# Elevated Postoperative Endogenous GLP-1 Levels Mediate Effects of Roux-en-Y Gastric Bypass on Neural Responsivity to Food Cues

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## 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 exendin 9-39 (Ex9-39) and placebo were assessed in 10 women before and after RYGB. We used functional MRI to investigate CNS activation in response to visual food cues (pictures) and gustatory food cues (consumption of chocolate milk), comparing results with Ex9-39 versus 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 in response 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 midjejunum, bypassing the duodenum and proximal jejunum. This may lead to reduced ingestive capacity and

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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 MRI (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 highly 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 the 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 antagonist, that endogenous GLP-1 mediates the 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 METHODS

### 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 40–65 years old, had a BMI $>35$ kg/m$^2$, had a stable body weight during the previous 1 month (i.e., $<5\%$ reported change), and were right handed. Subjects were not on a formal calorie-restricted diet prior to 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 8 weeks to 2 weeks before RYGB, and the final two visits were scheduled 4 weeks after RYGB (Fig. 1*A*). All patients had laparoscopic RYGB procedures. After an overnight fast, participants arrived at 8:30 A.M. at the research unit. During each visit, two fMRI scans were performed: one while the participant was fasted and one 30 min after intake of a standardized liquid meal. The liquid meal was consumed over a 25-min interval. The first four participants received 200 mL Nutridrink (Nutricia, Zoetermeer, the Netherlands; 300 kcal, carbohydrate 37.5 g, fat 11.6 g, and protein 12.0 g) 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 150 mL 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

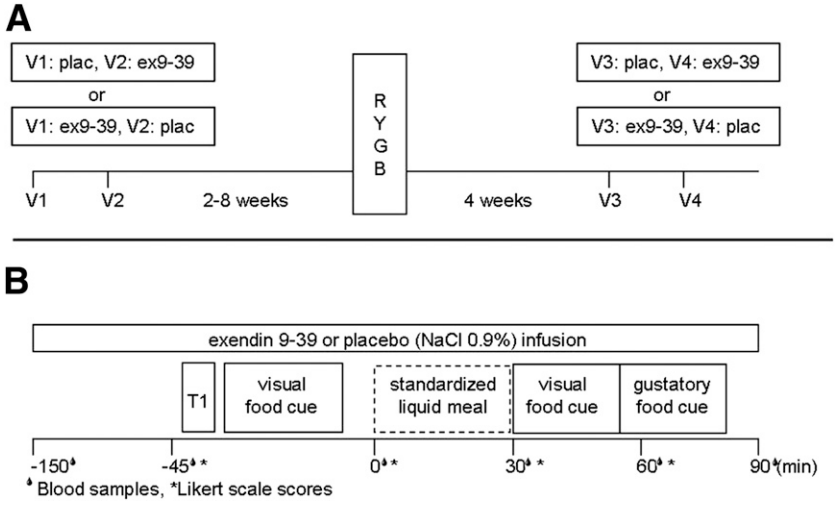


**Figure 1**—Study protocol. *A*: Study design. Ten candidates for RYGB were studied in an acute intervention study. All participants underwent four test visits: two before RYGB and two 4 weeks after RYGB. During two visits (one before and one after RYGB), the GLP-1 receptor antagonist Ex9-39 was infused in order to block actions of endogenous GLP-1. During the other visits, only placebo (saline) was infused. *B*: Test visit. The infusion started 1 h 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 of a standardized meal. During both the fMRI scans, visual food cues were presented, whereas 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. Plac, placebo; T1, structural MRI T1 weighted sequence; V1–4, test visits 1–4.

(Clinalfa; Bachem, Bubendorf, Switzerland; used to block effects of endogenous GLP-1), using MRI-compatible infusion pumps (MRidium 3850 IV Pump; Iradimed, Winter Park, FL). Ex9-39 was diluted in 0.9% sodium chloride solution containing 0.5% human serum albumin and infused at a rate of 600 pmol/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 1 h before the start of the MRI and was continued during the whole MRI 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 Fig. 1*B*.

### 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 the fasted condition and in the postprandial condition. The gustatory food-cue task was performed only in the 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 Eprime 1.2 (Psychology Software Tools, Pittsburgh, PA).

#### *Visual Food Cues*

Details of this fMRI task have been described previously (5,13,16). In brief, the fMRI task consisted of pictures selected from three different categories: *1*) high-calorie food, *2*) low-calorie food, and *3*) nonfood items. Pictures were presented in a block design. In total, 42 pictures per category were presented, divided in six blocks of 21 s (Supplementary Fig. 1*A*). 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 2.5 mmol/L $NaHCO_3$ and 25 mmol/L 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 0.4 mL 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 6 s and to refrain from swallowing until the sign "swallow" was presented afterward (Supplementary Fig. 1*B*).

