Neurobiological aspects of obesity: dopamine, serotonin, and imaging
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DOPAMINE D_{2/3} RECEPTOR AVAILABILITY AND AMPHETAMINE-INDUCED DOPAMINE RELEASE IN OBESITY

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Submitted
ABSTRACT

Background

The neurotransmitter dopamine is important in the regulation of food intake and might play a role in the pathophysiology of obesity. Food intake induces dopamine release in the striatum. It is hypothesized that obese people experience less reward from food due to a lower striatal dopamine release, which consequently leads to overeating. This study is the first to assess whether obese subjects have a blunted striatal dopamine release.

Methods

We measured the amphetamine-induced striatal dopamine release in 15 obese and 15 age-matched, normal-weight, women using [123]IBZM SPECT imaging (on a brain-dedicated system, using the bolus/constant infusion technique): the first scan was acquired before (baseline) and the second after intravenous administration of 0.3 mg/kg d-amphetamine. In addition, correlations of striatal dopamine D_{2/3} receptor (DRD_{2/3}) availability and dopamine release with food craving were examined.

Results

Baseline striatal DRD_{2/3} availability was lower in obese subjects (0.91 ± 0.16) compared to controls (1.09 ± 0.16; p = 0.006). However, amphetamine induced dopamine release did not significantly differ between groups (obese: 1.2% ± 17.7, control: 7.5% ± 9.2, p = 0.145). There were no significant correlations between striatal DRD_{2/3} availability or dopamine release and food craving measures.

Conclusions

The current study replicates previous findings of lower striatal DRD_{2/3} availability in obese people, but does not provide support for the hypothesis that overeating in obesity is related to reward deficits due to lower dopamine release.
INTRODUCTION

The neurotransmitter dopamine is important in the regulation of food intake and has therefore been implicated in the pathophysiology of overeating and obesity (1). Food intake induces dopamine release in the striatum (2;3), which is crucial for its reinforcing effects. It has been postulated that obese people may experience less reward from food due to a lower striatal dopamine release after food intake (1). This could consequently lead to overeating behavior to compensate for the reward deficit and thus play a role in the pathophysiology of obesity.

This hypothesis has been adapted from the drug addiction field, where models on the role of a hypodopaminergic mesolimbic system and a reward deficiency syndrome have been proposed (4). Apart from similarities in behaviour, such as compulsive behavior, loss of control over intake, and craving, there are also neurobiological similarities. Similar to drug abusers, obese people have decreased dopamine D2 receptor (DRD2) availability in the striatum (6;7), which may also play a role in a reduced reward experience. In alcohol and drug dependence, it has been demonstrated that the striatal dopamine release (induced by psychostimulant administration) is blunted (alcohol: (8), heroin: (9), cocaine: for review (10)). Whether this is also true for obesity has not yet been demonstrated though.

There are indications that the striatal dopamine release might also be blunted in obesity. One animal study tested this hypothesis and indeed found that both amphetamine-induced dopamine release in the nucleus accumbens (ventral striatum) and dopamine release induced by food were lower in diet-induced obese (DIO) rats (11). In another study it was found that lower dopamine release in the nucleus accumbens in rats on a high-fat diet was related to insulin-resistance (12). However, one study in humans that tested the difference in striatal dopamine release between normal-weight and overweight/obese subjects following intravenous glucose administration, did not show a difference between groups (13). On the other hand, binge-eating does seem to affect the striatal dopamine release in obesity. Obese subjects with binge-eating disorder have a higher amphetamine-induced striatal dopamine release than obese subjects without binge eating disorder (14). This still does not answer the question though whether striatal dopamine release is blunted in people with obesity.

Therefore, this study aims to test the hypothesis that obese subjects without a binge-eating disorder have lower striatal dopamine release compared to controls during an amphetamine challenge. We will also assess whether the dopamine release is related to subjective feelings of craving for food, which would support the reward deficiency hypothesis.

