Neurobiological aspects of obesity: dopamine, serotonin, and imaging
van de Giessen, E.M.

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TRIPLE MONOAMINE INHIBITOR TESOFENSINE DECREASES FOOD INTAKE, BODY WEIGHT, AND STRIATAL DOPAMINE \(D_{2/3}\) RECEPTOR AVAILABILITY IN DIET-INDUCED OBESE RATS

Elsmarieke van de Giessen  
Kora de Bruin  
Susanne E. la Fleur  
Wim van den Brink  
Jan Booij

ABSTRACT
The novel triple monoamine inhibitor tesofensine blocks dopamine, serotonin and norepinephrine re-uptake and is a promising candidate for the treatment of obesity. Obesity is associated with lower striatal dopamine D2 receptor availability, which may be related to disturbed regulation of food intake. This study assesses the effects of chronic tesofensine treatment on food intake and body weight in association with changes in striatal dopamine D2/D3 receptor (D2/3R) availability of diet-induced obese (DIO) rats. Four groups of 15 DIO rats were randomized to one of the following treatments for 28 days: 1. tesofensine (2.0 mg/kg), 2. vehicle, 3. vehicle + restricted diet isocaloric to caloric intake of group 1, and 4. tesofensine (2.0 mg/kg) + a treatment-free period of 28 days. Caloric intake and weight gain decreased significantly more in the tesofensine-treated rats compared to vehicle-treated rats, which confirms previous findings. After treatment discontinuation, caloric intake and body weight gain gradually increased again. Tesofensine-treated rats showed significantly lower D2/3R availability in nucleus accumbens and dorsal striatum than both vehicle-treated rats and vehicle-treated rats on restricted isocaloric diet. No correlations were observed between food intake or body weight and D2/3R availability. Thus, chronic tesofensine treatment leads to decreased food intake and weight gain. However, this appears not to be directly related to the decreased striatal D2/3R availability, which is mainly a pharmacological effect.
INTRODUCTION
The novel drug tesofensine is a triple monoamine inhibitor which blocks dopamine, serotonin and norepinephrine re-uptake from the synaptic cleft. It is a promising candidate drug for the treatment of obesity and has shown to induce significant weight loss in rodents (1) and in humans, with an average weight loss in humans of ~10% in 24 weeks (2). However, the exact mechanism of action of the drug is not yet elucidated. The weight loss is at least partly caused by reduced food intake and appetite suppression by the drug, as was shown in rodents (1;3) and humans (4). The acute appetite suppressing effect of tesofensine may be mediated via dopamine D1 receptor and α1 adrenoreceptor signaling, which was demonstrated by a reversion of the appetite suppression by blocking these receptors with SCH23390 and prazosin, respectively (3). However, in chronic treatment, the appetite suppressing effect diminishes over time (1). Therefore, it is important to know more about the pharmacological effects of the drug during sustained use.

Dopaminergic neurotransmission in the mesolimbic system is thought to affect food intake based on its role in reward function. Food is able to induce a release of endogenous dopamine in the nucleus accumbens (5;6), which is a part of the ventral striatum, and thus to exert a rewarding effect. It is postulated that deficits in this part of the reward system play a role in the pathophysiology of obesity by inducing overeating (7). Changes in the dopaminergic mesolimbic system related to reward deficits might be reflected in a decreased striatal dopamine D2/D3 receptor (D2/3R) availability, which is observed in both genetic obesity models (8-10), in diet-induced obesity models (11;12), and in humans (13). Recently, it has been shown that D2R downregulation can be induced by a cafeteria diet and that D2R downregulation increases the susceptibility for reward deficits and compulsive eating behavior in rats (11). Human research has also found that the Taq1A allele of the gene encoding for D2R increases susceptibility for obesity and is associated with lower striatal D2/3R levels in humans (14;15). Moreover, targeting the D2R with D2R agonists results in reduction of hyperphagia and appetite (16;17). Thus, the dopaminergic reward system and striatal D2/3R availability are related to regulation of food intake and substances that affect this system and changing striatal D2/3R availability might lead to different food intake and body weight.

As a triple monoamine inhibitor, tesofensine exerts its effects on three monoaminergic systems which all modulate food intake (18). The interactions of the systems are complex and will both directly and indirectly exert an effect on the mesolimbic dopaminergic system and thus can influence the striatal D2/3R availability. Additionally, the weight loss and reduced food intake itself may affect the striatal D2/3R levels during tesofensine treatment. Therefore, this study is designed to investigate the effects of chronic treatment with tesofensine on food intake and body weight and on striatal D2/3R availability in diet-induced obese (DIO) rats. In addition, the possible relations between changes in food intake and body weight with changes in D2/3R availability will be assessed. During the study, the rats are offered a high fat choice diet, which enables us to study effects of tesofensine on food preference that might be related to different reward processing from food and striatal D2/3R availability. At last, the long-term effects on food intake, weight gain and striatal D2/3R availability after discontinuation of chronic tesofensine treatment are assessed.
EXPERIMENTAL PROCEDURES