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

### MRI Acquisition and Analyses

MRI acquisition and analyses have been described previously (5,13,16,17). MRI data were acquired on a 3.0 Tesla GE Signa HDxt scanner (GE Healthcare, Milwaukee, WI). Functional images were analyzed with SPM8 software (Wellcome Trust Centre for Neuroimaging, London, U.K.).

Functional scans were analyzed in the context of the general linear model. For the visual food-cue task, the high-calorie, low-calorie, and nonfood 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 >nonfood and high calorie >nonfood, which refer to the activity during viewing food or high-calorie food that is greater compared with during viewing nonfood pictures. These contrast images were entered into three-way ANOVA (5,13,16) with factors surgery (pre-RYGB and post-RYGB), infusion (placebo and Ex9-39), and state of feeding (fasted and postprandial) to assess effects of surgery and to compare the effect of Ex9-39 versus placebo infusion before and after RYGB in both meal states. For the gustatory food-cue task, the events of the consumption of solution were modeled 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 the 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 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 (Glucose Analyzer; HemoCue, Ängelholm, Sweden). Total GLP-1 was analyzed using a C-terminally directed radioimmunoassay for amidated GLP-1 (antibody 89390) (20).

### Questionnaires

The participants were asked to score their sensations of hunger, fullness, prospective food consumption, and nausea and their appetite for sweet, savory, 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*) 30 min after meal intake, and *4*) 60 min after meal intake.

### Statistical Analyses

Clinical group data were analyzed with SPSS version 20. Data are expressed as mean ± SEM or median [interquartile range]. Effects of RYGB on clinical characteristics were analyzed with the 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 Supplementary Table 1. After RYGB, body weight was reduced significantly (mean ± SD, −8.8 ± 1.7 kg, $P = 0.005$). Additionally, waist circumference, body fat mass, and lean mass were significantly reduced after RYGB ($P \leq 0.007$).

Supplementary Fig. 2 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 also did not differ significantly between participants receiving 200 mL of the standardized liquid meal compared with the participants receiving only 150 mL (before RYGB, $P = 0.2$; after RYGB, $P = 0.6$). During Ex9-39 infusion, GLP-1 levels were significantly higher compared with 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 with 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 the left caudate nucleus and

**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) |
|---|---|---|---|---|---|---|---|
| Visual food cues: effects of RYGB | | | | | | | |
| Food >nonfood | Effects in fasted state: pre-RYGB >post-RYGB (both placebo) | Caudate nucleus | L | 18 | 3.15 | 0.03 | −15, 23, −2 |
| | | Rolandic operculum | R | 13 | 3.11 | 0.03 | 54, −4, 10 |
| High calorie >nonfood | | 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 >nonfood | Effects in postprandial state: pre-RYGB >post-RYGB (both placebo) | — | — | — | — | — | — |
| High calorie >nonfood | | — | — | — | — | — | — |
| Visual food cues: effects of GLP-1 R blockade × RYGB | | | | | | | |
| Food >nonfood | Fasted state: effect of GLP-1 post-RYGB >pre-RYGB | Caudate nucleus | L | 26 | 3.34 | 0.02 | −3, 14, −2 |
| High calorie >nonfood | | Caudate nucleus | L | 5 | 3.02 | 0.08 | −6, 20, 1 |
| Food >nonfood | Postprandial state: effect of GLP-1 post-RYGB >pre-RYGB | — | — | — | — | — | — |
| High calorie >nonfood | | — | — | — | — | — | — |
| Gustatory food cues: effects of RYGB | | | | | | | |
| Chocolate >tasteless | Pre-RYGB >post-RYGB (both placebo) | Insula | R | 52 | 4.27 | 0.003 | 51, 2, −8 |
| Gustatory food cues: effects of GLP-1 R blockade × RYGB | | | | | | | |
| 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 describe the effect of RYGB on the effect of GLP-1 R blockade (GLP-1 R blockade × RYGB [before versus after]). For each comparison, the two contrasts for the visual food cues (activation during food >nonfood pictures and high-calorie food >nonfood 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 (P-FWE). The last column describes the coordinates of the peak voxel of the observed difference in MNI space. For a stepwise interpretation of the results described in this table, please see RESULTS. GLP-1 R, GLP-1 receptor; L, left; MNI, Montreal Neurological Institute; R, right; val, value.