METHODS AND MATERIALS

Subjects

We included obese women (BMI > 35) and normal-weight, age-matched control women (BMI 18.5 – 25). Inclusion criteria were age between 18 to 45 years old and Caucasian descent. Exclusion criteria were diabetes mellitus, dyslipidemia, hypertension requiring more than one type of medication, past or present history of severe neurological or psychiatric disorders (e.g., binge eating disorder, substance abuse, major depression, psychosis, bipolar disorder, dementia), use of psychotropic medication (including neuroleptics and methylphenidate), smoking, history of
use of any drug of abuse (including amphetamines), abnormal electrocardiogram and pregnancy (confirmed by urine pregnancy test) or breastfeeding. The obese subjects were recruited at the obesity policlinics of the Slotervaart Hospital Amsterdam and the Reinier de Graaf Group of Hospitals, Delft, The Netherlands. The normal-weight control subjects were recruited via advertisements in local newspapers. All subjects provided written informed consent. The study was approved by the medical ethics committee of the Academic Medical Center, Amsterdam.

Study Design
On the test day, subjects came to the Academic Medical Center in fasten state. They received an intravenous catheter and blood samples were taken for plasma measurements of metabolic parameters: glucose, insulin, HOMA-IR (i.e. homeostasis model assessment of insulin resistance based on plasma glucose and insulin concentrations), leptin, cholesterol, and triglycerides. A urine sample was requested for urine toxicology screening and pregnancy test. Next, subjects received a standard breakfast. After breakfast, \(^{[2]^{3}}\text{IBZM}\) was given intravenously as a bolus followed by a constant infusion for a total of 300 min (for details: see SPECT acquisition and analysis; (15)). Then the Beck Depression Inventory II (BDI-II) was administered and eating binges were assessed with an interview (Eating disorder Examination). Next, a baseline single photon emission computed tomography (SPECT) scan of striatal \(\text{DRD}_{2/3}\) binding was performed between 120 and 180 min after the start of the \(^{[2]^{3}}\text{IBZM}\) infusion (15). Just before the start of this scan, subjects filled out the General Food Craving Questionnaire State (G-FCQ-S) (16) and visual analogue scales (VAS) to assess how much they felt like eating and how hungry they were (food craving). After the first SPECT scan, dexamphetamine sulphate (0.3 mg/kg ideal body weight; Spruyt Hillen BV, IJsselstein, The Netherlands) was administered intravenously over 2 minutes. The dexamphetamine dose was based on ideal body weight instead of total body weight due to ethical reasons to prevent intoxication by overdosing in obese subjects. Ideal body weight for females is calculated as follows: 45.4 kg + 0.89 kg/cm above length of 152.4 cm (17). Electrocardiogram and vital functions were monitored continuously for 20 minutes after dexamphetamine administration. Psychological responses were monitored with a simplified version of the Amphetamine Interview Rating Scale (18), including the following four items (scale 1-10): feeling good (euphoric), energetic, restless, and anxious. Assessments took place at -2, +2, +6, +10, +14, +20, +40, +120 minutes before/after dexamphetamine administration. Between 240 and 300 min after the start of the infusion of \(^{[2]^{3}}\text{IBZM}\), i.e. about 45 minutes after dexamphetamine administration, the second SPECT scan was acquired to assess dopamine release due to dexamphetamine administration (15). Directly after the second SPECT scan, another venous blood sample was acquired to measure plasma dexamphetamine concentration. The subjects again filled out the G-FCQ-S and visual analogue scales to assess how much they felt like eating and how hungry they were. See figure 1 for an overview of the study design.

SPECT acquisition and analysis
In all participants, approximately 80 MBq \(^{[2]^{3}}\text{IBZM}\) (specific activity > 200 MBq/nmol; radiochemical purity > 95%) was given as an intravenous bolus, followed by continuous infusion of 20 MBq/hour for the duration of the experiment (300 minutes; (15)). To induce a state of sustained binding equilibrium after 120 minutes, the bolus to hourly infusion ratio was approximately four (15;19). SPECT scans were performed using a 12-detector brain-dedicated scanner (Neurofocus...
DRD₂/₃ AVAILABILITY AND DOPAMINE RELEASE IN OBESITY

Blood/urine sampling (metabolic parameters, toxicology screening, pregnancy test)

Standard breakfast

Questionnaires (BDI-II, Interview (eating binges))

Dexamphetamine administration

Subjective/cardiovascular effects

Craving measures/Plasma sampling (dexamphetamine)

0

start [¹²³]IBZM infusion

120

SPECT scan 1

180

240

300

minutes

SPECT scan 2

Figure 1. Schematic overview of study design.

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. SPECT data were acquired for approximately 60 minutes per scan, starting from 120 minutes and 240 minutes after the initiation of [¹²³]IBZM administration.

