistently shown to be involved in responses to food cues and are part of the central reward circuits(3-5). CNS activations were reported as significant when these survived family-wise error (FWE) correction for multiple comparisons on the voxel level using small volume correction (SVC) within the predefined ROIs, using 5-mm (for amygdala) or 10-mm (for insula, putamen, caudate nucleus, and OFC) radius spheres as described previously, comparing peak voxel on group level(5;13;16;17).

Blood sampling and assays

The measurement of blood glucose was performed using the glucose dehydrogenase method (GlucoseAnalyzer-HemoCue, Ängelholm, Sweden). Total GLP-1 was analyzed using a C-terminally directed radioimmunoassay for amidated GLP-1 (antibody89390)(20).

Questionnaires

The participants were asked to score their sensations of hunger, fullness, prospective-food-consumption and nausea and their appetite for sweet, savoury or fat food items on a 10-point-Likert scale at four fixed time points during visits: 1) before start of the first MRI session, 2) before intake of the meal, 3) 30min. after meal intake, 4) 60min. after meal intake.

Statistical analyses

Clinical group data were analyzed with the Statistical Package for the Social Sciences (SPSS) version20. Data are expressed as mean \pm SEM or median [interquartile range]. Effects of RYGB on clinical characteristics were analyzed with Wilcoxon-signed-rank test. To analyze the interaction of RYGB and the infusion of Ex9-39, and for the measurements with more than one time point per visit, repeated measurement analysis was used. Results were considered statistically significant when $P < 0.05$.

RESULTS

Clinical characteristics

Clinical characteristics before and after RYGB are presented in Supplemental-table 1. After RYGB, body weight was reduced significantly (mean \pm SD, -8.8 ± 1.7 kg, $P=0.005$). Additionally, waist circumference, body fat mass and lean mass were significantly reduced after RYGB ($P\leq 0.007$).

Supplemental-figure2 shows the GLP-1 and glucose levels during the different visits. After RYGB GLP-1 levels were significantly higher compared with *before* surgery ($P<0.001$), but the levels did not differ significantly while patients were fasted ($P=0.3$). GLP-1 levels did also not differ significantly between participants receiving 200mL of the standardized liquid meal compared to the participants receiving only 150mL (before RYGB $P=0.2$, after RYGB $P=0.6$). During Ex9-39 infusion, GLP-1 levels were significantly higher compared to placebo, both before and after RYGB ($P<0.001$), but GLP-1 levels were not significantly affected by Ex9-39 infusion while patients were fasted (before RYGB $P=0.8$, after RYGB $P=0.1$). The effect of Ex9-39 infusion on GLP-1 levels was larger after RYGB compared with before surgery (interaction $P=0.05$). Glucose levels also differed significantly after RYGB compared with before ($P<0.001$, during placebo infusion), but not while fasted ($P=0.3$). Glucose levels were higher during Ex9-39 compared to placebo infusion, both before and after RYGB ($P<0.001$), and this effect of Ex9-39 was also observed while patients were fasted (before RYGB $P<0.001$, after RYGB $P<0.004$). However, no significant interaction of RYGB with Ex9-39 infusion was observed ($P=0.5$).

RYGB reduces CNS activation in response to visual and gustatory food cues

We first investigated if RYGB resulted in a difference in CNS activation in response to food cues, i.e. to visual and gustatory food cues. We compared CNS activation during placebo infusion before and after RYGB. A detailed overview of the results is presented in Table 1.

Visual food cues: In the *fasted condition* during placebo infusion, RYGB resulted in lower activation in response to viewing food pictures in left caudate nucleus and right rolandic operculum (cluster size=18, t-value=3.15, P=0.03 and cluster size=13, t-value=3.11, P=0.03 respectively). In addition, the activation in response to high-calorie pictures was decreased after RYGB in left caudate nucleus (cluster size=19, t-value=3.11, P=0.03), right rolandic operculum (cluster size=9, t-value=2.64, P=0.09) and in left OFC (cluster size=13, t-value=3.13, P=0.03) (Figure 2A; effect of RYGB, visual food cues). No significant effects of RYGB were observed in the postprandial condition.

