CHAPTER 7

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

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Submitted
ABSTRACT

Background & Aims
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 hypothesised that postoperative elevated GLP-1 levels contribute to the improved satiety regulation after RYGB via effects on the CNS.

Methods
Effects of the GLP-1 receptor antagonist exendin 9-39 (Ex9-39) and placebo were assessed in ten women before and after RYGB on separate visits. We used functional magnetic resonance imaging (fMRI) to investigate CNS activation in response to visual food cues (i.e. food pictures) and palatable gustatory food cues (i.e. consumption of 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. ClinicalTrials.gov number, NCT01363609
INTRODUCTION

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 (RYGB) (2), which comprises the formation of a small gastric pouch, which is connected to the mid-jejunum, bypassing the duodenum and proximal jejunum. This leads 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 (3;4).

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) (5-8). Interestingly, weight loss after RYGB is paralleled by decreased responsiveness of the CNS to high-calorie visual food cues, measured with fMRI (9;10), 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 responsiveness 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 (11). RYGB is consistently associated with increased postoperative levels of the gut hormone glucagon-like peptide-1 (GLP-1) (12-14), 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 (15;16), which is at least partly mediated via effects in the CNS (17-19). 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 (20;21).

We have previously shown, by means of a GLP-1 receptor antagonists, that endogenous GLP-1 mediates satiating effects of meal intake on CNS responsiveness to food cues in humans (22). We therefore hypothesised 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 surgery by comparing the effects of the selective GLP-1 receptor antagonist exendin 9-39 (Ex9-39) with placebo before and after RYGB.

METHODS

Participants

The study (NCT 01363609) was approved by the Medical Ethics Review Committee of the VU University Medical Center (VUMC). Subjects were included after written informed consent was obtained. Ten female candidates for RYGB surgery were recruited from the Center for Bariatric
Surgery at the Slotervaartziekenhuis in Amsterdam, The Netherlands. Subjects were eligible if they were 40 to 65 years of age, had a body mass index (BMI) > 35 kg/m², and were right-handed. 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 (Figure 1A). All patients had laparoscopic RYGB procedures. Following an overnight fast, participants arrived at 8:30 AM at the research unit. During each visit, two fMRI scans were performed; one while the participant was fasted and one 30 minutes after intake of a standardised liquid meal. The liquid meal was consumed over a 25 min. interval. The first four participants received 200mL, Nutridrink yoghurt style, Nutricia®, Zoetermeer, The Netherlands (300kcal, carbohydrate 37.5g, fat 11.6g and protein 12.0g.). However, since these participants reported that this amount was very difficult to consume entirely during the visits after the RYGB surgery, the protocol was adapted during the study. The remaining six participants received only 150mL before and after surgery. At each visit, a catheter was inserted into a cubital vein for infusion (in random order) of either placebo (0.9% sodium chloride solution) or the GLP-1 receptor antagonist Ex9-39 (Bachem; Clinalfa products, Bubendorf, Switzerland: used to block the effects of endogenous GLP-1), using a MRI-compatible infusion pump (MRIdium3850 pump, Iradimed, WinterPark, USA). Ex9-39 was diluted in 0.9% sodium chloride solution 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 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 randomisation 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 Figure 1B.

**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 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 SoftwareTools, Pittsburg, PA).

Visual food cues: Details of this fMRI task have been described previously (7;22;23). Briefly, the fMRI task consisted of pictures selected from three different categories of pictures; 1) high-calorie food items, 2) low-calorie food items and 3) non-food items. Pictures were presented in a block design. In total, 42 pictures per category were presented divided in six blocks of 21 sec. each (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, shape and colour.
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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 standardised 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.

Gustatory food cues: Details of this fMRI task have been described previously (8). 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 mM NaHCO₃ and 25 mM KCl (6)). This solution should provide a better neutral stimulus than water, which has previously been shown to be able to activate the gustatory cortex (24;25). 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 sec. 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 taste solution delivery.