Attenuation correction of all images was performed as earlier described (20). Images were reconstructed in 3-D mode. For quantification, a region-of-interest (ROI) analysis was performed, with fixed ROIs for the striatum and occipital cortex, as described earlier (20). Mean striatal and mean occipital binding densities were averaged from right and left ROIs. The non-displaceable binding potential (BPND) was calculated as the ratio of specific to non-specific activity, i.e. (activity in ROI – activity in occipital cortex)/activity in occipital cortex.

Statistical analysis

We used ANCOVA to test for group differences in baseline BPND and repeated-measures ANOVA to assess the change in BPND, i.e. dopamine release. Regression analyses were used to test for correlations between BMI, food craving measures, plasma glucose, insulin, leptin or HOMA-IR and baseline BPND or change in BPND. Within the obese group, ANCOVA was used to test for differences in baseline BPND and change in BPND between subjects with and without (previous) eating binges. ANCOVA and regression analyses for baseline BPND included age as a covariate to correct for age effects (21).

Subject characteristics and scores on visual analogue scales, questionnaires, plasma and cardiovascular measures (blood pressure, heart rate) were compared between groups with unpaired t-tests.

For the primary outcome measures, i.e. group differences in baseline BPND and change in BPND between obese and control subjects, p-values <0.05 were considered significant. For the secondary outcome measures, i.e. associations of BMI, food craving measures, subjective effects on amphetamine, and metabolic parameters with baseline BPND or change in BPND, p-values < 0.0125 with Bonferroni correction were considered significant.
RESULTS

Subjects
Fifteen obese women (mean BMI: 42.9 ± 4.9; mean age: 36.3 ± 4.0 years) and fifteen normal-weight women (mean BMI: 21.8 ± 1.8; mean age: 38.5 ± 5.6 years) were included in the study (Table 1). Eight obese subjects reported to have suffered from eating binges in the past or present, whereas two normal-weight subjects did so, although none of the subjects was diagnosed with or currently fulfilled the criteria for Binge Eating Disorder. The scores on the BDI-II did not differ significantly between groups. One obese subject and one normal-weight subject fell in the category moderate depression based on BDI-II score, and two obese subjects and one normal-weight subject were in the category mild depression. None of the subjects had a clinical diagnosis of major depression.

With regard to plasma measures, there were significant group differences for glucose, insulin, HOMA-IR, leptin, LDL and HDL cholesterol, and triglycerides (Table 1). However, none of the obese subjects fulfilled criteria for diabetes mellitus or dyslipidaemia.

Food craving measures
Obese and normal weight subjects did not differ significantly in subjective food craving scores before or after the dexamphetamine challenge and they showed similar increases in subjective craving after the methamphetamine challenge (Table 2).

Subjective effects and cardiovascular measures after amphetamine administration
Obese and normal-weight subjects only differed in the baseline score on euphoria before dexamphetamine administration: obese subjects scored higher (8.6 ± 0.7) than the normal-weight subjects (7.4 ± 1.2; t = 3.28, p = 0.003). There were no group differences in subjective effects after dexamphetamine administration (figure 2).

Interestingly, seven obese subjects and three normal-weight subjects became quite emotional after dexamphetamine administration and cried. None of them reported underlying sadness or a reason to cry, but it was an overwhelming emotion that they could not withhold.

With regard to cardiovascular measures, there were only group differences at baseline. Obese subjects had significantly higher systolic and diastolic blood pressure (t = 3.98, p = 0.001 and t = 2.85, p = 0.008, respectively). The maximum increase in blood pressure and heart rate from baseline after dexamphetamine administration did not differ between groups, although there was a trend for a larger increase in diastolic blood pressure in the obese subjects (t = -2.04, p = 0.051).