Gustatory food cues: In the *postprandial condition* during placebo infusion, RYGB resulted in decreased CNS activation in response to the gustatory food cues, i.e. activation during chocolate milk consumption, in right insula (cluster size=52, t-value=4.27, P=0.003) (Figure 2B; effect of RYGB, gustatory food cues).

Effects of GLP-1 receptor blockade after RYGB are larger vs. before RYGB

Second, we investigated if endogenous GLP-1 contributed to the effects of RYGB on CNS activation in response to food cues (described above). We compared the effect of Ex9-39 infusion vs. placebo infusion before and after RYGB on CNS activation during the different food-cue tasks. A detailed overview of the results is presented in Supplemental-table 1.

Visual food cues: In the fasted condition, GLP-1 receptor blockade with Ex9-39 infusion resulted in a larger increase after RYGB than before surgery in activation in the left caudate nucleus in response to both food pictures and high-calorie food pictures (cluster size=23, t-

value=3.34, $P=0.02$ and cluster size=5, t -value=3.02, $P=0.08$ respectively) (Figure3A; effect of GLP-1receptor blockade x RYGB, visual food cues). In the postprandial condition, we did not observe any effect of Ex9-39 administration after RYGB compared to before RYGB.

Gustatory food cues: In the postprandial condition, comparing GLP-1 receptor blockade before and after RYGB the effect of Ex9-39 was significantly larger after RYGB in right insula (cluster size=59, t -value=4.42, $P=0.002$) (Figure3B; effect of GLP-1receptor blockade x RYGB, gustatory food cues).

Appetite related scores

RYGB significantly decreased feelings of hunger and prospective food consumption ($P<0.001$) during placebo infusion (Supplemental-figure3). Appetite for sweet and savory food items was also reduced after RYGB ($P=0.001$, $P=0.006$ and $P=0.003$, respectively). Feelings of nausea were increased after RYGB ($P<0.001$), but no differences in sensation of fullness were observed ($P=0.3$). The effects of GLP-1 receptor blockade on VAS-score before and after RYGB were not significantly different (Supplemental-figure3).

Adverse events

During the visits after RYGB, two patients complained about nausea shortly after intake of the meal during placebo. Two patients experienced periods of dizziness and palpitation lasting approximately 10minutes after intake of the meal during the visit after RYGB with placebo. When we excluded these patients from the analyses, the findings in the postprandial state on CNS activation remained similar. One patient had diarrhoea shortly after intake of the meal on both visits after RYGB.

CONCLUSIONS

In the present study we investigated the effects of RYGB on CNS activation in response to food cues, measured with fMRI. In addition, we evaluated the contribution of changes in GLP-1 levels after RYGB to these central effects. We found that RYGB reduced the responsivity in our predefined ROI's of the CNS (involved in reward and satiety circuits) to both visual and gustatory food cues. Using the GLP-1 receptor antagonist Ex9-39 we also observed that the effects of endogenous GLP-1 on CNS responses to both the viewing of food pictures and the consumption of palatable food were larger after RYGB compared to before. These findings indicate that the effects of RYGB on the CNS are at least partly explained by postoperative changes in endogenous GLP-1.

RYGB is known for its substantial associated weight loss, which is maintained in the long term(1). Postoperative neuroendocrine alterations are suggested to play an important role in these effects of RYGB (21). Decreased activations in areas part of the central reward circuits (o.a. ventral striatum, putamen) in response to visual food cues after RYGB have been described(6;7). RYGB is also associated with a decreased desire to eat high-palatable food(6;22). In accordance with these studies, we observed decreased CNS activation to both visual and gustatory food cues after RYGB, paralleled by decreased scores for hunger and appetite. We also observed increased feelings of nausea after RYGB. As hunger and nausea feelings may be related, it could be hypothesized that the decrease in hunger is due to an increase in nausea. However, the increase in nausea after RYGB is mainly present in the postprandial state (not in the fasted state), whereas the decrease in hunger was also present in the fasted state. We therefore believe that RYGB has an effect on hunger independent of increase in nausea.

