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

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Striatal dopamine receptor binding and insulin sensitivity in obese women before and after gastric bypass surgery

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CHAPTER 3

Abstract

**Background:** In cross-sectional studies, a reduction in striatal D$_{2/3}$ receptor (D2/3R) binding has been reported in obese humans. It is unknown whether this reflects a cause or consequence of the obese state. In addition, the relation between metabolic health and striatal D2/3R is unclear at present. We therefore studied striatal D2/3R availability two weeks before and six weeks after a hypocaloric metabolic state accompanied by weight loss in morbidly obese women undergoing Roux-en-Y gastric bypass surgery (RYGB).

**Methods:** In morbidly obese women, striatal D2/3R availability was assessed using a brain-dedicated SPECT scanner and [123I]IBZM. Insulin sensitivity was determined at baseline during a two-step hyperinsulinemic euglycemic clamp using a stable glucose isotope tracer.

**Results:** We included 19 women. After RYGB, BMI was reduced, but D2/3R availability did not change significantly. Glucose production and hepatic insulin sensitivity did not correlate significantly with D2/3R availability while peripheral insulin sensitivity tended to correlate positively with D2/3R availability.

**Conclusions:** A hypocaloric state and significant weight loss following RYGB in morbidly obese women did not increase D2/3R availability. Moreover, the D2/3R did not correlate to measures of insulin sensitivity. This finding does not support an important role for striatal D2/3R in short-term changes in energy balance or weight loss in obesity.
Introduction

Unravelling the association between obesity and disturbances in lipid and glucose metabolism is necessary to improve future treatment modalities. The relationship between the increase in fat mass and the metabolic perturbations is complex and probably depends on the nature of the adaptive response to the hypercaloric milieu. Some of the metabolic adaptations are orchestrated by the brain. Areas that are involved include the hypothalamus, the prefrontal cortex and striatum (1,2). There is indeed evidence that these brain areas are functionally altered in obesity. In obese humans, we and others reported a reduction in dopamine D$_{2/3}$ receptor (D2/3R) binding in the striatum, an important component of the brain reward system (3,4). In addition, a hypercaloric diet for two days in lean healthy subjects resulted in a different activity pattern to visual food cues in the prefrontal cortex and hypothalamus, measured with functional MRI (fMRI), implying a central adaptation to the hypercaloric milieu (5).

Food is a powerful primary reinforcer, and individual differences in the reinforcing efficacy of food may provide a mechanism to explain the excess food intake and positive energy balance responsible for obesity (3,6). The neurotransmitter dopamine is important for the reinforcing value of food and it has been shown that food can induce a release of endogenous dopamine in the striatum (7). Obese subjects are thought to be more sensitive to food reinforcement than those who are non-obese. Using fMRI, it was shown that obese subjects show a greater hemodynamic response to visual food stimuli in dopamine-rich regions such as the nucleus accumbens/ventral striatum, caudate nucleus and putamen (8,9). This may underlie the notion that obese humans experience increased craving for food (10). In addiction, striatal D2/3R availability has been linked to craving and diet induced obesity (11,12). Similarly, the trait impulsiveness has been linked to striatal D2/3R availability (13-15). Therefore, it is plausible that the dopamine related mechanisms underlying craving and impulsiveness play a role in the development and pathophysiology of obesity. Dopamine deficiency in obese subjects may constitute a compensatory eating pattern to make up for decreased activation in the reward circuitry (3). It remains difficult, however, to dissect what components contribute to lower striatal D2/3R availability during the transition of the lean towards the obese state. Does lower D2/3R availability predispose to obesity, or is D2/3R availability reduced through a positive energy balance or an increase in fat mass? In addition, it is unknown at present whether reduced D2/3R availability is reversible after losing clinically significant fat mass or during a hypocaloric state. Therefore we studied D2/3R availability before and 6 weeks after Roux-en-Y gastric bypass (RYGB) surgery in morbidly obese women. We hypothesized that the reduction in striatal D2/3R availability observed in a hypercaloric condition, i.e. the obese state, would be restored by a negative energy balance and weight loss after bariatric surgery. Insulin receptors are widely expressed in the human brain (16) and a relationship between insulin sensitivity and central dopamine signaling has been suggested (17,18). Whether lower striatal D2/3R availability contributes to this observation is currently unknown. To study the possible coherence between striatal D2/3R and glucose metabolism, we correlated the
striatal D2/3R binding potential to hepatic and peripheral insulin sensitivity in these morbidly obese women. We expected striatal D2/3R availability to be positively correlated to measures of insulin sensitivity.

