An obese brain and an inflamed body: Central and peripheral consequences of obesity

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Citation for published version (APA):
Striatal dopamine $D_{2/3}$ receptor availability increases after long-term bariatric surgery–induced weight loss

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Submitted
Abstract

**Background:** Reduced striatal dopamine D$_{2/3}$ receptor (D$_{2/3}$R) availability was reported in obese subjects compared to lean controls. Recently we determined the effect of short-term bariatric surgery-induced weight loss on striatal D$_{2/3}$R availability in 20 morbidly obese women and found reduced striatal D$_{2/3}$R availability at baseline, which remained unaltered 6 weeks after surgery, despite significant weight loss. To determine whether long-term bariatric surgery-induced weight loss normalizes striatal D$_{2/3}$R availability.

**Methods:** In 14 morbidly obese women who participated in our previous short-term study and age-matched lean controls. Changes in striatal D$_{2/3}$R binding was measured using [123I]IBZM SPECT and were correlations with changes in body weight/composition, eating behaviour and fasting plasma levels of leptin, ghrelin, insulin and glucose.

**Results:** Mean body mass index declined from 46 ± 7 kg/m$^2$ to 32 ± 6 kg/m$^2$ and this was accompanied by a significant increase in striatal D$_{2/3}$R availability (p=0.031). D2/3R remained significantly reduced compared to the age-matched controls (BMI 22 ± 2 kg/m$^2$; p = 0.01). Changes in striatal D$_{2/3}$R availability did not correlate with changes in body weight/fat, insulin sensitivity, ghrelin or leptin levels. Although food craving measures improved, they were not related to the observed changes in striatal D2/3R availability.

**Conclusions:** Striatal D$_{2/3}$R availability increases after long-term weight loss independent of changes in body weight, metabolic hormones or food craving measures. Our data show that reduced D$_{2/3}$R availability in obesity is a reversible phenomenon.
Introduction

The prevalence of obesity and its health consequences is rising, necessitating fundamental insight into the regulation of energy balance with the aim to improve future treatment modalities (1). Previous studies have implicated the brain dopamine system in the hedonic and motivational aspects of food intake and, similar to findings in addiction, obese subjects exhibited reduced striatal dopamine D_{2/3} receptor (D_{2/3}R) availability compared to lean controls in some (2, 3, 4), but not all studies (5, 6, 7, 8, 9). It remains unknown whether lower D_{2/3}R availability reflects a cause or a consequence of obesity (or both). In support of a causal role, it has been hypothesized that overeating in subjects susceptible to obesity constitutes a compensatory response to make up for decreased dopaminergic signalling in the reward circuitry caused by reduced expression of dopamine receptors due to genetic factors (10, 11). In contrast, downregulation of striatal D_{2/3} R occurring after the onset of obesity in animal studies suggests changes in the striatal dopaminergic system to be a consequence of a persistent increase in palatable food consumption, positive energy balance and/or fat mass (12, 13).

To study the reversibility of reduced striatal D_{2/3} R binding in obesity, we previously determined D_{2/3}R availability in 20 morbidly obese women 2 weeks before and 6 weeks after Roux-en-Y gastric bypass surgery (RYGB) using [^{123}I]IBZM single photon emission computed tomography (SPECT). In that study, striatal D_{2/3} R availability was reduced by ~20% compared to lean controls and did not significantly change 6 weeks after surgery, despite significant weight loss (14). However, reductions in striatal D_{2/3} R availability caused by addiction to drugs of abuse may persist for several months after cessation of drug use, and in one study, self-administration of cocaine caused reductions in striatal D_{2/3} R availability that persisted up to 1 year in some, but not all monkeys (15). Furthermore, body weight following bariatric surgery only stabilizes after approximately 1 year (16). Therefore, we re-invited the subjects that were included in the study on the short-term effects of RYGB to repeat the striatal D_{2/3} R measurements at least 2 years after RYGB.