Sixty male Wistar rats (Horst, Harlan, The Netherlands; weight 225 ± 10 grams) were individually housed in a temperature- (21-23 °C) and light-controlled (lights on 7:00 am – 7:00 pm) room. They were allowed to adapt to their environment for 7 days. All experimental procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

Experimental design

Rats were randomized into four groups of 15 animals: group 1 treated with tesofensine (T), group 2 treated with vehicle (V), group 3 treated with vehicle combined with a restricted diet that is isocaloric to the caloric intake of group T to correct for feeding effects (V-RD), and group 4 treated with tesofensine followed by a period without treatment to observe chronic post-treatment effects (T-C) (Table 1). Before start of the treatment, all rats were offered an ad libitum high-fat (HF) choice diet for 28 days to induce obesity. The HF choice diet consisted of a dish of saturated fat (Beef tallow (Ossewit/Blanc de Boeuf), Vandemoortele, Belgium) presented in the cage on a metal receptacle in addition to normal standard chow (special diet service (SDS), England) and a water bottle (19). The HF choice diet was continued throughout the whole experiment.

At day 29, treatment with tesofensine (administered as a citrate; NeuroSearch A/S, Ballerup, Denmark) or vehicle started and lasted 28 consecutive days. Groups T and T-C received 2.0 mg/kg (3.8 μmol/kg) tesofensine daily between 10-11 am by oral gavage. This dose has previously been shown to induce weight loss and reduce food intake (3). Tesofensine was dissolved in 0.9% NaCl as a vehicle. Groups V and V-RD received a similar volume (1.0 ml) of 0.9% NaCl daily between 10-11 am by oral gavage.

During the 28-day treatment period, the V-RD group received a restricted HF choice diet, based on the caloric intake of group T. Every day, the V-RD rats were offered the average amount of fat and chow that the group T rats had consumed at the same day of the treatment schedule. To make sure that the all V-RD rats would have at least a similar caloric intake as the T rats they received 0.5 mg fat and chow more than the average consumption of group T.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Pre-treatment (day 1–28)</th>
<th>Treatment (day 29-56)</th>
<th>Post-treatment (day 57-84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>15</td>
<td>ad libitum HF choice diet</td>
<td>Tesofensine treatment + ad libitum HF choice diet</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>ad libitum HF choice diet</td>
<td>Vehicle treatment + ad libitum HF choice diet</td>
<td>-</td>
</tr>
<tr>
<td>V-RD</td>
<td>15</td>
<td>ad libitum HF choice diet</td>
<td>Vehicle treatment + restricted HF choice diet isocaloric to caloric intake group T</td>
<td>-</td>
</tr>
<tr>
<td>T-C</td>
<td>15</td>
<td>ad libitum HF choice diet</td>
<td>Tesofensine treatment + ad libitum HF choice diet</td>
<td>ad libitum HF choice diet</td>
</tr>
</tbody>
</table>

HF = high fat
At request of the health and safety department, filter tops were placed on the cages of all T and T-C rats during treatment. For consistency, we consequently tried to place filter tops on the cages of all animals during treatment. However, due to a limited number of filter tops, all T and T-C, but a minority of V and V-RD rats were in cages with filter tops.

On the day following the last treatment dose, i.e. day 57, 28 – 30 hours after the last administration, rats in groups T, V, and V-RD were sacrificed for striatal D2/3R measurements. Rats of group T-C were sacrificed on day 85 after a treatment-free period of 29 days.

**Behavioral measurements**

Body weight and food intake (fat and chow) were measured every two days for each individual animal throughout the whole experiment. Food intake for the V-RD group was measured daily during the treatment period (day 29-56). Food spillage in the cage was collected and weighed to correct for an overestimation of food intake.

Nocturnal locomotor activity of the rats was recorded in the fourth week of the pre-treatment and treatment period and for the T-C rats also in the fourth week of the post-treatment period. During recording, a piezoelectronic stabilimeter (20) was placed under the rat cage for 48 hours. The nocturnal activity was determined by calculating the average activity of the hours during the dark period (7:00 pm – 7:00 am) and was measured in arbitrary units proportional to the voltage output.

The abdominal fat stores of the rats were measured by dissecting and weighing the epidydimal and perirenal fat pads after sacrifice.