**Material and methods**

**Subjects**

Twenty obese women, who were scheduled for bariatric surgery, were studied two weeks before and six weeks after RYGB surgery. They participated in a study on the short term metabolic effects of RYGB surgery (NTR1548) (19). We reported earlier on the imaging findings of the preoperative data of 15 patients of this study population (4). None of these subjects had a history of neuroleptic or other dopaminergic treatment, childhood onset obesity, current or past psychiatric disease, lifetime history of alcohol/drug abuse, concomitant or past severe medical conditions, including diabetes mellitus. 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.

**SPECT acquisition**

All subjects were studied after a 12-h fast from 22:00 PM the day before and all scans were scheduled at the same time in the morning. SPECT studies were performed using a 12-detector brain-dedicated scanner (Neurofocus 810, Inc.,Medfield, Massachusetts, USA) with a full-width at half-maximum (FWHM) resolution of 6.5 mm, throughout the 20 cm field-of-view. After positioning of the subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps (300 sec scanning time per slice). The energy window was set at 135–190 keV. In all participants, approximately 80 MBq [123I]IBZM was given as an intravenous bolus, followed by continuous infusion of 20 MBq/h to achieve unchanging regional brain activity levels. Acquisition of the images was started 2 h after the bolus injection, the infusion continued until the scan was finished (after approximately 60 minutes). The SPECT scan was repeated under the same conditions and following the same protocol at 6 weeks after surgery. This timeframe was chosen based on the estimated turnover time of striatal D2/3 receptors, and this timeframe was used in previous preliminary studies (20,21).

**SPECT processing**

Attenuation correction of all images was performed as described earlier (22). Images were reconstructed in 3-D mode. For quantification, two techniques were used. In the first analysis, a classic region-of-interest (ROI) analysis was performed with fixed ROIs for the striatum and occipital cortex, as described earlier (4). Briefly, on four consecutive transverse slices representing the most intense striatal binding, the average striatal and occipital binding (representing nonspecific binding) was measured by positioning ROIs manually. Then the non-displaceable binding potential ($BP_{ND}$) was calculated as follows: (total striatal binding--
occipital binding)/occipital binding. In the second analysis, the individual SPECT images were registered with the individual MRI images as described earlier (23). Then ROIs were manually drawn on the MR images for the whole striatum, caudate nucleus and putamen and occipital cortex. Just like the first analysis, we then calculated the \( \text{BP}_{\text{ND}} \).

**MRI acquisition**

Prior to the SPECT scan, a MRI scan of the brain was completed to exclude anatomic abnormalities and to enable anatomic mapping. Brain scans were performed on an open 1.0 Tesla MR scanner with a 160 cm-wide patient aperture and a height of 45 cm (Panorama HFO, Philips Healthcare, Best, The Netherlands) using the sense head coil (scan time 6 minutes). Images were acquired using a T1 weighted 3D gradient echo sequence with full brain coverage and high spatial resolution (voxelsize: 0.88 x 0.88 x 0.80 mm\(^3\), 256 x 256 x 160 matrix) with TE/TR=25/6.9 ms.

**Surgical procedure**

The surgical procedures were carried out in two medical centers (Rijnstate Hospital, Arnhem and Slotervaart Hospital, Amsterdam, the Netherlands) and performed by experienced bariatric surgeons. During surgery, the gastric volume was reduced by stapling off a 30-mL proximal gastric pouch and connecting the antecolic alimentary limb in a gastroenterostomy. The biliopancreatic limb with a length of 45–50 cm from the ligament of Treitz was connected to this alimentary limb at a distance of 100–150 cm as an enteroenterostomy. This procedure resulted in a bypass of the distal stomach, duodenum, and proximal part of the jejunum.

**Hyperinsulinemic euglycemic clamp**

The subjects were studied two weeks before and two weeks after RYGB. The differences in glucose metabolism before versus 2 weeks after surgery are reported separately (19). They were admitted to the Metabolic Clinical Research Unit of the AMC after an overnight fast and were studied in the supine position. After a 10-h fast from 22:00 PM the day before, a catheter was inserted into the dorsal vein of each hand or distal vein of each arm. One catheter was used for sampling of arterialized blood using a heated hand box (60°C). The other catheter was used for infusion of [\( ^6,^6\)-\( ^2\)H\(_2\)]glucose, glucose 20%, and insulin. At T=09:00 h AM (t= -2), after drawing a blood sample for background enrichment of plasma glucose, a continuous infusion of [\( ^6,^6\)-\( ^2\)H\(_2\)]glucose (99% enrichment; Cambridge Isotopes, Andover, MA), Andover, MA was started at a rate of 0.11 µmol/kg*min after a priming dose equivalent to 2 hours of infusion. After 110, 115 and 120 min, blood samples were drawn for determination of glucose enrichment, and insulin. Subsequently, at T=11:05 h AM (t=0), a continuous infusion of insulin (Actrapid 100U/ml; Novo Nordisk Farma, Alphen a/d Rijn, the Netherlands) was started for 2 h at a rate of 20 mU/m\(^2\) body surface area. At T=2 h PM, the infusion rate of insulin was increased to 60 mU/m\(^2\) body surface area min\(^{-1}\). Plasma glucose was measured every 10 min and glucose 20% was infused at a variable rate to maintain plasma glucose at 5.0 mmol/L.
\[\text{[6,6-^2\text{H}_2]\text{glucose was added to the 20\% glucose solution to achieve glucose enrichments of 1\% to minimize changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. At } t = 2 \text{ h and } t = 4 \text{ h, blood samples with a 5 minutes interval were drawn to measure glucose enrichment and 2 samples were drawn to measure insulin. During the study the participants were only allowed to drink water.} \]

