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van de Giessen, E.M.

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HIGH FAT/CARBOHYDRATE RATIO BUT NOT TOTAL ENERGY INTAKE INDUCES LOWER STRIATAL DOPAMINE D$_{2/3}$ RECEPTOR AVAILABILITY IN DIET-INDUCED OBESITY

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

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ABSTRACT

High-energy diets that induce obesity decrease striatal dopamine D2/3 receptor (DRD2/3) availability. It is however poorly understood which components of these diets are underlying this decrease. This study assessed the role of saturated fat intake on striatal DRD2/3 availability. Forty rats were randomized to a free-choice high-fat high-sugar diet (HFHS) or a standard chow diet for 28 days. Striatal DRD2/3 availability was measured using [123I]-IBZM storage phosphor imaging at day 29. The HFHS group was split in a HFHS-high-fat (HFHS-hf) and HFHS-low-fat (HFHS-lf) group based on the percentage energy intake from fat. Rats of both HFHS subgroups had increased energy intake, abdominal fat stores and plasma leptin levels compared to controls. DRD2/3 availability in the nucleus accumbens was significantly lower in HFHS-hf than in HFHS-lf rats, whereas it was similar for HFHS-lf and control rats. Furthermore, DRD2/3 availability in the nucleus accumbens was positively correlated with the percentage energy intake from sugar. Total energy intake was lower for HFHS-hf than for HFHS-lf rats. Together these results suggest that a diet with a high fat/carbohydrate ratio, but not total energy intake or the level of adiposity, is the best explanation for the decrease in striatal DRD2/3 availability observed in diet-induced obesity.
INTRODUCTION

High-fat (HF) and cafeteria diets induce obesity in rodents and decrease striatal dopamine D_{2/3} receptor (DRD_{2/3}) levels (1-3). Imaging studies in humans also demonstrate that striatal DRD_{2/3} availability is lower in obese subjects (4;5). However, the mechanisms of action underlying this decrease remain poorly understood. Midbrain leptin receptors may play a role, as they mediate the decrease in extracellular dopamine levels in the nucleus accumbens by a HF diet (6). In addition, knockdown of the dopamine D_{2} receptor in the striatum is associated with increased reward deficiency and compulsive eating behavior in rats (1). This can be related to the alternative hypothesis that low DRD_{2/3} levels might be a down-regulation due to overstimulation of the dopamine system by repetitive food intake in obesity (4). It remains unclear though whether increased total energy intake by high-energy diets or specific dietary components (e.g. saturated fat, carbohydrates) underlie the induction of lower striatal DRD_{2/3} levels.

We previously showed that rats on a choice diet with saturated fat and a sugar solution (HFHS) become hyperphagic and obese (7). We observed that some rats prefer saturated fat whereas others prefer the sugar solution although they all become obese. This variety allows us to determine whether diet composition or total energy intake is involved in DRD_{2/3} down-regulation. As we recently demonstrated that rats on a HF diet, consuming large quantities of saturated fat, had lower striatal DRD_{2/3} levels (3), we hypothesized that high intake of saturated fat might play an essential role in decreasing DRD_{2/3} levels.

METHODS AND PROCEDURES

Forty male Wistar rats (Horst, Harlan, The Netherlands), weighing 250-300 grams, were individually housed in a temperature- (21-23°C) and light-controlled (lights on 7:00 am – 7:00 pm) room. Experimental procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

Experimental design

After a 7-day habituation period, rats were randomized into 2 groups that were fed different diets for 28 days:

1. free-choice high-fat high-sugar (HFHS) diet (n = 24) (7): a dish of saturated fat (beef tallow (Ossewit/Blanc de Boeuf), Vandemoortele, Belgium) and a bottle of 30% sugar water (1.0M sucrose) were presented in the cage in addition to standard pellet chow (AB diets, Woerden, The Netherlands);

2. standard pellet chow diet (chow; n = 16) (AB diets).

Body weight and food intake were measured three times a week. At sacrifice on day 29, blood samples were collected for plasma leptin measurement (as described previously (8)) and abdominal fat stores were measured by dissecting and weighing the epidydimal and perirenal fat mass after sacrifice.
**DRD\textsubscript{2/3} measurements**