In the current study, we focused on the role of enhanced postoperative GLP-1 in the decreased CNS responses to food cues after RYGB. GLP-1 and treatment with GLP-1

receptor agonists reduce food intake and body weight(11) via effects in the CNS(5;12;13; 16;17). In the present study, we observed that the effect of endogenous GLP-1 on responsivity in the caudate nucleus to viewing food pictures was larger *after* RYGB in the fasted state. In the postprandial condition, we found a larger effect of GLP-1 on responsivity in the insula to the consumption of palatable food *after* RYGB, although responses to viewing food pictures after RYGB were not affected by GLP-1. The fact that we only found effects in the postprandial condition on gustatory food cues suggests a larger role for GLP-1 in the central rewarding evaluation of taste perception than in the evaluation of visual food cues.

Interestingly, receptors for GLP-1 were reported to be present in mammalian taste buds and GLP-1 receptor knock-out mice were shown to have reduced sweet taste sensitivity, pointing towards an important role for GLP-1 in taste perception in rodents(23). It is however unknown whether this mechanism is also operative in humans.

As expected, GLP-1 levels were higher after RYGB, which may be related to rapid entry and absorption of nutrients to the more distal small intestine postoperatively(24), which may stimulate an enhanced release of GLP-1. In addition, an increased density of GLP-1-immunoreactive cells has been observed after RYGB(25). We demonstrated that the enhanced GLP-1 secretion may explain the decreased CNS activation in response to consumption of palatable food after RYGB. Noteworthy, although fasting GLP-1 levels were not significantly altered after RYGB, the effects of endogenous GLP-1 on responses to viewing food pictures in the fasted condition were increased. It could be speculated that this might be due to an increase in GLP-1 sensitivity, as suggested by a study in rats, showing that administration of GLP-1 receptor agonist exendin-4 decreased food intake more in RYGB than in sham-operated rats, indicating a higher sensitivity to GLP-1 after RYGB(26). In accordance, lower BMI in humans was correlated with an increased incretin effect(27). This observational finding is compatible with the hypothesis that reductions in BMI may enhance effects of and

sensitivity to incretin hormones, such as GLP-1. An increased sensitivity after RYGB has also been described for other hormones, i.e. insulin and thyroxin, independent of weight reduction(28;29). Although it could be considered contradictory to the often observed effect that increased hormonal levels leads to desensitization of the corresponding hormonal receptor, as postprandial GLP-1 levels are increased after RYGB, we do speculate that increased sensitivity for GLP-1 may explain our findings in the fasted state.

Although previous studies have investigated the effect of RYGB on CNS responses to the viewing of food pictures, only one recent pilot study has investigated CNS activation in response to sweet taste after RYGB in humans, showing a significant decrease in activation in response to sweet taste in the OFC after surgery(30). However, this finding was not conclusive, as this effect was also observed in control subjects. We also found that RYGB *decreased* the CNS responses in the insula in response to chocolate milk consumption, which was accompanied by weight reduction. At first sight, this finding may be considered to be at odds with previous studies, as several(4;17), but not all(31;32), previous studies demonstrated that leaner individuals have *increased* responsivity to the consumption of chocolate milk in comparison to obese individuals. However, in general, both lean and obese individuals are presumed to 'like' the palatable gustatory food cue, but seem to differ in the central responses and process of the reward evaluation of this cue. In contrast, RYGB is associated with changes in food preferences(22) and taste perception(33) with higher susceptibility for sweet taste perception(34;35). Studies reported that patients after RYGB have decreased interest in sweet food, finding it less enjoyable or even unpleasant(34). Therefore, the 'liking' of the chocolate milk consumption in our current study may be altered postoperatively, and chocolate milk may even be experienced as unpleasant. According to this, we observed a significant decreased in the appetite related scores for sweet food items after RYGB. This may explain the decreased responsivity of the insula to the consumption of chocolate milk

observed after RYGB in our study. In line with this, we found that blockade of endogenous GLP-1 effects after RYGB increased the CNS activations in response to chocolate milk consumption. These increased CNS activations may be interpreted as increased liking of chocolate milk, suggesting that endogenous GLP-1 decreased the liking of sweet taste, which may lead to reduced sweet palatable food consumption.