**D2/3R measurement**

On the last day of the experiment, rats were anesthetized with ketamine/xylazine 2:1 followed by immediate intravenous administration of approximately 37 MBq (1 mCi) of \( ^{123} \text{I}-\text{IBZM} \), which mainly binds to D2/3R and shows good sensitivity for displacement (Jongen et al., 2008). The majority of \(^{123} \text{I}-\text{IBZM} \) binding in the striatum is to D2R, as a result of substantially higher D2R density than D3R density in the striatum (21;22). \(^{123} \text{I}-\text{IBZM} \) (GE Healthcare, Eindhoven, the Netherlands) had a specific activity of 550 MBq/mol and a radiochemical purity of >95%. Ninety minutes after the \(^{123} \text{I}-\text{IBZM} \) injection (23), animals were sacrificed by bleeding through heart puncture under anesthesia. Brains were removed, immediately frozen on dry ice and sliced horizontally into 50 μm slices in a microtome cryostat at -21°C. Storage phosphor imaging was then performed as described previously (24). In short, every one in four slices was exposed to a Fuji BAS-MS IP for approximately 16 hours. The images were scanned at 50 μm resolution with 16-bit pixel depth using the Fuji FLA-3000 phosphor imager. Regions of interest (ROIs) were drawn accordingly to the standard rat brain atlas of Paxinos and Watson (25) and analyzed using AIDA image analysis software version 3.2 (Fig 1).

For both left and right dorsal striatum the twelve consecutive slices with highest binding were selected, and for the right and left nucleus accumbens (NAcc) the four consecutive slices with highest binding. Eight consecutive slices with highest binding for the cerebellum were selected as area of non-specific binding (26). Ratios of specific dorsal striatum-to-cerebellum and NAcc-to-cerebellum binding were obtained by dividing the average uptake per pixel of combined left and right dorsal striatum/NAcc by the average uptake per pixel of the cerebellum.
Figure 1. Examples of regions of interest: dorsal striatum (A), nucleus accumbens (B), and cerebellum (C).

Statistical analysis

Data were analyzed using SPSS version 16.0.2. ANOVAs were performed for differences between treatment groups (T, V, and V-RD) in weight of abdominal fatpads and D2/3R availability. Data for abdominal fatpad weight and D2/3R availability of the T-C group were not included in these ANOVAs, as these data were acquired at a different time point and therefore could not be validly compared to the V group. For treatment effects on caloric intake (total, from chow, from fat and percentage calories from fat) and weight gain, ANCOVAs were performed with change in caloric intake/change in weight gain between treatment and pre-treatment period as dependent variables, treatment group (T, V, V-RD, and T-C) as independent variable and caloric intake of the pre-treatment period or weight gain of the pre-treatment period, respectively, added as a covariate, to control for overestimation of the effects. The choice for change in caloric intake and change in weight gain between treatment and pre-treatment period (Δ treatment – pre-treatment) to test medication effects was based on the observation that caloric intake and weight gain differed between groups during the pre-treatment period in spite of randomization on day 1. Both ANOVAs and ANCOVAs were performed in the mixed model module of SPSS to be able to assess the effects on the T, V-RD, and (in ANCOVAs) T-C groups compared to the V group, which was regarded as the reference group. For locomotor activity, two-way repeated measures ANOVA was performed.

For the T-C group, paired t-tests were performed to analyze differences in food intake and weight gain between the treatment and post-treatment period. Finally, correlations between D2/3R availability and caloric intake, weight gain, and weight of abdominal fatpads were determined with Pearson’s correlation.

For the AN(C)OVAs, t-tests and correlations a probability value of 0.05 was considered significant.
RESULTS

All animals survived until the last day. Two rats (1 V, 1 T-C) were lost for D2/3R measurement, because of premature death after anesthesia before 123I-IBZM injection.

Food intake

In spite of randomization, the caloric intake during the pre-treatment period differed between groups (F(3,55) = 3.41, p = 0.024), with lower caloric intake by the T rats than the V rats (t = 2.21, p = 0.031). Caloric intake curves are shown in figure 2. During treatment, all groups had lower caloric intake per day during treatment compared to pre-treatment, but the decrease in caloric intake was different between groups (F(3,55) = 12.46, p < 0.001). T, T-C, and V-RD rats had a significantly larger decreases in caloric intake than V rats (T: t = -4.76, T-C: t = -5.64, V-RD: t = -4.14, all p < 0.001) (Table 2, Fig 3). These differences were based both on differences in decrease in caloric intake from chow (F(3,55) = 5.33, p = 0.003) and on the differences in decrease in caloric intake from fat (F(3,55) = 8.05, p < 0.001). The decrease in chow intake was larger in T and T-C groups compared to V rats (T: t = -2.58, p = 0.013, T-C: t = -3.32, p = 0.002), whereas the decrease in fat intake was larger for both T, T-C and V-RD groups compared to V rats (T: t = -3.77, p < 0.001, T-C: t = -3.51, p = 0.001, V-RD: t = -4.47, p < 0.001). T-tests showed that the total caloric intake per day (t = 0.78, p = 0.444), caloric intake from chow (t = 1.16, p = 0.257)