The dopamine system also appears to play a role in glucose control, as e.g. dopamine agonists previously showed insulin-sensitizing effects in obese diabetic subjects (17). Moreover, dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra (SN) pars compacta express receptors for insulin, leptin and ghrelin (18, 19) and insulin sensitivity and fasting plasma levels of ghrelin and leptin were previously associated with striatal D_{2/3} R availability (20). Therefore, we additionally studied whether long-term changes in plasma levels of leptin, insulin, glucose and ghrelin and the quantitative insulin sensitivity check index (QUICKI) correlated with changes in striatal D_{2/3} R availability.

Finally, healthier eating behavior was previously reported after bariatric surgery, with reductions in hunger, disinhibition and food craving (21, 22). As dopamine signaling is known to be involved in drug craving (23) and changes in striatal D_{2/3} R are linked to the emergence of compulsive feeding behavior in obese rats (12), we hypothesized that changes in food craving after RYGB would correlate with changes in D_{2/3} R availability. To investigate this,
we compared the results of eating behavior questionnaires before and after RYGB in these subjects.

Material and methods

Subjects

Twenty women previously participated in the study on the short-term effects of RYGB on striatal D<sub>2/3</sub>R availability and insulin sensitivity (14) (NTR1548) and were therefore eligible for this follow-up study (NTR3684). One subject was excluded due to claustrophobia, one due to pregnancy, one because she had started using anti-dopaminergic drugs (after completion of the short-term study), one did not wish to participate, and two were lost to follow up. The average age of the 14 remaining subjects was 40.5 ± 8 years (range 26 - 50). They were age-matched to non-obese historical controls that participated in a previous study and similarly examined after an overnight fast (3).

RYGB surgery had been carried out between December 2009 and December 2011 in two hospitals (Rijnstate Hospital, Arnhem and Slotervaart Hospital, Amsterdam, the Netherlands) as described previously (14). Informed consent was obtained in all subjects and the study was approved by the local medical ethics committee of the Academic Medical Center in Amsterdam.

Study protocol

After an overnight fast from 22:00 PM the day before, all subjects were admitted for one day to the Metabolic Clinical Research Unit of the AMC. Subjects were weighed and body composition determined using bioelectrical impedance analysis (Maltron BF-906, Rayleigh, UK). Blood samples were drawn after insertion of a catheter into a distal arm vein.

Striatal D<sub>2/3</sub>R availability was assessed with [123 I]IBZM SPECT, using the same protocol and brain-dedicated SPECT system (Neurofocus, Inc., Medfield, Massachusetts, USA) as described for the short-term study (14). A classic region-of-interest (ROI) analysis was performed by manually positioning fixed ROIs for the striatum and occipital cortex on four consecutive transverse slices representing the most intense striatal binding. Non-displaceable binding potential (BP<sub>ND</sub>) was calculated as follows: \[(\text{striatal binding} - \text{occipital binding})/\text{occipital binding}\]. Additional exploratory analyses of striatal subregions were performed in a similar manner using separate standardized ROIs for the putamen and caudate nucleus.

During the 2 hours waiting time between start of the administration of [123 I]IBZM and the acquisition of the SPECT data, subjects filled out the questionnaires described below (these were similarly administered at baseline in the short-term study). Until completion of the scan, participants were only allowed to drink water.

Plasma measurements

Leptin was measured with 125I radioimmunoassay (Millipore; intra-assay variation 3.4-8.3%; total assay variation 3.6-6.2%; detection limit 0.5 ng/ml). Ghrelin was determined with 125I
radioimmunoassay (Millipore; intra-assay variation 6.5-9.5%; total assay variation 9.6-16.2%; detection limit 10 pg/ml). Plasma glucose concentrations were measured with the glucose oxidase method using a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barleben/Magdeburg, Germany). Insulin was measured with a chemiluminescent immunometric assay (intra-assay variation of 3–6%; inter-assay variation of 4%; detection limit of 15 pmol/l). The quantitative insulin sensitivity check index (QUICKI) was calculated using the formula: \( I / (\log(\text{fasting insulin} \, \mu\text{U/mL}) + \log(\text{fasting glucose} \, \text{mg/dL})) \).