GLP-1 may affect the CNS directly via access through areas with a permeable blood-brain barrier or via secretion by GLP-1 producing neurons(36). However, central effects of GLP-1 may also indirectly be mediated via activation of vagal afferents. In our current study we used Ex9-39 which is able to cross the blood-brain barrier(37). We are therefore not able to distinguish if the observed effects of GLP-1 in our study are mediated directly or also partly indirectly.

In our current study we focused on the effects of the exaggerated GLP-1 response after RYGB on the CNS. It could be suggested that changes in levels of other hormones, such as insulin may also play a role. However, we do not believe that insulin levels can explain our findings for several reasons. First, in our previous study we did not find a significant difference in insulin levels between placebo and Ex9-39 infusion(13). Second, we previously demonstrated, using a pancreatic clamp, that the effects of the GLP-1 receptor agonist on the CNS responses to food stimuli were independent of changes in insulin levels(5). Third, although others have shown that insulin levels may increase at 3months after RYGB , this was not observed 1 week after RYGB(38). It also could be suggested that changes in glucose levels after RYGB may affect our observed findings. However, the effect of Ex9-39 on glucose level did not differ significantly before compared to after RYGB. In addition, we have demonstrated in our previous studies that the effects of GLP-1 on the brain are independent of glucose and/or insulin levels(5;13).

The sample size of the current study is relatively small. However, we used a longitudinal, within-subjects design with >90% power to detect the expected difference in CNS activation(5;6;13;39). It should, however, be emphasized that this was a pilot study with only female patients between the age of 40-65, which limits the generalizability to men and other age groups. In addition, we investigated patients four weeks after surgery, comparable to previous studies(6;39). However in this phase after surgery, patients may still have complaints of the intestinal anastomoses and may have problems with a number of food products, which they can tolerate more than a year after surgery. Others have found reduced CNS responses several years after RYGB(7;40), but further research is needed to determine the role for GLP-1 in these longer-term CNS changes.

In conclusion, similar to previous studies, we found that the effects of RYGB on food intake may be mediated by decreased activation in feeding regulating areas in the CNS in response to food stimuli. In addition, our findings using the GLP-1 receptor antagonist suggest that these effects of RYGB may be partly explained by postoperative changes in the levels of endogenous GLP-1 and/or possible changes in sensitivity to GLP-1. These findings provide further insights in the weight lowering mechanisms of RYGB and may ultimately lead to further development of treatment strategies for obesity.

Authors Contribution: J.S.t.K. designed the study, conducted the experiments, designed the fMRI paradigm, performed data analysis, and wrote the manuscript. D.J.V. designed the fMRI paradigm, performed data analysis, and wrote the manuscript. V.E.A.G. contributed to the design and the performance of the study and contributed to the writhing of the manuscript. L.v.B. designed the fMRI paradigm and contributed to writing the manuscript. F.B. performed analyses of all structural MRI scans and contributed to writing the manuscript. C.F.D. performed laboratory analyses and contributed to writing the manuscript. J.J.H. performed laboratory analyses and contributed to writing the manuscript. M.L.D. contributed to the design of the study and to the writing the manuscript. M.D. designed the study. R.G.IJ. designed the study, performed data analysis, and wrote the manuscript. J.S.t.K. and R.G.IJ. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have seen and approved the final version of the manuscript.