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment (day 1–28)</th>
<th>Treatment (day 29–56)</th>
<th>Post-treatment (day 57–84)</th>
<th>Δ treatment - pre-treatment</th>
<th>Δ post-treatment - treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total caloric intake per day (kcal; mean ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>111 ± 10*</td>
<td>89 ± 9</td>
<td>-22 ± 8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>104 ± 9</td>
<td>97 ± 9</td>
<td>-7 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-RD</td>
<td>103 ± 10</td>
<td>87 ± 6</td>
<td>-15 ± 9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-C</td>
<td>110 ± 8</td>
<td>87 ± 5*</td>
<td>100 ± 7</td>
<td>-23 ± 7*</td>
<td>+13 ± 5</td>
</tr>
<tr>
<td><strong>Percentage caloric intake from fat (%; mean ± SD)</strong></td>
<td></td>
<td></td>
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<tr>
<td>T</td>
<td>18.2 ± 9.7</td>
<td>18.2 ± 8.3</td>
<td>0 ± 3.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>25.7 ± 10.3</td>
<td>27.0 ± 9.1</td>
<td>1.2 ± 6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-RD</td>
<td>26.5 ± 15.1</td>
<td>20.7 ± 5.4*</td>
<td>-5.8 ± 11.4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-C</td>
<td>22.6 ± 12.0</td>
<td>21.6 ± 10.5</td>
<td>19.7 ± 11.0</td>
<td>-1.1 ± 5.1</td>
<td>-1.9 ± 3.4</td>
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<tr>
<td><strong>Caloric intake from chow (kcal; mean ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T</td>
<td>90 ± 13</td>
<td>73 ± 11</td>
<td>-18 ± 7*</td>
<td></td>
<td></td>
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<tr>
<td>V</td>
<td>77 ± 11</td>
<td>71 ± 10</td>
<td>-6 ± 6</td>
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<tr>
<td>V-RD</td>
<td>75 ± 14</td>
<td>69 ± 7</td>
<td>-6 ± 9</td>
<td></td>
<td></td>
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<tr>
<td>T-C</td>
<td>85 ± 12</td>
<td>68 ± 10</td>
<td>80 ± 12</td>
<td>-17 ± 8*</td>
<td>+12 ± 6</td>
</tr>
<tr>
<td><strong>Caloric intake from fat (kcal; mean ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>21 ± 11</td>
<td>16 ± 7</td>
<td>-5 ± 5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>27 ± 11</td>
<td>26 ± 10</td>
<td>-1 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-RD</td>
<td>28 ± 18</td>
<td>18 ± 5*</td>
<td>-16 ± 6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-C</td>
<td>25 ± 10</td>
<td>19 ± 9*</td>
<td>20 ± 10</td>
<td>-6 ± 6*</td>
<td>+1 ± 6</td>
</tr>
</tbody>
</table>

*significantly different from V (p < 0.001), †significantly different from V (p < 0.01), * significantly different from V (p < 0.05). All groups are n = 15.
and caloric intake from fat \((t = -0.86, p = 0.398)\) did not differ significantly between T and V-RD rats, confirming that the diet of the V-RD rats was isocaloric to the food intake of the T rats.

To test whether food preference, i.e. preference for fat or chow, was changed by treatment, the percentage calories from total caloric intake that comes from fat was compared between groups and this also showed significant differences \((F(3,55) = 4.43, p = 0.007)\). Whereas the V rats where the only group with an increase in percentage calories from fat intake during treatment, the T-C rats had a trend for a lower change in percentage calories from fat than V rats with a decrease in percentage calories from fat \((t = -1.94, p = 0.058)\), and the T rats and V-RD rats had significantly lower changes in percentage calories from fat compared to V rats \((T: t = -2.32, p = 0.024, V-RD: t = -3.58, p = 0.001)\).

Post-treatment, the caloric intake of T-C rats was higher than during tesofensine treatment \((t = 9.62, p < 0.001)\). This was primarily based on an increased caloric intake from chow \((t = 7.98, p < 0.001)\). In contrast, caloric intake from fat did not increase significantly, which resulted in a decrease in the percentage fat intake post-treatment \((t = -2.17, p = 0.047)\).

**Locomotor activity**

Data were incomplete for some animals (1 V, 1 V-RD, 2 T-C), due to data loss during a brief power outage. Analysis included only complete datasets. The nocturnal locomotor activity was not different between groups \((F = 0.565, p = 0.641)\), but treatment \((F = 36.6, p < 0.001)\) and treatment \(\times\) group interaction \((F = 10.9, p < 0.001)\) showed a significant effect. This was based on a general decrease in locomotor activity during treatment, which was larger for the T (pre-treatment: \(1465 \pm 203\), treatment: \(1014 \pm 236\)) and T-C (pre-treatment: \(1345 \pm 136\), treatment: \(1242 \pm 209\)) rats compared to V (pre-treatment: \(1238 \pm 203\), treatment: \(1242 \pm 209\)) and V-RD (pre-treatment: \(1298 \pm 239\), treatment: \(1249 \pm 206\)) rats. With respect to these results, it should be noted that the cages with the filter tops were slightly lowered. We cannot exclude that this might have had an effect on the locomotor activity during treatment of the rats in cages with filter tops.