On day 29, rats were anesthetized with ketamine/xylazine mix followed by intravenous administration of approximately 37 MBq (1 mCi) of the selective DRD\textsubscript{2/3} tracer \textsuperscript{123}I-IBZM (GE Healthcare, Eindhoven, the Netherlands). Ninety minutes later, animals were sacrificed by bleeding through heart puncture under anesthesia. Brains were removed, frozen on dry ice and sliced horizontally into 50 μm slices in a microtome cryostat at -21°C. Storage phosphor imaging was performed as described previously (9). Every one in four slices were exposed to a Fuji BAS-MS IP for approximately 16 hours. The images were scanned using the Fuji FLA-3000 phosphor imager. Regions of interest (ROIs) were drawn for dorsal striatum and nucleus accumbens (NAcc) (Figure 1a; for detailed description see (10)). ROIs drawn for the cerebellum were used to assess non-specific binding (9;10). Ratios of dorsal striatum-to-cerebellum/NAcc-to-cerebellum binding were obtained by dividing the average uptake per pixel in the dorsal striatum/NAcc parts by the average uptake per pixel in the cerebellum.

**Statistical analysis**

Due to logistic reasons, this study contains 3 subgroups that were started at different time points all with their own chow controls. All parameters concerning body weight and feeding behavior were comparable between the controls of all subgroups. However, the imaging analysis is more prone to variation between studies. We therefore standardized all measurements on the average of the chow controls, which was set at 100%. The analysis results are reported for the standardized data.

To determine the role of diet composition on DRD\textsubscript{2/3} availability, in particular the role of saturated fat intake, we split the HFHS group in two halves with the cut off at the median fat intake, i.e. 21% saturated fat intake, resulting in a HFHS-high-fat (HFHS-hf) and HFHS-low-fat (HFHS-lf) group.

ANOVAs were used to assess group differences. When appropriate, post-hoc tests (Tukey) were performed. Correlations between DRD\textsubscript{2/3} availability and energy intake, weight gain, abdominal fat mass, and plasma measurements were determined with Pearson’s correlation. A probability value of <0.05 was considered significant.

**RESULTS**

One animal from the HFHS-lf group was excluded from all analysis, because DRD\textsubscript{2/3} availability could not reliably be determined.

Total energy intake per day was different between groups (F(2,36) = 90.8, p <0.001). HFHS-hf (p <0.001) and HFHS-lf (p <0.001) rats had higher energy intake than chow rats. In addition, HFHS-lf rats ate more calories than HFHS-hf rats (p = 0.039). Repeated measures ANOVA showed that energy intake per week also differed between groups (F(2,36) = 41.4, p <0.001) and there was a trend for a group x time interaction (F(6,36) = 2.1, p = 0.066; figure 1). Post-hoc tests for week 4 showed that there was also a trend for lower energy intake in the HFHS-hf compared to HFHS-lf rats (p = 0.066). Supplementary data show that the HFHS-hf rats significantly decreased energy intake from fat and chow over time compared to HFHS-lf rats (see supplementary information). Percentages energy intake from fat, sucrose and chow per group are displayed in table 1.
High fat/carbohydrate ratio lowers DRD\textsubscript{2/3} binding

**Figure 1.** (A) Examples of dopamine \( \text{D}_\text{2/3} \) receptor (DRD\textsubscript{2/3}) binding in brain slices with regions of interest (ROIs) drawn for: dorsal striatum (A), nucleus accumbens (B), and cerebellum (C). (B) Energy intake per day for each week. Data are mean ± s.e.m. # marks significantly lower energy intake than both HFHS-hf and HFHS-lf groups (post-hoc, \( p < 0.005 \)). * marks trend for lower energy intake than in HFHS-lf group (post-hoc, \( p = 0.066 \)). (C) DRD\textsubscript{2/3} availability in the nucleus accumbens standardized to the chow group (100%). Horizontal lines display means per group. * marks outlier. Different letters (a, b) represent significant differences between groups for analysis including HFHS-lf outlier (\( p<0.05 \)).
Body weight gain was also different between groups ($F(2,36) = 3.5, p = 0.039$), i.e., HFHS-lf rats gained more weight than chow rats ($p = 0.036$), but HFHS-hf rats did not differ from HFHS-lf ($p = 0.620$) or chow rats ($p = 0.244$). The weight of perirenal and epidydimal fat mass as percentage from total body weight also showed between-group differences ($F(2,36) = 25.2, p < 0.001$). HFHS-hf ($p < 0.001$) and HFHS-lf ($p < 0.001$) rats had a higher percentage of abdominal fat mass than chow rats, whereas fat mass of HFHS-hf and HFHS-lf rats did not differ ($p = 0.208$). Plasma leptin concentrations also differed ($F(2,36) = 16.0, p < 0.001$), as HFHS-hf ($p < 0.001$) and HFHS-lf ($p < 0.001$) rats had higher plasma leptin levels than chow rats, but did not differ from each other ($p = 0.675$).