Baseline DRD$_{2/3}$ availability
Obese subjects had significantly lower baseline BP$_{ND}$ than normal-weight subjects (0.91 ± 0.16 vs. 1.09 ± 0.16; F(1,27) = 8.85, p = 0.006; table 2/figure 3). Obese subjects with and without (previous) eating binges did not significantly differ in baseline BP$_{ND}$(0.94 ± 0.13 vs. 0.88 ± 0.20; F (1,12) = 0.661, p = 0.432). Regression analyses showed that there were no significant correlations between baseline BP$_{ND}$ and BMI, food craving measures and plasma glucose, insulin, HOMA-IR, or leptin, neither in the obese, nor in the normal-weight group.
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal-weight</th>
<th>Obese</th>
<th>p</th>
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<tbody>
<tr>
<td>Number of subjects</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 1.8</td>
<td>42.9 ± 4.9</td>
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<tr>
<td>Range</td>
<td>18.5 – 24.9</td>
<td>36.3 – 56.5</td>
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<tr>
<td>Age (years)</td>
<td>38.5 ± 5.6</td>
<td>36.3 ± 4.0</td>
<td>0.216</td>
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<tr>
<td>Subjects with eating binges (n)</td>
<td>2</td>
<td>5</td>
<td></td>
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<tr>
<td>BDI-II</td>
<td>5.5 ± 6.2</td>
<td>9.0 ± 5.7</td>
<td>0.123</td>
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Plasma measures

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<th>p</th>
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</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.7 ± 0.3</td>
<td>5.0 ± 0.4</td>
<td>0.047</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>2.9 ± 1.3</td>
<td>11.0 ± 9.0</td>
<td>0.005</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.6 ± 0.3</td>
<td>2.4 ± 1.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.8 ± 6.2</td>
<td>46.8 ± 10.0</td>
<td>&lt;0.001</td>
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Cholesterol

<table>
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<th>Normal-weight</th>
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</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>4.6 ± 0.8</td>
<td>4.9 ± 0.5</td>
<td>0.218</td>
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<tr>
<td>HDL</td>
<td>1.9 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>2.4 ± 0.7</td>
<td>3.0 ± 0.5</td>
<td>0.008</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.7 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data are displayed as mean ± standard deviation, unless otherwise specified. BMI = body mass index.

Dexamphetamine-induced effects on DRD<sub>2/3</sub> availability

Repeated measures ANOVA showed that the time x group interaction was not significant (F(1,28) = 1.79, p = 0.192), indicating that the change in BP<sub>No</sub> i.e. dopamine release, did not differ between groups (see also figure 3). Although dopamine release was lower in obese subjects compared to normal-weight controls, the percentage change did not differ significantly between groups: obese: -1.2% ± 17.7; normal-weight: -7.5% ± 9.2 (t = 1.23, p = 0.233). Obese subjects with and without (previous) eating binges did not have a significantly different change in BP<sub>No</sub> (F(1,13) = 0.949, p = 0.348).

Due to some technical problems the dexamphetamine plasma concentration was only available for 11 obese subjects and 13 normal-weight subjects. In this subgroup, plasma dexamphetamine concentrations were not significantly different between obese and control subjects (89.4 ± 62.7 vs. 70.1 ± 58.7; t = -0.773, p = 0.448). There were no significant correlations between change in BP<sub>No</sub> and food craving measures, subjective effects of amphetamine, BMI, plasma glucose, insulin, HOMA-IR, leptin, or plasma dexamphetamine concentration, neither overall nor in the obese or in the control group.

DISCUSSION

The current study does not show significantly lower striatal dopamine release in obese compared to normal-weight women and, therefore, we cannot confirm the hypothesis that the dopamine release is blunted in obesity. We also did not observe significant differences in food craving and subjective responses to the dexamphetamine challenge between obese and normal-weight women. Together, these results suggest that the striatal dopaminergic reward
Figure 2. Subjective effects of dexamphetamine administration. There are no significant group differences in subjective effects after dexamphetamine administration. * marks significant difference between groups at baseline (p < 0.05). Displayed are mean and standard deviation at each time point. AMF indicates dexamphetamine administration, which is time point 0.

Figure 3. Striatal DRD<sub>2/3</sub> availability before and after dexamphetamine administration. The difference between baseline and post-amphetamine measurements reflects the striatal dopamine release. Displayed are mean and standard deviation. * marks significant difference in baseline BP<sub>No</sub> between obese and normal-weight group (p = 0.006).
Table 2. Effects of dexamphetamine administration on striatal DRD$_{2/3}$ availability, food craving and cardiovascular measures