**Weight gain and abdominal fatpad weight**

The average weights on day 1 of the experiment did not differ significantly between groups \((T: 265 \pm 10.1 \text{ g}, V: 260 \pm 7.7 \text{ g}, V-RD: 263 \pm 11.0 \text{ g}, T-C: 267 \pm 11.7 \text{ g}, F(3,56)=1.33, p = 0.275)\). However, the weight gain during the pre-treatment period was significantly different between groups
in spite of randomization ($F(3,56) = 6.30, p = 0.001$), with higher weight gain of the T-C group compared to V group ($t = 2.59, p = 0.012$). Weight curves for the four groups are shown in figure 4.

All groups showed a diminished weight gain from pre-treatment to treatment period, but the change differed between groups ($F(3,55) = 12.69, p < 0.001$). The T rats ($t = -4.57, p < 0.001$), T-C rats ($t = -5.89, p < 0.001$), and V-RD rats ($t = -3.07, p = 0.003$) had significantly larger decreases in weight gain than the V rats (Table 3), which is also reflected in the cumulated weight gain curves during treatment (Fig 5). After discontinuation of tesofensine treatment, the change in weight gain of T-C rats significantly increased compared to the treatment period ($t = 4.38, p = 0.001$).

Weights of the epididymal and perirenal fatpads are shown in table 4. There were significant differences between groups for epididymal ($F(2,41) = 3.71, p = 0.033$), but not for perirenal

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**Table 3. Weight gain (g; mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment (day 1–28)</th>
<th>Treatment (day 29-56)</th>
<th>Post-treatment (day 57-84)</th>
<th>Δ treatment - pre-treatment</th>
<th>Δ post-treatment - treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>165 ± 26.5</td>
<td>46 ± 14.5*</td>
<td>- 119 ± 21.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>156 ± 32.5</td>
<td>69 ± 19.5</td>
<td>- 87 ± 24.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-RD</td>
<td>141 ± 23.3</td>
<td>48 ± 12.7</td>
<td>- 92 ± 24.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-C</td>
<td>180 ± 17.8*</td>
<td>41 ± 16.0*</td>
<td>63 ± 9.2</td>
<td>-140 ± 27.3*</td>
<td>+ 22.9 ± 20.2</td>
</tr>
</tbody>
</table>

*significantly different from V (p < 0.001), †significantly different from V (p < 0.01), * significantly different from V (p < 0.05). All groups are n = 15.

**Table 4. Abdominal fatpad weight (grams; mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>Epididymal</th>
<th>Perirenal</th>
<th>% fatpad weight of total body weight</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>6.98 ± 2.30*</td>
<td>6.65 ± 2.52</td>
<td>2.8 ± 0.8</td>
<td>15</td>
</tr>
<tr>
<td>V</td>
<td>9.48 ± 3.93</td>
<td>8.23 ± 4.33</td>
<td>3.6 ± 1.3</td>
<td>14</td>
</tr>
<tr>
<td>V-RD</td>
<td>6.89 ± 2.17*</td>
<td>6.23 ± 2.35</td>
<td>2.9 ± 0.8</td>
<td>15</td>
</tr>
<tr>
<td>T-C</td>
<td>11.81 ± 2.52*</td>
<td>11.76 ± 4.39*</td>
<td>4.3 ± 1.2</td>
<td>15</td>
</tr>
</tbody>
</table>

*significantly different from V (p < 0.05)
(F(2,41) = 1.60, p = 0.251) fatpad weights. Both T and V-RD rats had reduced epididymal fatpads compared to V rats (t = -2.41, p = 0.020 and t = -2.49, p = 0.016, respectively. To correct for differences in total body weight, also the percentage of fatpad weight (epididymal + perirenal fatpad weight) of total body weight was compared between groups (F(2,41) = 2.64, p = 0.085). However, T (t = -1.97, p = 0.054) and V-RD (t = -1.85, p = 0.70) rats only showed trends for lower abdominal fat percentage than V rats.

**D2/3R availability**

For a few animals we were unable to accurately determine the NAcc-to-cerebellum ratio (1 T, 1 V, 2 V-RD) and dorsal striatum-to-cerebellum ratio (1T, 1V-RD), due to unreliable data read-out. As a result, these animals were removed from the analyses.

ANOVA showed that there were between-group differences in D2/3R availability in both NAcc (F(2,537) = 6.79, p = 0.003) and dorsal striatum (F(2,39) = 3.76, p = 0.032) (Table 5).
Tesofensine-treated rats (T) had substantially and significantly lower D2/3R availability than V rats in both NAcc (t = -3.02, p = 0.004) and dorsal striatum (t = -2.53, 0.015), with 13.5% lower D2/3R binding in the NAcc and 9.3% lower D2/3R binding in the dorsal striatum. V and V-RD rats did not differ in D2/3R availability.