### Table 1. Adiposity measures and dopamine D$_{2/3}$ receptor availability per group

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>HFHS-hf</th>
<th>HFHS-lf</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>16</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td><strong>Energy intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (kcal/day)</td>
<td>$88.7 \pm 6.4^1$</td>
<td>$114.7 \pm 8.0^2$</td>
<td>$119.8 \pm 10.8^2$</td>
</tr>
<tr>
<td>Total (standardized)</td>
<td>$100 \pm 4.3^1$</td>
<td>$131 \pm 10.2^1$</td>
<td>$139 \pm 9.7^2$</td>
</tr>
<tr>
<td>energy intake from fat (mean; range)</td>
<td>$28.5% (22-43%)$</td>
<td>$13.8% (5-21%)$</td>
<td>$41.7% (28-51%)$</td>
</tr>
<tr>
<td>energy intake from sucrose (mean; range)</td>
<td>$28.4% (17-37%)$</td>
<td>$41.7% (28-51%)$</td>
<td>$41.7% (28-51%)$</td>
</tr>
<tr>
<td>energy intake from chow (mean; range)</td>
<td>$100%$</td>
<td>$43.2% (36-58%)$</td>
<td>$44.5% (36-56%)$</td>
</tr>
<tr>
<td><strong>Abdominal fat mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% from body weight</td>
<td>$2.0 \pm 0.4^1$</td>
<td>$3.4 \pm 0.6^2$</td>
<td>$3.6 \pm 0.8^2$</td>
</tr>
<tr>
<td>% from body weight (standardized)</td>
<td>$100 \pm 21.5^1$</td>
<td>$166 \pm 29.3^1$</td>
<td>$192 \pm 53.3^1$</td>
</tr>
<tr>
<td><strong>Plasma leptin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ng/ml</td>
<td>$5.9 \pm 1.2^1$</td>
<td>$10.7 \pm 2.6^2$</td>
<td>$13.6 \pm 6.6^2$</td>
</tr>
<tr>
<td>standardized</td>
<td>$100 \pm 13.9^1$</td>
<td>$191 \pm 43.1^2$</td>
<td>$210 \pm 88.3^2$</td>
</tr>
<tr>
<td><strong>Body weight gain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (grams)</td>
<td>$109 \pm 10.8^1$</td>
<td>$117 \pm 14.3^1$</td>
<td>$124 \pm 16.2^1$</td>
</tr>
<tr>
<td>Total (standardized)</td>
<td>$100 \pm 9.6^2$</td>
<td>$107 \pm 11.7$</td>
<td>$112 \pm 15.2^1$</td>
</tr>
<tr>
<td><strong>Dopamine D$_{2/3}$ receptor availability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens (standardized)</td>
<td>$100 \pm 10.8^2$</td>
<td>$91 \pm 6.4^1$</td>
<td>$103 \pm 14.9^2$</td>
</tr>
<tr>
<td>Dorsal striatum (standardized)</td>
<td>$100 \pm 11.0$</td>
<td>$97 \pm 9.2$</td>
<td>$104 \pm 10.3$</td>
</tr>
</tbody>
</table>

Data are displayed as mean ± standard deviation, unless otherwise indicated. Different numbers ($^1$,$^2$) represent significant differences between groups (post-hoc, $p<0.05$).

**DRD$_{2/3}$ availability**

The DRD$_{2/3}$ availability in the NAcc differed between groups ($F(2,36) = 4.1, p = 0.025$; figure 1) due to a lower DRD$_{2/3}$ availability in HFHS-hf compared to HFHS-lf rats ($p = 0.027$). There was also a trend for lower DRD$_{2/3}$ availability in HFHS-hf than in chow rats ($p = 0.087$). There was one extreme outlier (>2.5 standard deviations) in the HFHS-lf group. When this outlier was removed from the analyses, groups still differed significantly ($F(2,35) = 4.4, p = 0.020$). By this analysis, HFHS-hf rats had significantly lower DRD$_{2/3}$ availability than chow rats ($p = 0.023$) and a trend for lower DRD$_{2/3}$ availability compared to HFHS-lf rats ($p = 0.071$).
In the dorsal striatum, no significantly differences in DRD<sub>2/3</sub> availability between groups were found (F(2,36) = 1.3, p = 0.291).

Within the chow, HFHS-hf, and HFHS-If groups, there were no significant correlations between DRD<sub>2/3</sub> availability and total energy intake, energy intake from fat or sugar, weight gain, abdominal fat mass, or plasma leptin concentrations. When HFHS-hf and HFHS-If were combined to one group, there was one significant correlation between NAcc DRD<sub>2/3</sub> availability and percentage energy intake from sugar (r = 0.546, p = 0.007), which became borderline significant after exclusion of the outlier (r = 0.424, p = 0.050).