**Questionnaires**

To compare different aspects of eating behavior with measures obtained at baseline, subjects were asked to repeat the following questionnaires:

1. **Dutch eating behavior questionnaire (DEBQ):** 33 items, divided into three subscores/ eating behavior patterns (24) - (1) restrained eating (i.e. the degree of conscious food restriction); (2) external eating (i.e. eating in response to food-related stimuli); (3) emotional eating (i.e. eating in response to negative emotions in order to relieve stress while disregarding internal physiological signals of satiety).

2. **Eating disorder examination questionnaire (EDEQ):** 30 items with four subscores (25) - (1) Restraint; (2) Eating Concern; (3) Shape Concern; (4) Weight Concern.

3. **General food craving questionnaire - trait (GFCQ-T):** 21 items to measure food craving occurring in general, containing four factors (26) - (1) preoccupation with food (i.e., obsessively thinking about food and eating); (2) loss of control (i.e., experiencing difficulties in regulating eating behavior when exposed to food cues); (3) positive outcome expectancy (i.e., believing eating to be positively reinforcing); and (4) emotional craving (i.e., the tendency to crave food when negative emotions are present).

**Statistical analysis**

SPSS version 20.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Outliers were defined as values more than 1.5 times the interquartile range beyond the quartiles. Changes in all measured parameters were analyzed using parametric tests (paired student’s t-test, two-sided), except for leptin and the GFCQ-T, which were tested using the Wilcoxon signed rank test (two-sided), as they failed to pass the Kolmogorov-Smirnov test for normality. Comparisons were considered statistically significant when \( p \) was <0.05 for the primary outcome measures (changes in D\(_{2/3}\)R availability, plasma measurements and total scores on eating behavior questionnaires). Correlations between striatal D\(_{2/3}\)R availability and changes in body mass index (BMI), %body fat, plasma measurements, QUICKI and scores on the GFCQ-T were determined with Spearman’s rank order. These secondary outcome measures were considered statistically significant at a p-value of <0.01 (to correct for multiple testing). Other Spearman’s rank order correlations are presented as exploratory analyses, as are the changes in the subscores of the questionnaires. Unless otherwise specified, data are presented as mean ± standard deviation (minimum - maximum).
Results

Body weight, body composition, plasma measurements and QUICKI

Table 1 summarizes the changes in measures of body weight/composition and metabolic parameters of the 14 subjects included. Time since RYGB ranged from 2.1 to 3.6 years (average 3.1 years). All subjects reported that body weight had stabilized by the second year after RYGB. One outlier was excluded from the analysis for ghrelin levels (baseline ghrelin of 2860 pg/ml). Changes in plasma leptin levels correlated with percentage weight loss ($\rho = -0.70$, $p=0.005$) and change in BMI ($\rho =0.58$, $p=0.03$), but not with absolute weight loss ($\rho = 0.45$, $p=0.1$). There was a trend for correlation of changes in insulin with changes in %body fat ($\rho=0.48$, $p=0.08$) and of changes in QUICKI with changes in BMI ($\rho = -0.54$, $p=0.07$). Plasma level changes in ghrelin and glucose were not correlated with any measure of body weight loss ($p> 0.3$).

Table 1. Descriptive characteristics of the 14 participants at baseline and long-term follow-up after RYGB (2.1-3.6 years). Data are shown as mean ± SD (range). ** $p < 0.01$.

<table>
<thead>
<tr>
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<th>BASELINE</th>
<th>LONG-TERM FOLLOW-UP</th>
<th>P-VALUE</th>
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<tr>
<td>BMI (KG/M$^2$)</td>
<td>45.2 ± 6.7 (38.7 – 61.3)</td>
<td>31.2 ± 5.7 (24.1 – 43.7)</td>
<td>&lt; 0.001**</td>
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<tr>
<td>% BODY FAT</td>
<td>54.0 ±3.4 (48.7 - 60)</td>
<td>40.3 ±6.6 (26.9-52.3)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>% LEAN BODY MASS</td>
<td>46.1 ± 3.4 (40 - 51.3)</td>
<td>59.7 ± 6.6 (47.7 - 73.1)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>LEPTIN (NG/ML)</td>
<td>69.3 ± 22.7 (37.3 - 107.8)</td>
<td>43.1 ± 23.3 (12.2 - 76.8)</td>
<td>0.001**</td>
</tr>
<tr>
<td>GHRELIN (PG/ML)</td>
<td>894 ± 330 (499-1456)</td>
<td>1069 ± 384 (572-1779)</td>
<td>0.23</td>
</tr>
<tr>
<td>GLUCOSE (MG/DL)</td>
<td>101.2 ± 15.2 (81.1 - 127.9)</td>
<td>83.5 ± 4.0 (77.5 - 91.9)</td>
<td>0.001**</td>
</tr>
<tr>
<td>INSULIN (MU/L)</td>
<td>11.7 ± 5.1 (2.8-20.4)</td>
<td>7.2 ± 2.8 (2.2 - 11.1)</td>
<td>0.001**</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.33 ± 0.03 (0.30 - 0.40)</td>
<td>0.37 ± 0.03 (0.33 -0.44)</td>
<td>0.002**</td>
</tr>
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</table>