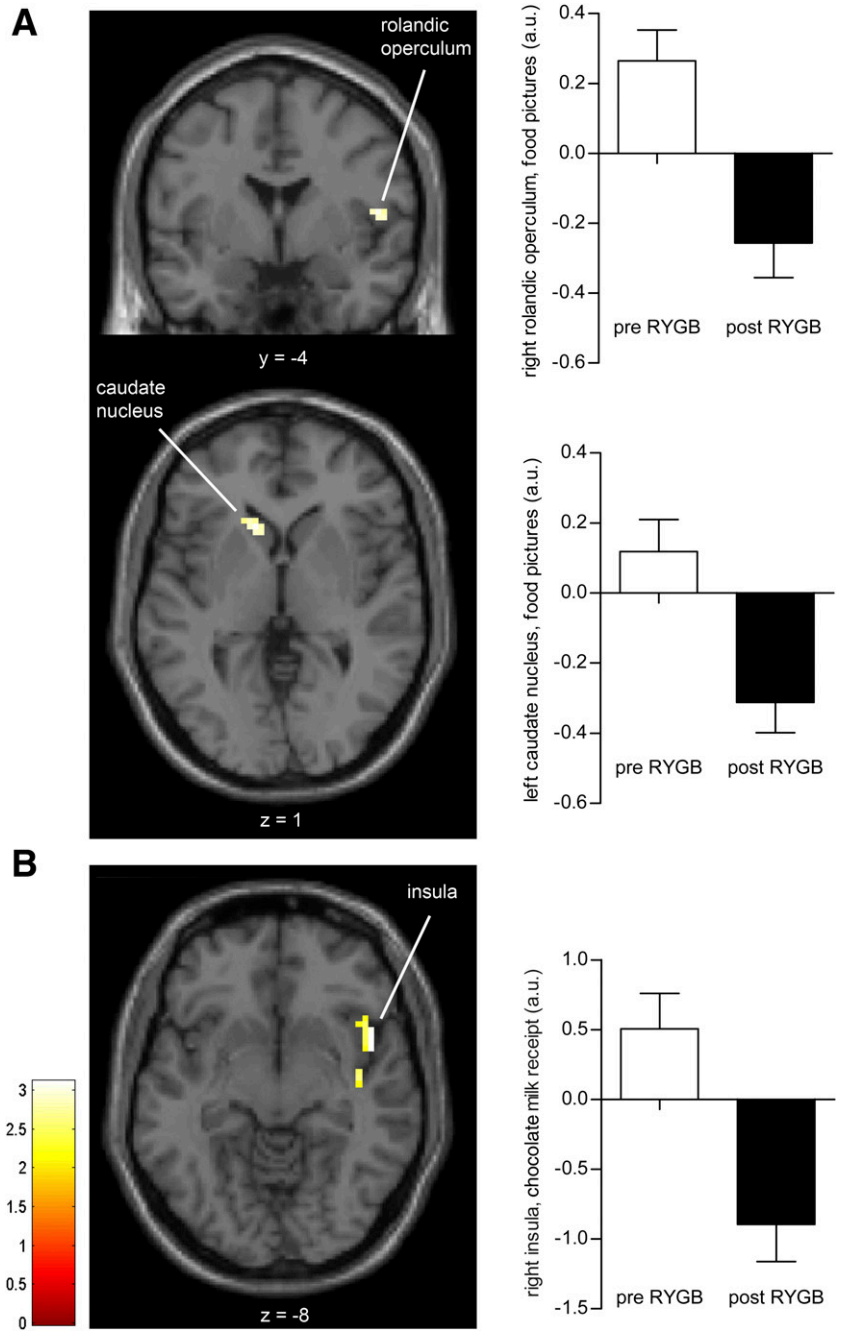


**Figure 2**—Effects of RYGB on CNS activation in response to visual (*A*) and gustatory (*B*) food cues. Coronal and axial slices showing the difference between the group averages for the 10 participants regarding activation in areas of the CNS where activation in response to viewing food pictures was decreased after RYGB compared with before (*A*) and activation in response to chocolate milk consumption was lower after RYGB compared with before (*B*). 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 units [a.u.]); mean and SEM are shown.

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 the 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 left OFC (cluster size = 13, *t* value = 3.13, *P* = 0.03) (effect of RYGB, visual food cues) (Fig. 2*A*). 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 the right insula (cluster size = 52, *t* value = 4.27, *P* = 0.003) (effect of RYGB, gustatory food cues) (Fig. 2*B*).

### Effects of GLP-1 Receptor Blockade After RYGB Are Larger Versus 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 versus placebo infusion before and after RYGB on CNS activation during the different food-cue tasks. A detailed overview of the results is presented in Supplementary 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) (effect of GLP-1 receptor blockade × RYGB, visual food cues) (Fig. 3*A*). In the postprandial condition, we did not observe any effect of Ex9-39 administration after RYGB compared with 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 the right insula (cluster size = 59, *t* value = 4.42, *P* = 0.002) (effect of GLP-1 receptor blockade × RYGB, gustatory food cues) (Fig. 3*B*).