MRI acquisition and analyses

MRI acquisition and analyses have been described previously (7;8;22;23). MRI data were acquired on a 3.0 Tesla GE Signa HDxt scanner (GeneralElectric, Milwaukee, Wisconsin, USA). Functional images were analysed with SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK). Functional scans were analysed in the context of the general linear model. For the visual food-cue task, the high-calorie food block, low-calorie food block 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 pictures that is greater compared to during viewing non-food pictures. These contrast images were entered into three-way ANOVA with factors surgery (pre RYGB, post RYGB), infusion (placebo, Ex9-39) and satiety state (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 the postprandial state. A priori regions of interest (ROIs) were determined based on previous studies (i.e. insula (including the adjacent opercular cortices), striatum (i.e. putamen and caudate nucleus), amygdala and orbitofrontal cortex (OFC)) (5-7,26). 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, as described previously (7,8,22,23).

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 analysed using a C-terminally directed radioimmunoassay for amidated GLP-1 (antibody 89390) (27).

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 the visits: 1) before start of the first MRI session, 2) before intake of the meal, 3) 30 min. after meal intake, 4) 60 min. after meal intake.

Statistical analyses
Clinical group data were analysed with the Statistical Package for the Social Sciences (SPSS) version 20. Data are expressed as mean ± SEM or median [interquartile range]. Effects of RYGB surgery on clinical characteristics were analysed with Wilcoxon signed-rank test. To analyse the interaction of RYGB and the infusion of Ex9-39, and for the measurements with more than one time point per visit, a generalised estimating equation approach was used. Results were considered statistically significant when P < 0.05.

RESULTS
Clinical characteristics
Clinical characteristics before and after RYGB surgery are presented in Table 1. After RYGB, body weight was reduced significantly (mean ± SD, -8.8 ± 1.7 kg, P = 0.005). Additionally,
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waist circumference, body fat mass and lean mass were significantly reduced after RYGB surgery (P ≤ 0.007).

Figure 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). During Ex9-39 infusion, GLP-1 levels were significantly higher compared to placebo infusion, 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 surgery (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 surgery (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 surgery reduces CNS activation in response to visual and gustatory food cues

To investigate the effects of RYGB surgery on the CNS activation in response to visual and gustatory food cues, we compared CNS activation during placebo infusion before and after RYGB (Table 2). Visual food cues: In the fasted condition during placebo infusion, RYGB surgery resulted in lower activation in response to viewing food pictures in left caudate nucleus and right rolandic operculum (P = 0.03 for both). In addition, the activation in response to high-calorie pictures was decreased after RYGB surgery in left caudate nucleus (P = 0.03), right rolandic operculum (P = 0.09) and in left OFC (P = 0.03) (Figure 3A). No significant effects of RYGB surgery were observed in the postprandial condition.

Table 1: Clinical characteristics before and after RYGB.

<table>
<thead>
<tr>
<th></th>
<th>Before RYGB</th>
<th>4 weeks after RYGB</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>46.5 [40.0, 50.0]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>107.8 [101.2, 114.0]</td>
<td>99.3 [92.6, 104.9]</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39.9 [37.8, 42.5]</td>
<td>36.8 [34.6, 39.1]</td>
<td>0.005</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>118 [114, 123]</td>
<td>115 [108, 126]</td>
<td>0.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 [77, 90]</td>
<td>76 [68, 82]</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>72 [65, 79]</td>
<td>65 [60, 71]</td>
<td>0.06</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>118 [111, 121]</td>
<td>113 [107, 116]</td>
<td>0.005</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>52.7 [50.5, 58.0]</td>
<td>49.3 [42.6, 51.4]</td>
<td>0.005</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>54.9 [49.9, 57.9]</td>
<td>52.9 [48.9, 54.8]</td>
<td>0.007</td>
</tr>
<tr>
<td>HbA1c</td>
<td>37 [34, 40]</td>
<td>37 [34, 37]</td>
<td>0.2</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.1 [4.8, 5.4]</td>
<td>3.8 [3.2, 4.4]</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.0 [0.7, 1.6]</td>
<td>1.0 [0.8, 1.1]</td>
<td>0.1</td>
</tr>
<tr>
<td>Glucose fasting</td>
<td>4.8 [4.5, 5.3]</td>
<td>4.7 [4.4, 4.9]</td>
<td>0.6</td>
</tr>
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</table>

Data are median (interquartile range).
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 ($P = 0.003$) (Figure 3B).