SPECT analysis

Unfortunately, acquisition of SPECT images failed in two subjects due to technical difficulties. These were excluded from the analyses, in addition to an outlier with a baseline BP$_{ND}$ of 1.15 (showing an unexplainable decrease to 0.69). Figure 1 depicts the changes in BP$_{ND}$ compared to baseline (delta BP$_{ND}$). A significant increase in striatal D$_{2/3}$R availability was observed for the entire striatum (0.76 ± 0.11 to 0.88 ± 0.13, $p=0.031$). Additional exploratory analyses of striatal subregions revealed that the increase in BP$_{ND}$ was slightly more pronounced within the caudate nucleus (0.79 ± 0.12 to 0.90 ±0.13; $p=0.027$) than the putamen (0.76 ± 0.11 to 0.85 ± 0.18; $p=0.052$). Striatal D$_{2/3}$R availability remained significantly lower than age-matched lean controls (1.05 ± 0.12; $p = 0.01$; Figure 2), however BMI of the RYGB subjects also remained significantly higher than that of controls (31.8 ± 6.1 vs 21.9 ± 2.0 kg/m$^2$ respectively, $p < 0.001$).
There were no significant correlations between striatal BP
ND and BMI at baseline (p=0.85), at long-term follow-up (p=0.59) or in the control group (p=0.42; Summarized in figure 3). In addition, there were no significant correlations between changes in D2/3R availability and changes in BMI (p=0.54), % body fat (p=0.67), plasma levels of leptin (p=0.42), ghrelin (p=0.20), glucose (p=0.15), insulin (p=0.54) and QUICKI (p=0.47; Figure 4).

Figure 1. Change in D2/3R availability measured with [123I]IBZM SPECT, represented as change in BPND compared to baseline (Delta BPND) for (A) the total striatum; (B) the caudate nucleus (squares) and putamen (triangles). Line and whiskers represent median and interquartile range.

Figure 2. Striatal D2/3R availability measured with [123I]IBZM SPECT, represented as BPND for: obese subjects before RYGB (filled circles), at long term follow-up after RYGB (open circles), and age-matched lean controls (triangles). Line and whiskers represent median and interquartile range. *p= 0.03 (paired t-test); **p< 0.01 (t-test).
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Figure 3. Correlation between BMI and striatal D_{2/3}R availability measured with [123I]IBZM SPECT (BP_{ND}) for obese subjects before RYGB (filled circles), at long term follow-up after RYGB (open circles), and age-matched lean controls (triangles).

Figure 4. Correlation between changes in (A) BMI, (B) leptin, (C) ghrelin, (D) QUICKI, (E) glucose, and (F) insulin with changes in total striatal D_{2/3}R availability, represented as change in BP_{ND} compared to baseline. ▲ = two overlapping data points.

**Questionnaires**

Total scores and subscores of the questionnaires are summarized in Table 2. (Sub)scores for the GFCQ-T could not be calculated for 2 subjects, due to missing values.

The lower score on the DEBQ was due to lower values on all subscores (external eating, emotional eating and restrained eating). The trend for a decrease in score on the EDEQ
(p=0.07) was accompanied by decreases in all subscores, especially ‘weight concern’. The reduction in total score for the GFCQ-T was mainly due to reductions in factor 2 (loss of control) and factor 4 (emotional craving), and to a lesser degree reductions in factor 1 (preoccupation with food) and factor 3 (positive outcome expectancy). There were no significant correlations between changes in striatal D_{2/3}R availability and the total GFCQ-T score (p=0.35, p=0.29) or any subscore (p>0.2).