<table>
<thead>
<tr>
<th></th>
<th>Normal-weight</th>
<th>Obese</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td><strong>Striatal DRD$_{2/3}$ availability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP$_{ND}$ Baseline</td>
<td>1.09 ± 0.16</td>
<td>0.91 ± 0.16</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>1.01 ± 0.14</td>
<td>0.89 ± 0.18</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>-7.5 ± 9.2</td>
<td>-1.17 ± 17.7</td>
<td>0.233</td>
</tr>
<tr>
<td>Plasma amphetamine concentration</td>
<td>89.4 ± 62.7</td>
<td>70.1 ± 58.7</td>
<td>0.4448</td>
</tr>
<tr>
<td><strong>Measures for food craving</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS - feel like eating pre-scan session</td>
<td>30.9 ± 22.8</td>
<td>30.2 ± 25.8</td>
<td>0.935</td>
</tr>
<tr>
<td>post-scan session</td>
<td>38.2 ± 31.9</td>
<td>40.8 ± 29.5</td>
<td>0.819</td>
</tr>
<tr>
<td></td>
<td>7.2 ± 37.5</td>
<td>10.6 ± 27.4</td>
<td>0.783</td>
</tr>
<tr>
<td>VAS - hunger pre-scan session</td>
<td>21.5 ± 18.0</td>
<td>27.1 ± 25.1</td>
<td>0.484</td>
</tr>
<tr>
<td>post-scan session</td>
<td>33.0 ± 32.7</td>
<td>37.3 ± 26.5</td>
<td>0.693</td>
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<tr>
<td></td>
<td>11.5 ± 32.9</td>
<td>10.2 ± 24.2</td>
<td>0.900</td>
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<tr>
<td>G-FCQ-S pre-scan session</td>
<td>31.1 ± 12.4</td>
<td>31.2 ± 10.8</td>
<td>0.988</td>
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<tr>
<td>post-scan session</td>
<td>33.5 ± 13.7</td>
<td>32.7 ± 14.7</td>
<td>0.878</td>
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<tr>
<td></td>
<td>2.3 ± 14.0</td>
<td>1.5 ± 12.6</td>
<td>0.860</td>
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<tr>
<td><strong>Cardiovascular measures</strong></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>119.3 ± 7.3</td>
<td>135.1 ± 13.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Δ max</td>
<td>57.1 ± 15.5</td>
<td>56.7 ± 23.4</td>
<td>0.964</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.2 ± 9.6</td>
<td>82.9 ± 70</td>
<td>0.008</td>
</tr>
<tr>
<td>Δ max</td>
<td>29.0 ± 12.4</td>
<td>19.0 ± 14.3</td>
<td>0.051</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>63.7 ± 12.2</td>
<td>71.3 ± 8.7</td>
<td>0.065</td>
</tr>
<tr>
<td>Δ max</td>
<td>29.3 ± 27.3</td>
<td>16.7 ± 13.7</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Data are displayed as mean ± standard deviation.
Baseline = 2 minutes before dexamphetamine administration, Δ max = maximum increase from baseline.
BP$_{ND}$ = non-displaceable binding potential, VAS = visual analogue scale, G-FCQ-S = General Food Craving Questionnaire State, bpm = beats per minute.

The system does not respond differently to a dexamphetamine challenge in obese compared to normal-weight women. However, we confirmed the finding of previous studies that baseline striatal DRD$_{2/3}$ availability is lower in obese compared to normal-weight women.

Based on these findings the theory can be questioned that obesity is related to a reward deficit due to lower dopamine release following food intake. We did not find associations between dopamine release and food craving measures, in spite of the fact that the anorexigenic effects of dexamphetamine are well-known. Wang et al. also found no correlation between dopamine
release and self-reported hunger in obese subjects (14). Only Small et al. (2) previously observed that dopamine release in response to a meal correlated with meal pleasantness, although not with hunger, but this study included only healthy lean subjects. One fMRI study showed lower striatal brain activation in response to tasting a milkshake in subjects with a higher BMI though (22). This finding was interpreted as a reflection of lower striatal dopamine release at higher BMI. However, BOLD (blood-oxygen-level-dependent) response cannot be linked directly to a dopamine release (23). Therefore, there is no empirical support for the reward deficiency hypothesis and its relation to lower dopamine release in obese humans yet.