**Association between behavioral data and D2/3R availability**

There were no significant correlations between D2/3R availability in the NAcc or dorsal striatum and weight gain, and caloric intake in any of the groups, except for a negative correlation in the T-C group of D2/3R availability in the striatum with percentage caloric intake from fat after treatment discontinuation (r = -0.616, p = 0.019). Thus, rats with higher striatal D2/3R availability had a smaller percentage of their caloric intake from fat after discontinuation of treatment. This was also reflected in a positive correlation between striatal D2/3R availability and caloric intake from chow after treatment stop (r = 0.602, p = 0.023), in contrast to a negative correlation between striatal D2/3R availability and caloric intake from fat after treatment discontinuation (r = -0.611, p = 0.020) in the T-C rats.

**DISCUSSION**

This study demonstrates that chronic tesofensine treatment leads to decreased food intake and weight gain and to decreased D2/3R availability in DIO rats. The tesofensine-treated rats with treatment-abstinent period show that these decreases are mostly reversed after treatment discontinuation. However, no relation between the food intake, weight gain and striatal D2/3R availability could be observed.

**Food intake**

This study confirms previous findings that tesofensine reduces appetite (1;3). In the previous study on the acute effects of tesofensine (3), blocking the D2/3R did not affect appetite suppression. One might hypothesize that a chronic increase in dopamine levels in the NAcc by chronic tesofensine treatment may lead to increased reward signaling, which could reduce craving for food and thus food intake. However, we did not find any correlation between the D2/3R availability in the different groups and caloric intake. Thus, this study provides no indication for a direct relation between D2/3R availability or the dopaminergic effects of tesofensine and regulation of food intake.

Hansen et al. (1) and Axel et al. (3) showed that tesofensine induced an initial strong decrease in appetite in rodents, which diminishes over time: after 14 days of treatment the caloric intake

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**Table 5.** Dopamine D<sub>2/3</sub> receptor availability (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Nucleus Accumbens (n)</th>
<th>Dorsal striatum (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T</strong></td>
<td>2.77 ± 0.33 (14)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.51 ± 0.49 (14)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>V</strong></td>
<td>3.20 ± 0.30 (13)</td>
<td>4.96 ± 0.45 (14)</td>
</tr>
<tr>
<td><strong>V-RD</strong></td>
<td>3.22 ± 0.44 (13)</td>
<td>4.94 ± 0.54 (14)</td>
</tr>
<tr>
<td><strong>T-C</strong></td>
<td>3.04 ± 0.41 (14)</td>
<td>4.65 ± 0.41 (14)</td>
</tr>
</tbody>
</table>

*significantly different from V (p < 0.05)
of the tesofensine-treated rats was not different anymore from vehicle-treated rats. Although we used the same treatment dose, we did not observe this pattern in the tesofensine-treated rats in this study. This could well be due to the use of different rat strains. In addition, the diet used to induce obesity was different, i.e. a synthetic high fat diet versus a HF choice diet, which has been shown to lead to different phenotypes (Mercer and Archer, 2008). A choice diet is hypothesized to be more reinforcing, thus resulting in overconsumption, which is reduced in rats treated with tesofensine. In humans, the appetite suppressing effect of tesofensine was also reported to be long lasting (2).

We cannot exclude that increasing extracellular monoamine levels systemically by tesofensine could have affected autonomic and gastrointestinal systems. This may have induced aversion/malaise states, thereby possibly affecting food intake. However, we do not reckon this likely, as Axel et al. (2010) reported that in both normal (acute treatment) and DIO rats (acute or chronic treatment), tesofensine administration did not cause side effects, which could potentially influence feeding behavior, for example negative reinforcement or gastrointestinal upset.

Post-treatment, the caloric intake of the tesofensine-treated rats gradually increased again. We did not observe a clear peak suggesting a binge effect on food intake directly after treatment discontinuation. Thus, the low D2/3R levels after chronic tesofensine treatment do not directly lead to a large increase in food intake after treatment discontinuation. A limitation of this study is the lack of a vehicle-treated group with four week post-treatment period to compare food intake post-treatment.

Interestingly, tesofensine had a small effect on the food preference of the rats resulting in a decreased preference for fat. In the post-treatment period of the T-C rats, the percentage caloric intake from fat negatively correlated with the D2/3R availability. This may indicate that the T-C rats who had higher D2/3R availability and ate in percentage less fat, experienced less reward from the fat, possibly due to lower dopamine levels. However, the correlation is not very strong and this hypothesis should be tested in additional studies. Overall, the data on food preference indicate that tesofensine has a positive effect with a reduced preference for the ‘unhealthy’ fat and increased preference for the ‘healthy’ chow.

Locomotor activity
As noted at the results section, the cages with the filter tops were slightly lowered. Therefore, we can, unfortunately, not exclude that the filter tops might have had an effect on the locomotor activity during treatment of the rats in cages with filter tops. Consequently, it cannot be concluded from this study that tesofensine would lead to a decrease in locomotor activity. This would also not be in line with results of other monoamine inhibitors, who led to an increase in locomotor activity (Billes and Cowley, 2008). In spite of this, the results on locomotor activity do show that the significant decrease in weight gain by tesofensine treatment is existent although locomotor activity decreases. So, the decrease in weight gain in the tesofensine treated rats is primarily from reduced food intake and possibly from higher thermogenesis, but not from increased locomotor activity, and may be rather underestimated than overestimated.