Table 2 Total scores and subscores for the Dutch Eating Behavior Questionnaire (DEBQ), Eating Disorder Examination Questionnaire (EDEQ) and General Food Craving Questionnaire-Trait (GFCQ-T) at baseline and long-term follow-up. Data are shown as mean ± SD. ** p < 0.01; *p < 0.05;  #p < 0.1.

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<th>BASELINE</th>
<th>FOLLOW-UP</th>
<th>P-VALUE</th>
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<tbody>
<tr>
<td>DEBQ-TOTAL</td>
<td>2.87 ± 0.42</td>
<td>2.36 ± 0.43</td>
<td>0.004**</td>
</tr>
<tr>
<td>DEBQ-EMOTIONAL EATING</td>
<td>2.53 ± 0.86</td>
<td>2.10 ± 0.72</td>
<td>0.034*</td>
</tr>
<tr>
<td>DEBQ-EXTERNAL EATING</td>
<td>2.98 ± 0.76</td>
<td>2.39 ± 0.56</td>
<td>0.001**</td>
</tr>
<tr>
<td>DEBQ-RESTRAINED EATING</td>
<td>3.10 ± 0.72</td>
<td>2.59 ± 0.52</td>
<td>0.06*</td>
</tr>
<tr>
<td>EDEQ-TOTAL</td>
<td>2.97 ± 0.65</td>
<td>2.24 ± 0.84</td>
<td>0.07*</td>
</tr>
<tr>
<td>EDEQ-RESTRAINT</td>
<td>2.54 ± 0.99</td>
<td>1.79 ± 0.85</td>
<td>0.10</td>
</tr>
<tr>
<td>EDEQ-EATING CONCERN</td>
<td>1.87 ± 0.82</td>
<td>1.49 ± 0.58</td>
<td>0.14</td>
</tr>
<tr>
<td>EDEQ-SHAPE CONCERN</td>
<td>3.86 ± 1.24</td>
<td>3.11 ± 1.13</td>
<td>0.24</td>
</tr>
<tr>
<td>EDEQ-WEIGHT CONCERN</td>
<td>3.61 ± 0.79</td>
<td>2.59 ± 1.21</td>
<td>0.05*</td>
</tr>
<tr>
<td>GFCQ-T TOTAL</td>
<td>2.84 ± 1.02</td>
<td>2.13 ± 0.62</td>
<td>0.024*</td>
</tr>
<tr>
<td>GFCQ-T PREOCCUPATION WITH FOOD</td>
<td>2.70 ± 0.83</td>
<td>2.10 ± 0.78</td>
<td>0.076*</td>
</tr>
<tr>
<td>GFCQ-T LOSS OF CONTROL</td>
<td>2.88 ± 1.18</td>
<td>1.86 ± 0.63</td>
<td>0.006**</td>
</tr>
<tr>
<td>GFCQ-T POSITIVE OUTCOME EXPECTANCY</td>
<td>2.85 ± 1.04</td>
<td>2.38 ± 0.70</td>
<td>0.099*</td>
</tr>
<tr>
<td>GFCQ-T EMOTIONAL CRAVING</td>
<td>2.97 ± 1.37</td>
<td>2.14 ± 0.80</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. ** p < 0.01; *p < 0.05;  #p < 0.1.