### Appetite-Related Scores

RYGB significantly decreased feelings of hunger and prospective food consumption ($P < 0.001$) during placebo infusion (Supplementary Fig. 3). 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 visual analog scale (VAS) score before and after RYGB were not significantly different (Supplementary Fig. 3).

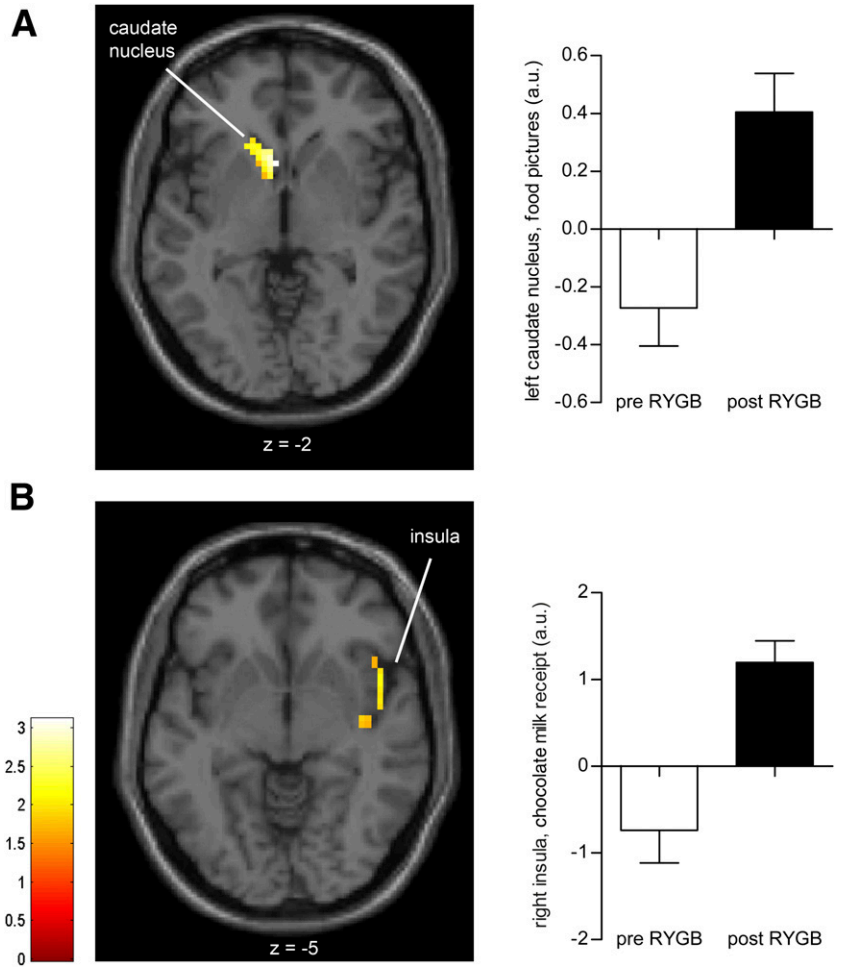


**Figure 3**—Effects of GLP-1 blockade (using Ex9-39), comparing before versus after RYGB, on CNS activation in response to visual (*A*) and gustatory (*B*) food cues. Axial slices showing the difference in the group averages in activation in areas of the CNS, depicting the difference of the effect of GLP-1 blockade by infusion of Ex9-39 (versus placebo). *A*: GLP-1 receptor blockade resulted in a larger increase in activation in response to viewing food pictures after RYGB compared with before. *B*: 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 units [a.u.]); mean and SEM are shown.

### 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 10 min 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 diarrhea shortly after intake of the meal on both visits after RYGB.

## CONCLUSIONS

In the current 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 ROIs 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 with 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 that are part of the central reward circuits (among others, ventral striatum and putamen) in response to visual food cues after RYGB have been described (6,7). RYGB is also associated with a deceased desire to eat highly palatable food (6,22). In accordance with these studies, we observed decreased CNS activation in response 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 current 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 knockout mice were shown to have reduced sweet taste sensitivity, pointing toward 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 the 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 with 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 decrease 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 be indirectly 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 the 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 3 months 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 with 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 ages of 40 and 65 years, which limits the generalizability to men and other age-groups. In addition, we investigated patients 4 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.

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**Author Contributions.** 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 writing 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. and J.J.H. performed laboratory analyses and contributed to writing the manuscript. M.L.D. contributed to the design of the study and to writing the manuscript. M.D. designed the study. R.G.I. designed the study, performed data analysis, and wrote the manuscript. All authors have seen and approved the final version of the manuscript. J.S.t.K. and R.G.I. 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.

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