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

In order to determine the effects of endogenous GLP-1 after RYGB surgery, we compared the effect of Ex9-39 infusion vs. placebo infusion before and after RYGB surgery on CNS activation during the different food cue tasks.

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 ($P = 0.02$ and $P = 0.08$ respectively) (Figure 4A). 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 after RYGB, Ex9-39 infusion resulted in increased activation to chocolate milk consumption in right insula, putamen and OFC compared with placebo ($P = 0.007$, $P = 0.01$ and $P = 0.04$, respectively), but Ex9-39 had no effect before RYGB. Comparing GLP-1 receptor blockade before and after RYGB, the effect of Ex9-39 was significantly larger after RYGB in right insula ($P = 0.002$) (Figure 4B).

Appetite related scores

RYGB surgery decreased feelings of hunger and prospective food consumption significantly ($P < 0.001$) during placebo infusion. Appetite for sweet, savory and food items was also reduced after RYGB surgery ($P = 0.001$, $P = 0.006$ and $P = 0.003$, respectively). Feeling of nausea were increased after RYGB ($P < 0.001$), but no differences in sensation of fullness were observed ($P = 0.3$). Comparing the effect of GLP-1 receptor blockade before and after RYGB, the effect of Ex9-39 infusion was not significantly different.
### Table 2: Effects of RYGB surgery and GLP-1 receptor blockade in response to visual and gustatory food cues

<table>
<thead>
<tr>
<th>Contrast used</th>
<th>Comparison</th>
<th>Region</th>
<th>Side</th>
<th>Cluster</th>
<th>Max T-val</th>
<th>FEW P value</th>
<th>MNI coordinates (x, y, z)</th>
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<tbody>
<tr>
<td><strong>Visual food cues: effects of RYGB</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Food &gt; non-food</td>
<td>Effects in fasted state</td>
<td>Caudate nucleus</td>
<td>L</td>
<td>18</td>
<td>3.15</td>
<td>0.03</td>
<td>-15, 23, -2</td>
</tr>
<tr>
<td>Pre RYGB &gt; post RYGB (both placebo)</td>
<td>Rolandic Operculum</td>
<td>R</td>
<td>13</td>
<td>3.11</td>
<td>0.03</td>
<td>54, -4, 10</td>
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<tr>
<td>High-calorie &gt; non-food</td>
<td>Caudate nucleus</td>
<td>L</td>
<td>19</td>
<td>3.11</td>
<td>0.03</td>
<td>-13, 23, -2</td>
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<tr>
<td>OFC</td>
<td>L</td>
<td>13</td>
<td>3.13</td>
<td>0.03</td>
<td>-33, 47, -8</td>
<td></td>
<td></td>
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<tr>
<td>Rolandic Operculum</td>
<td>R</td>
<td>9</td>
<td>2.64</td>
<td>0.09</td>
<td>48, -1, 10</td>
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<tr>
<td>Food &gt; non-food</td>
<td>Effects in postprandial state</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td></td>
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<tr>
<td>High-calorie &gt; non-food</td>
<td>Pre RYGB &gt; post RYGB</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Visual food cues: effects of GLP-1 R blockade x RYGB</strong></td>
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<tr>
<td>Food &gt; non-food</td>
<td>fasted state</td>
<td>Caudate nucleus</td>
<td>L</td>
<td>26</td>
<td>3.34</td>
<td>0.02</td>
<td>-3, 14, -2</td>
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<td>High-calorie &gt; non-food</td>
<td>Effect of GLP-1 post RYGB &gt; pre RYGB</td>
<td>Caudate nucleus</td>
<td>L</td>
<td>5</td>
<td>3.02</td>
<td>0.08</td>
<td>-6, 20, 1</td>
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<tr>
<td>Food &gt; non-food</td>
<td>Postprandial state</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>High-calorie &gt; non-food</td>
<td>Effect of GLP-1 post RYGB &gt; pre RYGB</td>
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<td><strong>Gustatory food cues: effects of RYGB</strong></td>
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<tr>
<td>Chocolate &gt; tasteless</td>
<td>Pre RYGB &gt; post RYGB (both placebo)</td>
<td>Insula</td>
<td>R</td>
<td>52</td>
<td>4.27</td>
<td>0.003</td>
<td>51, 2, -8</td>
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<td><strong>Gustatory food cues: effects of GLP-1 R blockade x RYGB</strong></td>
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</tr>
<tr>
<td>Chocolate &gt; tasteless</td>
<td>Effect GLP-1 blockade:</td>
<td>Insula</td>
<td>R</td>
<td>59</td>
<td>4.42</td>
<td>0.002</td>
<td>48, -7, -11</td>
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</table>
Figure 3: Effects of RYGB on CNS activation in response to visual and gustatory food cues. 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 surgery and B) activation in response to chocolate milk consumption was lower after RYGB compared to before surgery.