Discussion

To our knowledge, this is the first study to determine changes in striatal D_{2/3}R availability after long-term weight loss in obese subjects. Our data show that, more than 2 years after RYGB, striatal D_{2/3}R availability increased compared to pre-operative measures. Weight loss after RYGB was also accompanied by improvements in insulin sensitivity and eating behaviour, although these did not correlate with changes in D_{2/3}R availability.
Previous imaging studies assessing short-term changes in striatal D2/3R binding after RYGB surgery have produced conflicting results: one preliminary positron emission tomography (PET) study in 5 women reported a decrease ± 7 weeks after bariatric surgery (27), another preliminary PET study in 5 women reported an increase 6 weeks after RYGB (9), whereas our previous SPECT study reported no significant changes 6 weeks after RYGB (14). Similar to previous reports, body weight loss in the present study was initially rapid, but stabilized in the second year after surgery (16). Therefore, the increase in striatal D2/3R availability observed in this study provides a reliable estimate of the effect of long-term weight loss following RYGB. Although striatal D2/3R availability increased after long-term weight loss in this study, it remained significantly lower than age-matched controls. However, there was considerable variability in the degree of long-term weight loss after RYGB, and average BMI remained in the overweight/obese range (31.2 ± 5.7 kg/m²). Therefore, D2/3R availability may have normalized completely if the all subjects had reached a BMI within the normal range.

At present, it remains uncertain to what extent reduced striatal D2/3R availability precedes or follows development of obesity in humans, as there is data supporting both hypotheses (10; 12; 11; 13). In addition, it remains a matter of debate whether lower striatal D2/3R availability in obese humans reflects a hyperdopaminergic or hypodopaminergic state. This is partly due to the fact that striatal D2/3R availability is not only influenced by D2/3R expression but also by synaptic dopamine levels, which in turn are influenced by both tonic and phasic dopamine release. Importantly, increased basal dopaminergic tone is accompanied by reduced phasic dopamine release (e.g. triggered by food-stimuli), because it is partly influenced by activation of pre-synaptically located autoreceptors (28). Thus, interpretation of the mechanisms underlying the reduced striatal D2/3R availability observed in obesity is not straightforward. As reduced expression of striatal D2 receptors was reported following diet-induced obesity in rodents (12), the increase in BPND observed in the present study could reflect an upregulation of striatal D2 receptors (i.e. reversal of downregulation). Alternatively, the increase in BPND could also be due to a reduction in basal dopaminergic tone (as subjects in this study were scanned in a fasted state). It was recently hypothesized that subjects in the morbidly obese range exhibit a higher basal dopaminergic tone with a subsequent decrease in phasic dopamine release (28). Reversal of this phenomenon after weight loss would cause a reduction in basal dopaminergic tone, and thus explain the increase in BPND observed in this study. Interestingly, we recently showed blunted phasic dopamine release in severely obese women (3). However, due to the present study design we were unable to quantify any changes in dopamine release after RYGB-induced weight loss in this cohort.

The lack of correlation between delta BPND and body weight loss after RYGB suggests the change in striatal D2/3R availability might not be determined solely by (changes in) body weight. Interestingly, reduced striatal D2/3R mRNA expression was observed following long-term exposure of rats to ‘junk food’ even in the absence of body weight gain (12). Furthermore, studies in which rats are exposed to different obesogenic diets suggest that the reduction in striatal D2/3R availability depends on dietary composition, especially a high fat/carbohydrate...
ratio (13; 29). Although we did not assess dietary caloric content or diet composition before versus after RYGB, previous studies reported lower caloric intake after bariatric surgery with reduced preference for high fat and high sucrose food (30; 31). Therefore the increase in striatal D_{2/3}R availability observed in this study might be (partially) explained by changes in diet composition and caloric intake.

Our additional exploratory analysis of striatal subregions revealed a slightly more pronounced increase in D_{2/3}R availability in the caudate nucleus compared to the putamen. Interestingly, obese subjects showed increased hemodynamic responses and glucose uptake in the caudate nucleus while viewing appetizing versus bland foods (32). Moreover, in a recent fMRI study, RYGB patients showed lower activation of brain reward systems (including the caudate nucleus) compared to gastric banding patients, with lower palatability scoring and appeal of high-calorie foods (33). Although changes in blood flow and glucose consumption do not necessarily reflect changes in dopamine signalling, taken together with our findings, this suggests that the caudate nucleus may play an important role in the hedonic aspects of food intake in obese subjects and that this may be altered by RYGB-induced weight loss. This might result from decreased phasic dopamine release in response to food-related stimuli, however, as mentioned above, it was not possible to determine changes in phasic dopamine release in this cohort.