Weight gain and abdominal fatpad weight
The body weight reducing effect of tesofensine is confirmed in this study. Because of the age at the start of the study, all rats still gained weight during treatment, but the decrease in weight gain
of the tesofensine-treated rats was highly significantly larger compared to the vehicle-treated rats. The absolute weight gain during treatment is comparable for the tesofensine-treated rats (T) and vehicle-treated rats on the isocaloric diet (V-RD) as is their caloric intake. Thus, the larger decrease in weight gain in the tesofensine-treated rats can be explained for the major part by the decreased food intake, although a minor effect of increased energy expenditure, as reported previously (1;3;4), is still possible. After treatment discontinuation, the weight gain of the rats gradually increases again towards the (extrapolated) original weight curve.

The decreased weight gain is in accord with lower weight of the abdominal fat pads and a trend for lower percentage fatpad weight from body weight, i.e. a reduction of the obesity. The abdominal fatpad weight as a percentage of body weight is comparable for the tesofensine- and vehicle-treated rats on isocaloric diet. Therewith, we are unable to replicate the previous finding that tesofensine leads to a decrease in abdominal fat mass that is larger than in pair-fed rats (1). The previous studies (1;3) also observed that weight loss as percentage of total body weight was 8-10% by chronic tesofensine treatment of comparable dosage and length. In this study, the weight at the end of the tesofensine treatment period is 5.6% (T rats) to 7.8% (T-C rats) lower than the estimated weight when treated with vehicle. These differences might be explained by the fact that in the previous studies an inbred strain of obesity-prone rats was utilized, who had been pre-treated with a high-fat diet for 10 to 13 weeks instead of 4 weeks. Therefore, these rats might have had a higher level of obesity, which could subsequently result in a higher responsiveness to tesofensine.

D2/3R availability
This study shows that chronic tesofensine treatment decreases D2/3R availability in the NAcc and in the dorsal striatum of diet-induced obese (DIO) rats. The decrease in the NAcc is 13.5% compared to vehicle-treated rats, whereas the dorsal striatum in the tesofensine-treated rats showed 9.3% lower availability. The lower D2/3R availability is an effect of the tesofensine treatment and not a result of reduced food intake or weight gain, because vehicle-treated rats with a diet isocaloric to that of tesofensine-treated rats had similar D2/3R availability as vehicle treated rats with ad libitum diet, but similar weight gain and caloric intake as the tesofensine-treated rats. The data on D2/3R availability after the discontinuation of tesofensine treatment (T-C rats), suggest that the D2/3R expression tends to normalize again after discontinuation of treatment with tesofensine. The observed ratios for the vehicle treated rats are comparable with those previously reported with this technique (27;28), and similar to unpublished data from our laboratory that indicate a decrease in striatal D2/3R availability in rats on ad libitum HF choice diet compared to standard chow diet.

The decrease in D2/3R availability by chronic tesofensine administration is most probably due to an indirect pharmacological effect of the medication. The current storage phosphor technique measures the availability of free D2/3R, i.e the D2/3Rs that are not occupied by endogenous dopamine but available to bind to the radiopharmaceutical IBZM. Thus, the results indicate either a tesofensine-induced down-regulation of D2/3R, a tesofensine related increase of synaptic endogenous dopamine levels (and consequently less D2/3R available to bind to the radiopharmaceutical due to competition) or a combination of these two effects. The dopamine transporter (DAT) inhibiting qualities of tesofensine will almost certainly lead to increased endogenous dopamine levels, which subsequently may result in a down-regulation
of the postsynaptic D2/3R by internalization of the D2/3R and a chronic down-regulation of the D2/3R levels by chronic exposure to increased synaptic dopamine levels. It is not likely that the observed decrease in NAcc D2/3R binding ratios of 13.5% reflects only a lower binding due to increased levels of endogenous dopamine. A fivefold increase of extracellular dopamine, induced by amphetamine administration, is required to produce 10% decrease in \(^{11}C\)-raclopride binding ratios to the synaptic D2/3R in the striatum of monkeys (29) and we do not believe that tesofensine will lead to an increase of dopamine levels similar to that of amphetamine, in particular not after chronic treatment. In addition, it has been reported that tesofensine is unlikely to display any abuse potential in humans (30), unlike amphetamine, due to the slow absorption and elimination rate of tesofensine, which is associated with a slow rate of DAT blockade and subsequent no to low ‘high’ induction (31). To conclude, we reckon it likely that at least part of the 13.5% lower D2/3R availability in the NAcc is explained by indirect D2/3R down-regulation, which may be induced by higher levels of dopamine.