The colour 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.
During the visits after RYGB surgery, two patients complained about nausea shortly after intake of the meal during placebo infusion. Two patients experienced periods of dizziness and palpitation lasting approximately 10 minutes after intake of the meal during the visit after RYGB surgery with placebo. One patient had diarrhoea shortly after intake of the meal on both visits after RYGB.

**Figure 4:** Effects of Ex9-39 administration in CNS activation to food cues before and after RYGB. 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 surgery. Panel B shows a comparable effect in response to chocolate milk consumption. The colour 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.
DISCUSSION

In the present study we investigated the effects of RYGB surgery 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 areas of the CNS involved in reward and satiety circuits to both visual and gustatory food cues. 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 surgery. 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 (28). Decreased activation in the CNS reward pathway in response to visual food cues after RYGB surgery has been described (9;10), and RYGB is associated with a deceased desire to eat high-palatable food items (9;29;30). 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.

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 (15;16) via effects in the CNS (7;8;17-19;22;23). 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 (31). 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 (32), which may stimulate an enhanced release of GLP-1 (33). In addition, an increased density of GLP-1-immunoreactive cells has been observed after RYGB (34). 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 from a study in rats (35). According to this, BMI is correlated with impaired incretin effect of GLP-1 in humans (36), suggesting that reductions in BMI may improve the sensitivity and the effects of GLP-1.

To our knowledge, although previous studies have investigated the effect of RYGB on CNS responses to the viewing of food pictures, none have investigated CNS activation in response to palatable food consumption after RYGB in humans. In response to chocolate milk consumption, we found that
RYGB decreased the CNS responses in the insula, which was accompanied by weight reduction. At first sight, this finding may be considered to be at odds with previous studies, as several (6;8), but not all (37-39), 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 surgery is associated with changes in food preferences (29) and taste perception (30), with higher susceptibility for sweet taste perception (40;41). Studies reported that patients after RYGB have decreased interest in sweet food, finding it less enjoyable or even unpleasant (41;42). 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.

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 (7;9;22;43-46). It should, however, be emphasised that this was a pilot study with only female patients between the age of 40 and 65, which limits the generalisability to men and other age groups. In addition, we investigated patients four weeks after surgery, comparable to previous studies (9;46). 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 (10;47), 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 surgery are at least partly explained by postoperative changes in the levels of endogenous GLP-1. These findings provide further insights in the weight lowering mechanisms of RYGB surgery and may ultimately lead to further development of treatment strategies for obesity.
REFERENCES


ELEVATED POSTOPERATIVE ENDOGENOUS GLP-1 LEVELS MEDIATE EFFECTS OF ROUX-EN-Y GASTRIC BYPASS


**Supplemental Figure**

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:** Subjects 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.