**Glucose and insulin measurements**

Plasma glucose concentrations were measured with the glucose oxidase method using a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barbleben/Magdeburg, Germany). \[\text{[6,6-^2\text{H}_2]\text{glucose enrichment (tracer-to-tracer ratio) was measured as described earlier (24). The } [6,6-^2\text{H}_2]\text{glucose intra-assay variation was 0.5 \text{ – 1\% with an inter-assay variation of 1\% and a detection limit of 0.04\%. Insulin was determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA). Insulin was measured with a chemiluminescent immunometric assay with intra-assay variation of 4\text{–}5\%, inter-assay variation of 5\% and detection limit of 15 \text{ pmol/l.} \]

**Resting energy expenditure (REE)**

REE was measured during the final 10 min of the basal state of the hyperinsulinemic euglycemic clamp by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, USA).

**Statistical analysis**

Data were analyzed using parametric tests. Comparison of the data before surgery compared to the data obtained six weeks after surgery were analyzed using the paired student’s \(t\)-test. SPSS version 16.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Data are presented as mean ± sd (minimum - maximum). Comparisons were considered statistically significant when \(P\) was <0.05 and as a trend with \(P < 0.1\). Correlations between striatal D2/3R availability and insulin sensitivity were determined with Pearson’s correlation. Resting energy expenditure (REE), was calculated from VO\(_2\) and VCO\(_2\) as reported previously (25).

**Results**

In one subject, acquisition of the SPECT failed due to claustrophobia during the scanning procedure and she was excluded from the analyses. The descriptive characteristics for the 19 remaining obese women are shown in table 1. Weight loss 6 weeks after RYGB was 13.8 ± 4.5 kg [range 8-24 kg] which resulted in a significant reduction in BMI after surgery (Table 1). No correlation could be found between BMI and D2/3R availability before surgery (\(p = 0.59; r^2 0.017\)).
**SPECT analysis**

The region-of-interest analysis showed no significant change in D2/3R availability before versus 6 weeks after RYGB ($0.81 \pm 0.23$ [0.39 – 1.28] vs. $0.79 \pm 0.16$ [0.54-1.07]) respectively; $p = 0.666$; Fig. 1a). Also, in the MRI-driven analysis, the D2/3R availability in the whole striatum as well as in subregions of the striatum (caudate nucleus and putamen) did not significantly change after surgery (Fig. 1b).

**Glucose metabolism**

Since one subject was already excluded from the entire analyses due to failure of the SPECT system, scan 19 subjects remained. The entire hyperinsulinemic euglycemic clamp was unsuccessful in two subjects and the second step was unsuccessful in one subject due to technical failures with the iv-lines. As a consequence, the correlation between hepatic insulin sensitivity and D2/3R availability was performed in 17 subjects and between peripheral insulin sensitivity and D2/3R availability was performed in 16 subjects. Weight loss 2 weeks after RYGB was $7.8 \pm 3.2$ kg [range 6.2-9.4kg].

As reported earlier (19) basal endogenous glucose production (EGP) was $13.6\pm1.8$ (10.3-18.1) µmol/kg FFM*min. Hepatic insulin sensitivity expressed as percentage suppression of EGP by insulin was assessed during the first step of the hyperinsulinemic clamp and was 79±14 (55-99)%.

Insulin-mediated peripheral glucose uptake (Rd) was $25.4\pm9.5$ (11.6-42.5) µmol/kg*min. The REE measurement failed in a total of five subjects. The results of the 15 remaining subjects show that the mean REE significantly decreased from $1889\pm247$ (1530-2413) kcal/day to $1718\pm230$ (1339-2050) kcal/day in the basal state ($p=0.008$). The metabolic parameters (EGP, Rd, basal glucose, basal insulin) were correlated to the striatal D2/3R availability and revealed a clear trend between D2/3R availability and peripheral insulin sensitivity ($p=0.06$; $r^2=0.022$; fig 2.).