The concept of lower dopamine release related to reward deficiency comes from the addiction field, where it has been found in alcohol, opiate, and cocaine addicts (8;9;24;25). While the lower striatal DRD_{2/3} availability is a clear similarity between obesity and addiction, this does not seem the case for blunted dopamine release. An explanation for this difference could be that drugs of abuse often have a direct and large pharmacologic effect on the dopaminergic system, whereas the relation between food intake and the dopaminergic system is more complex. In addition, the dopamine release induced by food is far lower than for example amphetamine-induced release (26;27). It is interesting that it has recently been shown that there is no lower amphetamine-induced dopamine release in cannabis dependence (28). The authors of this study suggest that this may be due to the non-measurable (29) or lower dopamine release (30) observed on a delta-9-tetrahydrocannabinol (i.e. psychoactive compound in cannabis) challenge compared to an alcohol (31) or a low-dose amphetamine (32) challenge. Thus, the lower dopamine release induced by food or cannabis could possibly be related to limited damage to the striatal dopaminergic system at high intake, at least compared to some other drugs of abuse. This might be reflected in the findings that amphetamine-induced dopamine release is not decreased in obesity and cannabis dependence. Overall, the parallels between addiction and obesity with regard to reward deficiency are an interesting model, but might turn out to be limited (33). The present findings reveal a difference between obesity and different forms of addiction or at least suggest that similar mechanisms are less outspoken in obesity than in substance abuse.

An important finding in this study is that baseline striatal DRD_{2/3} availability was significantly lower in obese compared to normal-weight women. Therewith, we replicate previous findings (6;7) in an independent sample. Together these studies clearly show that lower striatal DRD_{2/3} availability in obesity is a robust finding. Whereas the present study cannot support a role for lower dopamine release in relation to reward deficits in obesity, the finding of lower striatal DRD_{2/3} availability in obesity could still be related to reward deficits, as there is lower capacity for reward signal transduction in the striatum. However, we found no significant correlations between the food craving measures and striatal DRD_{2/3} availability and these correlations were not reported in other studies, as well. In a recent study, viral knockdown of striatal DRD_{2} in rats on a cafeteria diet led to compulsive food seeking and reward deficits (34). However, there is no convincing evidence that these findings can be translated to the human situation. The pathophysiology behind lower striatal DRD_{2/3} availability in obesity is not yet elucidated. Animal studies show that high-fat and cafeteria diets can induce lower DRD_{2} levels in the striatum (34;35). On the other hand, striatal DRD_{2/3} availability can predict body weight in rats (36). Wang et al. (6) reported a correlation between BMI and striatal DRD_{2/3} availability, but we (and others) did not find such correlation. We also did not find correlations with food craving measures or plasma glucose, insulin, HOMA-IR
or leptin. The latter findings contradict a recent study reporting correlations between DRD$_{2/3}$ availability in ventral regions of the striatum and insulin sensitivity, leptin and ghrelin (37). Various outcomes between studies may be due to different scan technique (PET versus SPECT) and a different tracer to measure DRD$_{2/3}$ ([F]allypride versus [I]IBZM). In short, the present study confirms that striatal DRD$_{2/3}$ availability is lower in obesity, but provides no evidence for a direct relationship with BMI, food craving, insulin resistance or leptin signaling.

There are several limitations in this study that we would like to discuss. First of all, the administered dose of dexamphetamine was based on ideal body weight instead of real body weight resulting in a lower dexamphetamine dose per kilogram in obese compared to normal-weight subjects. This was done to prevent administration of toxic doses to the obese subjects. At the same time, this is another reason why we think that there really is no blunting of the dexamphetamine-induced dopamine release in obese compared to control subjects, because the dopamine release in the obese subjects was rather underestimated than overestimated and still there was no significant difference in dopamine release between the groups. Moreover, plasma dexamphetamine concentrations did not differ between the obese and normal-weight group. Further, dexamphetamine was administered intravenously and total blood volume is not different between obese and controls, which is of importance when studying the acute effects of dexamphetamine administration. In addition, subjective and cardiovascular effects on dexamphetamine were not significantly different between groups. Thus, it is unlikely that dosing on ideal body weight has largely influenced the dopamine release.