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REFERENCES

1. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrback K, Schoelles K: Bariatric surgery: a systematic review and meta-analysis. *JAMA* 2004;292:1724-1737
2. Rao RS: Bariatric surgery and the central nervous system. *Obes Surg* 2012;22:967-978
3. Stoeckel LE, Weller RE, Cook EW, III, Twieg DB, Knowlton RC, Cox JE: Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *Neuroimage* 2008;41:636-647
4. Stice E, Spoor S, Bohon C, Small DM: Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science* 2008;322:449-452
5. Bloemendaal L. v, IJzerman RG, ten Kulve JS, Barkhof F, Konrad RJ, Drent ML, Veltman DJ, Diamant M: GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes* 2014;63:4186-4196
6. Ochner CN, Kwok Y, Conceicao E, Pantazatos SP, Puma LM, Carnell S, Teixeira J, Hirsch J, Geliebter A: Selective reduction in neural responses to high calorie foods following gastric bypass surgery. *Ann Surg* 2011;253:502-507
7. Frank S, Wilms B, Veit R, Ernst B, Thurnheer M, Kullmann S, Fritsche A, Birbaumer N, Preissl H, Schultes B: Altered brain activity in severely obese women may recover after Roux-en Y gastric bypass surgery. *Int J Obes (Lond)* 2014;38:341-348
8. Murphy KG, Bloom SR: Gut hormones and the regulation of energy homeostasis. *Nature* 2006;444:854-859
9. Borg CM, le Roux CW, Ghatei MA, Bloom SR, Patel AG, Aylwin SJ: Progressive rise in gut hormone levels after Roux-en-Y gastric bypass suggests gut adaptation and explains altered satiety. *Br J Surg* 2006;93:210-215
10. Falken Y, Hellstrom PM, Holst JJ, Naslund E: Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab* 2011;96:2227-2235
11. Vilsboll T, Christensen M, Junker AE, Knop FK, Gluud LL: Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2012;344:d7771
12. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996;379:69-72
13. ten Kulve JS, Veltman DJ, van BL, Barkhof F, Deacon CF, Holst JJ, Konrad RJ, Sloan JH, Drent ML, Diamant M, IJzerman RG: Endogenous GLP-1 mediates postprandial reductions in activation in central reward and satiety areas in patients with type 2 diabetes. *Diabetologia* 2015;58:2688-2698
14. Tadross JA, le Roux CW: The mechanisms of weight loss after bariatric surgery. *Int J Obes (Lond)* 2009;33 Suppl 1:S28-S32
15. Madsbad S, Dirksen C, Holst JJ: Mechanisms of changes in glucose metabolism and bodyweight after bariatric surgery. *Lancet Diabetes Endocrinol* 2014;2:152-164
16. ten Kulve JS, Veltman DJ, van BL, Barkhof F, Drent ML, Diamant M, IJzerman RG: Liraglutide Reduces CNS Activation in Response to Visual Food Cues Only After Short-term Treatment in Patients With Type 2 Diabetes. *Diabetes Care* 2016;39:214-221
17. Bloemendaal L. v, Veltman DJ, ten Kulve JS, Groot PF, Ruhe HG, Barkhof F, Sloan JH, Diamant M, IJzerman RG: Brain reward-system activation in response to anticipation and consumption of palatable food is altered by GLP-1 receptor activation in humans. *Diabetes Obes Metab* 2015;
18. O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F: Representation of pleasant and aversive taste in the human brain. *J Neurophysiol* 2001;85:1315-1321