| Table 1. Descriptive characteristics of the morbidly obese women before and 6 weeks after bariatric surgery. |
|----------------------------------|----------------------------------|-----------------|--------|
| **BEFORE SURGERY**               | **6 WEEKS AFTER SURGERY**        | *P*              |        |
| N                                | 19                              | 19              |        |
| AGE (YEARS)                      | $40.5 \pm 8$ (26 - 50)          |                 |        |
| BMI (KG/M²)                      | $45.5 \pm 6.3$ (38.7 – 61.3)    | $38.9 \pm 6.3$ (34.1 – 57.6) | < 0.001 |

Data are shown as: mean ± standard deviation (range).
Discussion

We studied the short-term effects of weight loss on striatal D2/3R availability in morbidly obese women after RYGB and found that despite being in a negative energy balance and after clinically significant weight loss, D2/3R availability did not change significantly. We analysed the data using two different techniques namely classic fixed ROI analysis and MRI-based analysis and the outcome of both analyses were consistent. This suggests that striatal D2/3R is not regulated by acute changes in energy balance nor influenced by fat mass per se. Glucose production rate and hepatic insulin sensitivity did not correlate with the D2/3R binding potential while there was a clear trend for a positive correlation with peripheral insulin sensitivity.
Earlier studies on D2/3R binding after bariatric surgery are contradictory and report either an increase (21) or a decrease in striatal D2/3R availability (20). It should be noted however that these two studies included less than 10 patients, whereas in this study 19 patients were included. Besides the difference in number of included patients, we used a different radioligand ([123I]IBZM SPECT versus [11C]raclopride and [18F]fallypride PET). However, this is unlikely to have affected the results because all three tracers are well-validated to measure D2/3R in-vivo in humans (26-28).

Whether an increase in D2/3R occurs after long-term weight loss is unknown. The low D2/3R availability in obesity has previously been compared to findings in drug abuse. It has been shown that drug abuse results in reductions in striatal D2/3R binding up to 4 months after the last drug abuse (29). In addition, self-administration of cocaine in monkeys depressed D2/3R binding up to one year in some but not all monkeys (30). Also, we cannot rule out that reduced D2/3R availability in our subjects was already present before the occurrence of obesity, although we excluded subjects with childhood onset obesity. Finally, striatal dopaminergic neurotransmission might be resistant to the metabolic signals involved in weight loss in chronically obese subjects as has been shown for leptin (31) and insulin in rodents. This might predispose these individuals to relapse of obesity after weight loss.

Insulin, leptin, and ghrelin are important neuroendocrine hormones which drive the homeostatic control of feeding behaviour by the hypothalamus (32; 33). In addition to this homeostatic control, these hormones also regulate non homeostatic control via receptors located on dopaminergic neurons in the striatum. Despite the fact that the dopamine system is insulin responsive (34), in this present study the striatal dopaminergic neurotransmission as assessed by D2/3R availability did not correlate convincingly with measures of insulin sensitivity besides a positive trend for peripheral insulin sensitivity. The latter is in line with the insulin sensitizing effects of dopamine agonists in obese diabetic subjects (35). In addition, dopamine antagonists are known for their diabetic side effects (36) and drug-naïve schizophrenic patients, known for their disturbed central dopamine metabolism (37), are characterized by hepatic insulin resistance (38). In a previous study (17) a correlation between peripheral insulin sensitivity, using the insulin sensitivity index (SI) and D2/3R availability in the ventral striatum was found. We measured hepatic and peripheral insulin sensitivity separately using the gold standard technique and found no correlation with hepatic insulin sensitivity but a trend for a positive correlation between D2/3R availability and peripheral insulin sensitivity. This suggests that peripheral glucose uptake, which occurs under hyperinsulinemic conditions predominantly in skeletal muscle, might be in part regulated by cerebral dopamine metabolism. These observations suggest a biological relationship between cerebral dopamine and glucose metabolism. On the other hand, systemic treatment with modulators of whole body dopamine metabolism might exert pharmacological effects on glucose metabolism without altering striatal D2/3R availability (39). Basal EGP and hepatic insulin sensitivity were not correlated to D2/3R availability making a functional connection between the striatal dopaminergic transmission and hepatic glucose metabolism unlikely, at
least in chronically obese female subjects. Although a clear difference in D2/3R availability was found between lean and obese subjects (3; 4), within our obese group no clear correlation between BMI and D2/3R availability was found. This suggests that fat mass per se is not the main determinant of D2/3R availability in obesity. This is in line with the unchanged D2/3R availability despite clinically significant weight loss. In conclusion, surgery-induced weight loss does not significantly increase striatal D2/3R availability in morbidly obese women. This suggests that short-term changes in energy balance in morbidly obese humans do not induce profound alterations in striatal dopaminergic neurotransmission and might predispose obese individuals to weight gain after a hypocaloric diet. Moreover, the striatal dopamine receptor binding potential is not significantly correlated to hepatic insulin sensitivity but showed a trend for a positive correlation with peripheral insulin sensitivity. This confirms earlier findings on a potential role of cerebral dopamine in glucose metabolism.

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References