The effects on D2/3R binding due to the serotonin transporter (SERT) and norepinephrine transporter (NET) inhibiting qualities of tesofensine are probably minor compared to the DAT inhibiting effect. Previous studies on the effects of selective serotonin reuptake inhibitors (SSRIs) on striatal D2/3R levels in rodents indicate that repeated administration results in an upregulation of D2/3R levels in the NAcc and dorsal striatum (32-35). Similar effects are observed for NET inhibitors, which are able to increase D2/3R levels in the NAcc (32;33). Thus, the SERT and NET inhibiting effects of tesofensine could theoretically have a diminishing effect on the decrease in D2/3R availability that we presently observed. As the overall effect of tesofensine leads to decreased D2/3R availability in the NAcc, this suggests a major pharmacological role for DAT inhibition.

It has been hypothesized previously that a decrease in food intake and weight loss might lead to an increase in D2/3R levels in the NAcc and dorsal striatum. This hypothesis is based on the theory that the observed decrease in striatal D2/3R levels in obese humans and rodents is related to reward deficiency similar as in drug addiction (7). Reduced food intake and weight loss might lead to a normalization of the dopaminergic system, including an up-regulation/normalization of D2/3R levels. The observed decrease in caloric intake and decrease in weight gain in the tesofensine-treated rats would then counteract the pharmacological effect of the drug. However, the vehicle-treated rats on isocaloric diet did not show any indication for an effect of lower food intake or lower weight gain on striatal D2/3R availability, as they did not have higher D2/3R availability as the vehicle treated ad libitum fed rats. Although the tesofensine-treated rats have a somewhat larger decrease in food intake and decrease in weight gain than the vehicle-treated rats on isocaloric diet, an effect of food intake and weight gain on the D2/3R levels would be very minor if any.

**Strengths and limitations**

This study is the first to demonstrate the effects of chronic tesofensine treatment on the striatal D2/3R availability. The results are well translatable to the human situation, as the applied binding technique with *in vivo* injection of \(^{11}C\)-IBZM is largely comparable to human \(^{12}I\)-IBZM SPECT imaging of D2/3R. Due to binding of \(^{12}I\)-IBZM to both D2R and D3R, a possible minor effect of a change in striatal D3R availability on the data cannot be fully ruled out. Additional studies with measurements of endogenous dopamine levels, DAT density, and D2R protein or mRNA levels.
are necessary to provide more detailed insight in the effects of tesofensine on the dopaminergic system. Also, studies on the serotonergic and noradrenergic systems are required to elucidate the working mechanism of tesofensine, in particular because this study was unable to show direct relations between D2/3R availability and food intake and body weight.

The assignment to groups was determined by randomization at day 1 of the study, i.e. the day they started the four weeks of obesity induction. At that moment, body weights of the groups were very similar. Unfortunately, we observe a somewhat different responsivity in weight gain and caloric intake to the high fat choice diet during the pre-treatment period, which was an unexpected outcome. Due to organizational restrictions (e.g. V-RD rats started the study two days later than T rats to be able to determine their diet restriction during treatment), the rats did not all start the study at the same day, which possibly has contributed to the different responsivity. To correct for this limitation, we chose to analyze data on the changes (Δ) in food intake and weight gain between treatment and pre-treatment period and added caloric intake of the pre-treatment period or weight gain of the pre-treatment period, respectively, as a covariate in the ANCOVAs.

As previously mentioned, a limitation of the study is the lack of a vehicle-treated group with 28 day post-treatment period. However, the results of the tesofensine-treated rats with post-treatment period still show indications of the effects on food intake and weight gain after treatment discontinuation. These results also demonstrate that there are no large rebound effects on weight gain of food binges after treatment discontinuation. Thus, we do not expect that tesofensine would lead to undesirable adverse effects after treatment discontinuation, although the gradual increase in food intake and weight gain should be expected and managed in human studies.

CONCLUSION

The results on caloric intake and weight gain confirm previous findings that tesofensine reduces appetite and has a reducing effect on body weight. After treatment discontinuation, caloric intake and weight gain increase again, although there is no strong rebound effect directly after treatment discontinuation and no indication that caloric intake and weight gain reach higher levels than without treatment. Secondly, this study demonstrates that chronic treatment with the triple monoamine inhibitor tesofensine decreases D2/3R availability in the NAcc and dorsal striatum of DIO rats. After treatment discontinuation this decrease is mostly reversed. We were not able to find evidence for a relation between D2/3R availability and caloric intake or weight, though. The decreased striatal D2/3R availability appears to be mainly a pharmacological effect of tesofensine due to increased synaptic dopamine levels as a result of DAT inhibition and subsequent D2/3R down-regulation. Overall, this study shows that, first, a decrease in striatal D2/3R availability can co-occur with a decrease in food intake and weight gain when dopaminergic, serotonergic and noradrenergic systems are stimulated conjointly and, second, a further decrease in striatal D2/3R availability in obesity does not directly lead to undesirable increases in food intake and weight gain after treatment discontinuation.
REFERENCES


21. Booze RM, Wallace DR (1995): Dopamine D2 and D3 receptors in the rat striatum and nucle-