19. Zald DH, Pardo JV: Cortical activation induced by intraoral stimulation with water in humans. *Chem Senses* 2000;25:267-275
20. Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 1994;43:535-539
21. Berthoud HR, Morrison C: The brain, appetite, and obesity. *Annu Rev Psychol* 2008;59:55-92
22. Halmi KA, Mason E, Falk JR, Stunkard A: Appetitive behavior after gastric bypass for obesity. *Int J Obes* 1981;5:457-464
23. Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, Jang HJ, Mattson MP, Drucker DJ, Egan JM, Munger SD: Modulation of taste sensitivity by GLP-1 signaling. *J Neurochem* 2008;106:455-463
24. Jacobsen SH, Bojsen-Møller KN, Dirksen C, Jørgensen NB, Clausen TR, Wulff BS, Kristiansen VB, Worm D, Hansen DL, Holst JJ, van HG, Madsbad S: Effects of gastric bypass surgery on glucose absorption and metabolism during a mixed meal in glucose-tolerant individuals. *Diabetologia* 2013;56:2250-2254
25. Rhee NA, Wahlgren CD, Pedersen J, Mortensen B, Langholz E, Wandall EP, Friis SU, Vilmann P, Paulsen SJ, Kristiansen VB, Jelsing J, Dalbøge LS, Poulsen SS, Holst JJ, Vilsbøll T, Knop FK: Effect of Roux-en-Y gastric bypass on the distribution and hormone expression of small-intestinal enteroendocrine cells in obese patients with type 2 diabetes. *Diabetologia* 2015;
26. Abegg K, Schiesser M, Lutz TA, Bueter M: Acute peripheral GLP-1 receptor agonism or antagonism does not alter energy expenditure in rats after Roux-en-Y gastric bypass. *Physiol Behav* 2013;121:70-78
27. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E: Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* 2008;57:1340-1348
28. Moulin de Moraes CM, Mancini MC, de Melo ME, Figueiredo DA, Villares SM, Rascovski A, Zilberstein B, Halpern A: Prevalence of subclinical hypothyroidism in a morbidly obese population and improvement after weight loss induced by Roux-en-Y gastric bypass. *Obes Surg* 2005;15:1287-1291
29. Wickremesekera K, Miller G, Naotunne TD, Knowles G, Stubbs RS: Loss of insulin resistance after Roux-en-Y gastric bypass surgery: a time course study. *Obes Surg* 2005;15:474-481
30. Wang JL, Yang Q, Hajnal A, Rogers AM: A pilot functional MRI study in Roux-en-Y gastric bypass patients to study alteration in taste functions after surgery. *Surg Endosc* 2016;30:892-898
31. Stice E, Yokum S, Burger KS, Epstein LH, Small DM: Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. *J Neurosci* 2011;31:4360-4366
32. Szalay C, Aradi M, Schwarcz A, Orsi G, Perlaki G, Nemeth L, Hanna S, Takacs G, Szabo I, Bajnok L, Vereczkei A, Doczi T, Janszky J, Komoly S, Ors HP, Lenard L, Karadi Z: Gustatory perception alterations in obesity: an fMRI study. *Brain Res* 2012;1473:131-140
33. Tichansky DS, Boughter JD, Jr., Madan AK: Taste change after laparoscopic Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding. *Surg Obes Relat Dis* 2006;2:440-444
34. Bueter M, Miras AD, Chichger H, Fenske W, Ghatei MA, Bloom SR, Unwin RJ, Lutz TA, Spector AC, le Roux CW: Alterations of sucrose preference after Roux-en-Y gastric bypass. *Physiol Behav* 2011;104:709-721

35. Burge JC, Schaumburg JZ, Choban PS, DiSilvestro RA, Flancbaum L: Changes in patients' taste acuity after Roux-en-Y gastric bypass for clinically severe obesity. *J Am Diet Assoc* 1995;95:666-670
36. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C: Distribution of glucagon-like peptide-1 and other proglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 1997;77:257-270
37. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN: Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 2003;9:1173-1179
38. Jorgensen NB, Jacobsen SH, Dirksen C, Bojsen-Moller KN, Naver L, Hvolris L, Clausen TR, Wulff BS, Worm D, Lindqvist HD, Madsbad S, Holst JJ: Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. *Am J Physiol Endocrinol Metab* 2012;303:E122-E131
39. Ochner CN, Laferrere B, Afifi L, Atalayer D, Geliebter A, Teixeira J: Neural responsiveness to food cues in fasted and fed states pre and post gastric bypass surgery. *Neurosci Res* 2012;74:138-143
40. Scholtz S, Miras AD, Chhina N, Prechtel CG, Sleeth ML, Daud NM, Ismail NA, Durighel G, Ahmed AR, Olbers T, Vincent RP, Aghband-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* 2014;63:891-902

Table 1: Effects of RYGB surgery and GLP-1 receptor blockade in response to visual and gustatory food cues

Used contrast	Comparison	Region	Side	Cluster	Max t-val	P-FWE	MNI coordinates (x, y, z)
<u>Visual food cues: effects of RYGB</u>							
Food > non-food	Effects in fasted state	Caudate nucleus	L	18	3.15	0.03	-15, 23, -2
	Pre RYGB > post RYGB	Rolandic Operculum	R	13	3.11	0.03	54, -4, 10
High-calorie > non-food	(both placebo)	Caudate nucleus	L	19	3.11	0.03	-13, 23, -2
		OFC	L	13	3.13	0.03	-33, 47, -8
		Rolandic Operculum	R	9	2.64	0.09	48, -1, 10
Food > non-food	Effects in postprandial	-	-	-	-	-	-
High-calorie > non-food	state	-	-	-	-	-	-
	Pre RYGB > post RYGB						
<u>Visual food cues: effects of GLP-1 R blockade x RYGB</u>							
Food > non-food	fasted state	Caudate nucleus	L	26	3.34	0.02	-3, 14, -2
High-calorie > non-food	Effect of GLP-1 post RYGB > pre RYGB	Caudate nucleus	L	5	3.02	0.08	-6, 20, 1
Food > non-food	Postprandial state	-	-	-	-	-	-
High-calorie > non-food	Effect of GLP-1 post RYGB > pre RYGB	-	-	-	-	-	-
<u>Gustatory food cues: effects of RYGB</u>							
Chocolate > tasteless	Pre RYGB > post RYGB (both placebo)	Insula	R	52	4.27	0.003	51, 2, -8
<u>Gustatory food cues: effects of GLP-1 R blockade x RYGB</u>							
Chocolate > tasteless (ex9-39 > placebo):	Effect GLP-1 blockade: Post RYGB > pre RYGB	Insula	R	59	4.42	0.002	48, -7, -11