The unchanged ghrelin levels after RYGB in this study are in line with previous findings (34) but in contrast to the study by Dunn et al., we observed no correlations between changes in BP_{ND} and changes in plasma levels of ghrelin (20). This may be because we measured total ghrelin and not acylated ghrelin. In addition, no correlation was present between plasma leptin, glucose, insulin or the insulin sensitivity index (QUICKI) and changes in D_{2/3}R availability. However, given that circadian rhythms both affect these metabolic parameters as well as dopaminergic signalling, it is also possible that methodological differences played a role (35, 36). Subjects in the present study were scanned just before noon, after an overnight fast, whereas subjects in the study of Dunn et al. were scanned in the evening after a 6-hour fast (20). Furthermore, although in lean subjects acyl-ghrelin levels correlated with D_{2/3}R availability in the SN, in obese subjects it did not (37). Thus, the relationship between D_{2/3}R availability and levels of acyl-ghrelin and BMI does not appear straightforward and dependent on experimental circumstances. Finally, the lack of correlation between changes in BP_{ND} with changes in QUICKI, plasma glucose and insulin suggest that these changes are independent effects of RYGB and that lower basal insulin and glucose levels do not affect striatal D_{2/3}R availability or vice versa. Thus, although dopamine agonists may improve insulin sensitivity in obese diabetics, the beneficial effects of RYGB on insulin sensitivity do not appear to depend on changes in striatal D_{2/3}R availability (17).

The changes we observed in eating behavior questionnaire scores are in line with previous studies reporting reductions in hunger, restraint, food craving and disinhibition after RYGB (21, 22). The reductions we observed in the G-FCQ-T and DEBQ indicate a lower tendency
to experience food cravings and to eat in response to negative emotions, stress or external food-cues. Although the GFCQ-T score decreased after RYGB, no correlation was found between the observed changes in BPND and GFCQ-T scores, suggesting that increases in basal D2/3R availability did not play a role in the reductions in food craving and disinhibition. Interestingly, this is in line with a recent animal study that showed that low dopamine D2 receptor expression increased vulnerability to diet-induced obesity through changes in physical activity, and not through increased food motivation (38). Nevertheless, it remains possible that changes in feeding/food cue-induced striatal dopamine release underlie the reduction in food craving observed in this study.

To our knowledge, this is the first study to determine the effect of long-term weight loss on striatal D2/3 R availability in a human model. However, it has several limitations. Due to the study design and the relative inaccessibility of the human brain, we can only speculate on the mechanisms underlying the observed effect on striatal D2/3 R availability, as both D2/3 R expression and synaptic dopamine levels can influence this. Other limitations include the limited number of subjects and the fact that repeat scans were only performed on the obese subjects. Ideally repeat scans would also have been performed in the healthy controls or a group of obese subjects that did not undergo RYGB. However, striatal D2/3 R availability decreases with age (39), thus any age-related effect would only have reduced the probability to detect an increase in BPND after RYGB. Furthermore, good reproducibility was previously reported for striatal D2/3 R measurements using a similar IBZM-protocol (intraclass correlation coefficient of 0.74 (40) and the SPECT scans of this follow-up study were performed using the same protocol and scanner as for baseline measurements, with ROI analyses performed by the same researcher. Finally, the study was performed in obese females only and studies in males might yield different results.

In summary, this study shows that long-term weight loss after RYGB is accompanied by an increase in striatal D2/3 R availability, which suggests the reduction of striatal D2/3 R availability observed in obesity is reversible. Furthermore, although insulin sensitivity and eating behavior improved after RYGB, neither the changes in QUICKI nor the reductions in food craving and disinhibition correlated with changes in striatal D2/3 R availability, challenging an important role for altered basal D2/3 R availability in these effects of RYGB. Future studies that measure effects of weight loss on phasic dopamine release are needed to assess whether changes in feeding/food-cue-induced dopamine release might underlie the effects of RYGB on food motivation.

**Acknowledgements:** We would like to thank Michelle Panton, Ruth Versteeg, Mette Stam, Bastiaan Kee, Erik Knaap, Paul Groot, Murat Kilicarslan, Karin Koopman, Shreyas de Jong, and Martine van Vessem-Timmermans for their valuable assistance during the preparation and execution of this study.
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