Another issue that might limit our study is that BMI as the main selection criterion might result in a sample that is too heterogeneous. This might be reflected in the large variation in dopamine release that we observed within the obese group. In the study design, we have tried to reduce heterogeneity in the obese sample by excluding subjects with past or present psychiatric disorders (including eating disorders), and metabolically unhealthy subjects, i.e. subjects with diabetes mellitus or dyslipidaemia. However, this strategy could not prevent the observed high variability in dexamphetamine-induced dopaminergic responses. Possibly, it is better to identify subgroups of obese subjects based on eating behavior. For example, following food stimulation combined with methylphenidate, dopamine release in the caudate nucleus (but not ventral striatum) was higher in obese subjects with binge eating disorder than in non-binging obese subjects (14). In that study, binge eating scores even correlated with the dopamine release in the caudate nucleus. It has been hypothesized that obese subjects with binge eating might be considered a food-addiction phenotype within the obese population, which is most similar to drug addiction (33). In this respect, it is surprising that binge eating obese subjects have increased dopamine release as opposed to the blunted release in several addictions (14). The increased dopamine release in the obese subjects with binge eating disorder might thus reflect an increased drive or reinforcement of action to attain the reward from food. In the present sample we found no difference in striatal dopamine release between the obese subjects with and without (previous) eating binges, but the subgroups were small and participants with a binge eating disorder were excluded. More knowledge on the relation between eating behavior and dopamine release in humans is needed to better understand these mechanisms and to evaluate its role in obesity.

It is also still possible that it is better to stratify obese subjects in subgroups based on metabolic parameters when studying striatal dopamine release. For example, an animal study
showed that reduced dopamine release in DIO rats negatively correlated with insulin resistance (12) and other metabolic parameters such as ghrelin and leptin can also affect the dopamine release (38;39). In the present study we were not able to identify metabolic parameters that correlated with the striatal dopamine release. It should be noted, however, that metabolically unhealthy subjects (with diabetes mellitus or dyslipidaemia) were excluded in the current study. In spite of that, obese and control subjects differed in plasma measures on glucose, insulin, leptin, and triglycerides, but it is almost impossible to find a sample of (morbidly) obese subjects with the same metabolic profile as normal-weight control subjects.

Furthermore, it is possible that we were not able to detect a difference in striatal dopamine release between obese and control subjects due to technical limitations. Animal studies using microdialysis previously showed that dopamine release in the nucleus accumbens, i.e. ventral striatum, is lower in DIO rats (11;12;39). It is interesting that Geiger et al. (11) also assessed dopamine release after dexamphetamine administration in DIO rats, which is more comparable to the present study in humans. They report that basal and dexamphetamine-challenged extracellular dopamine levels were significantly lower in DIO rats. However, they found that the percentage increase in dopamine from baseline after dexamphetamine was higher in the cafeteria DIO rats than in the chow-fed rats. This percentage is a measure that comes closer to the percentage change in binding that we use for humans. Therefore, it cannot be excluded that we failed to detect an existing blunted response in obese subjects because the technique that we used does not allow direct measurement of dopamine levels in humans comparable to microdialysis in rodents. Instead, we can only measure the change in $\text{DRD}_2$ availability, i.e. the change in synaptic dopamine, and not the change in total extracellular dopamine (40). Still, a lower change in synaptic dopamine was detectable for several addictions (8;9;24;25), but apparently it is absent or too small to detect for obesity.

Another limitation of the study is that only females were included. Women tend to have a lower amphetamine-induced dopamine release than men (41). The dopamine release of on average 7.5% change in striatal $\text{DRD}_2$ binding in our female controls is comparable to what has previously been found in women (41). The study on dopamine release by intravenous glucose administration showed that there were gender differences, but no differences between lean and overweight/obese subjects (13). Therefore, we cannot directly apply the results of this study to the male obese population. However, lower striatal $\text{DRD}_2$ availability in obesity was found in female samples and in a mixed sample (6). Thus, we expect no large differences with a male population. One more limitation might be that some subjects could be classified as having moderate or mild depression according to the BDI-II. Since it has been reported that amphetamine-induced dopamine release is not abnormal in major depression (42), we believe it very unlikely that this has influenced our results. A final limitation is that we could not extend our analysis to subdivisions of the striatum, e.g. ventral and dorsal parts, due to the resolution of our SPECT images. Therefore, we cannot exclude that the dopamine release in obesity is affected in only a subregion of the striatum, although PET studies in cocaine and opiate addicts have shown that the whole striatum was affected (9;24).

In conclusion, we did not find evidence for a blunted striatal dopamine release in obesity, but we confirm previous findings that striatal $\text{DRD}_2$ availability is lower in obesity. Therewith, this study does not provide support for the hypothesis that overeating in obesity is related to a reward deficit due to lower dopamine release.
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