This table describes the areas where significant differences in activations were observed for the different comparisons. First we describe the effect of RYGB, i.e. the difference in CNS responses before and after RYGB. Second, we have described the effect of RYGB on the effect of GLP-1R blockade (GLP-1R blockade x RYGB (before vs after)). For each comparison, the two contrasts for the visual food cues (activation during food > non-food pictures and high-calorie food > non-food pictures) and the contrast for the gustatory food cues (activation during chocolate > tasteless solution) are presented. The areas with significant differences are listed, including the cluster size of this effect, the t-value and the FWE corrected P-value after small volume correction. The last column describes the coordinates of the peak voxel of the observed difference in MNI space. For a step wise interpretation of the results described in this table, please see 'Results' section in the manuscript. CNS, central nervous system ; FWE, family wise error; GLP-1 R, glucagon-like peptide-1 receptor; L, left; MNI, Montreal Neurological Institute; OFC, orbito frontal cortex ; R, right ; RYGB, roux-en-y gastric bypass.

Figure legends

Figure 1: *Study protocol*

A) Study design

Ten candidates for RYGB were studied in an acute intervention study. All participants underwent four test visits: two before RYGB and two four weeks after RYGB. During two visits (one before and one after RYGB), the GLP-1 receptor antagonist, Ex9-39 was infused in order to block actions of endogenous GLP-1. During the other visits, only placebo (saline) was infused.

B) Test visit

The infusion started one hour before the beginning of the scan and lasted until the end of the visit. During each visit, two fMRI scans were performed; one while fasted and one 30 min after intake a standardized meal. During both the fMRI scans, visual food cues were presented while a task with gustatory food cues was presented only during the postprandial fMRI scan. Blood samples were drawn and sensation of hunger, fullness and appetite were scored on a 10-point Likert scale at fixed time points.

V1-4, test visit 1-4; T1, structural MRI T1 weighted sequence.

Figure 2: *Effects of RYGB on CNS activation in response to visual (A) and gustatory food cues (B)*

Coronal and axial slices showing the difference between the group averages for the ten participants regarding activation in areas of the CNS where **A)** activation in response to viewing food pictures was decreased after RYGB compared to before and **B)** activation in response to chocolate milk consumption was lower after RYGB compared to before. The color scale reflects the T value of the functional activity. Results are presented at the threshold

of $P < 0.05$, FWE corrected (correction for multiple comparisons on the voxel level) on cluster extent. In the graphs, BOLD signal intensity is plotted (arbitrary unit), mean and SEM.

Figure 3: *Effects of GLP-1 blockade (using Ex9-39), comparing before vs after RYGB, on CNS activation to visual (A) and gustatory food cues (B)*

Axial slices showing the difference in the group averages in activation in areas of the CNS, depicting the difference of the effect of GLP-1 blockade by infusion of Ex9-39 (vs. placebo).

Panel **A** shows that GLP-1 receptor blockade resulted in a larger increase in activation in response to viewing food pictures after RYGB compared to before. Panel **B** shows a comparable effect in response to chocolate milk consumption. The color scale reflects the T value of the functional activity. Results are presented at the threshold of $P < 0.05$, FWE corrected (correction for multiple comparisons on the voxel level) on cluster extent. In the graphs, BOLD signal intensity is plotted (arbitrary unit), mean and SEM.

Supplemental table 1: Clinical characteristics before and after RYGB.

n = 10	Before RYGB	4 weeks after RYGB	P - value
Age (yr)	46.5 [40.0, 50.0]	-	-
Weight (kg)	107.8 [101.2, 114.0]	99.3 [92.6, 104.9]	0.005
BMI (kg/m ²)	39.9 [37.8, 42.5]	36.8 [34.6, 39.1]	0.005
Systolic blood pressure (mmHg)	118 [114, 123]	115 [108, 126]	0.4
Diastolic blood pressure (mmHg)	81 [77, 90]	76 [68, 82]	0.01
Heart rate (beats/min)	72 [65, 79]	65 [60, 71]	0.06
Waist circumference (cm)	118 [111, 121]	113 [107, 116]	0.005
Body fat mass (kg)	52.7 [50.5, 58.0]	49.3 [42.6, 51.4]	0.005
Lean mass (kg)	54.9 [49.9, 57.9]	52.9 [48.9, 54.8]	0.007
HbA1c (mmol/mol)	37 [34, 40]	37 [34, 37]	0.2
HbA1c (%)	5.5 [5.3, 5.8]	5.5 [5.3, 5.5]	0.2
Total cholesterol (mmol/l)	5.1 [4.8, 5.4]	3.8 [3.2, 4.4]	0.005
Triglycerides (mmol/l)	1.0 [0.7, 1.6]	1.0 [0.8, 1.1]	0.1
Glucose fasting (mmol/l)	4.8 [4.5, 5.1]	4.7 [4.4, 4.9]	0.6

Data are median [interquartile range].

Supplemental figure legends

Supplemental figure 1: *fMRI paradigms*

A) Visual food cues fMRI paradigm

One run comprised six blocks of each 21 seconds (7 pictures). Within one run, two blocks of each category were presented. The blocks were separated with a 9 second black screen with fixation cross. Each MRI session included three runs, resulting in the presentation of six blocks per category. The order of the categories was randomised per run and per session.

B) Gustatory food cues fMRI paradigm

This paradigm consisted of two types of trials: paired and unpaired trials, which were randomised in order and type.

Paired trials: Subject were presented a picture of an orange triangle or a blue star during 2 sec. The orange triangle was coupled to chocolate milk receipt and the blue star to tasteless solution receipt. After the presentation of the picture, the subjects waited 3 sec., while watching a fixation cross, until receiving the coupled solution (during 2 sec.). The subjects were instructed to keep the solution within the mouth during 6 sec. and to refrain from swallowing until the sign 'swallow' was presented afterwards. Between the trials a jitter of 1-7 seconds was used. In total 40 paired trials were presented and half of them included the orange triangle and chocolate milk receipt.

Unpaired trials: These trials were similar to the beginning of the paired trial, however without receiving the coupled solution. Between the trials a jitter of 1-7 seconds was used. At the beginning of the trials, subjects were unaware if it consisted a paired or unpaired trials. In total 32 paired trials were presented and half of them included the orange triangle.

Supplemental figure 2: *GLP-1 and glucose levels*

A) GLP-1 levels were significantly higher after RYGB compared to before ($P < 0.001$ during placebo infusion). GLP-1 levels were higher during Ex9-39 compared to placebo infusion, both before and after RYGB ($P < 0.001$). A significant interaction of RYGB with Ex9-39 infusion was observed ($P = 0.05$)

B) Glucose levels differed significantly after RYGB compared to before ($P < 0.001$ during placebo infusion). Glucose levels were higher during Ex9-39 compared to placebo infusion, both before and after RYGB ($P < 0.001$), but no interaction of RYGB with Ex9-39 infusion was observed ($P = 0.5$).

Supplemental figure 3: *VAS-scores*

VAS-scores for hunger, prospective food consumption, fullness, nausea, appetite for sweet and savory food items. Feelings of hunger, prospective food consumption and appetite for sweet and savory food items was significantly decreased after RYGB ($P < 0.001$). Feelings of nausea were significantly increased after RYGB ($P < 0.001$). Infusion of Ex9-39 did not significantly affect the VAS-scores and did not differ before compared with after RYGB.