**DISCUSSION**

This study showed that DRD<sub>2/3</sub> availability in the NAcc was lower in HFHS-hf rats compared to HFHS-If rats and chow rats. Moreover, in spite of the higher energy intake by the HFHS-If rats, they had similar DRD<sub>2/3</sub> availability as the chow rats. Finally, the percentage energy intake from sugar positively correlated with DRD<sub>2/3</sub> availability in the Nacc in the combined HFHS-hf and HFHS-If group. However, the percentage energy intake from fat and sugar are not independent but strongly negatively correlated (r = -0.81). Therefore, we conclude that a high fat/carbohydrate ratio is essential in the induction of lower DRD<sub>2/3</sub> availability in diet-induced obesity (DIO) rats and that total energy intake itself is not an underlying factor for the lower DRD<sub>2/3</sub> availability in DIO rats.

HFHS-hf rats and HFHS-If rats do not differ in abdominal fat mass, body weight gain, and plasma leptin, showing that the level of adiposity is similar. It is thus unlikely that these factors play a role in the DRD<sub>2/3</sub> down-regulation. HFHS-If rats tended to have a higher energy intake than HFHS-hf rats and whereas the energy intake tends to increase over time in HFHS-If rats, it tends to decrease in HFHS-hf rats based on a decrease in fat and chow intake. The DRD<sub>2/3</sub> down-regulation in the HFHS-hf might thus be linked to a mechanism that aims to curb food intake, although this is a preliminary assumption.

The high energy intake of HFHS-If rats, without inducing a decrease in DRD<sub>2/3</sub> availability, makes the hypothesis that DRD<sub>2/3</sub> down-regulation in obesity is due to overstimulation of the dopamine system by repetitive food intake unlikely (4). Similar for the sugar preference observed in HFHS-If rats which would predict lower DRD<sub>2/3</sub> availability since sugar is very rewarding (11) and induces a dopamine release (12). Moreover, diets high in fat reduce dopamine levels and decrease dopamine release in the NAcc (6;13;14). Leptin might play a role in this mechanism (6;15), although a role for the degree of insulin resistance is also possible (14). However, decreased dopamine release can not directly explain DRD<sub>2/3</sub> down-regulation, because, in case of low extracellular dopamine levels, one would expect a compensatory DRD<sub>2/3</sub> up-regulation. So, the effects of diet-induced obesity on the mesolimbic dopaminergic system are more complex than can be explained by this leptin or insulin resistance pathway. The lack of a correlation with plasma leptin in our sample also does not confirm a direct role for leptin.

It is interesting that the HFHS-hf rats consume on average a similar percentage of calories from fat (28.5±6.0%) as the rats on a HF diet (30.1±10.0%) that also had lower striatal DRD<sub>2/3</sub> availability (3). Therefore, it is plausible that a high fat/carbohydrate ratio with excessive fat intake is important for the induction of decreased striatal DRD<sub>2/3</sub> availability. A peptide that has been linked to fat preference and fat intake is galanin, in particular galanin levels in the
high fat/carbohydrate ratio lowers DRD<sub>2/3</sub> binding

hypothalamic paraventricular nucleus (PVN) (16;17). In addition, galanin knock-out mice have decreased fat preference, which is partly reversed by intracerebroventricular galanin administration (18). Galanin administration in the PVN can also induce a dopamine release in the NAcc (19). This peptide could possibly be a linking factor between fat preference and DRD<sub>2/3</sub> down-regulation in the NAcc.

In conclusion, we show that neither total energy intake nor adiposity level is important for the decrease in striatal DRD<sub>2/3</sub> in obesity, but that a high fat/carbohydrate ratio seems to be the driving factor. This outcome stresses the importance of diet composition and provides a new focus for the investigation of underlying mechanisms.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY INFORMATION

Energy intake from chow, fat, and sugar over time for HFHS-hf and HFHS-lf rats

HFHS-hf rats significantly decrease energy intake from fat and chow compared to HFHS-lf rats. The time x group interaction for energy intake from chow is significant (p = 0.015) and the time x group interaction for energy intake from fat is significant (p = 0.009), whereas the time x group interaction is not significant for energy intake from sugar (p = 0.831). Further, both HFHS-hf and HFHS-lf rats show an increase in percentage of sugar intake and decrease in percentage of chow intake over time, whereas percentage intake from fat only decreases for the HFHS-hf rats. Thus, the decrease in total energy intake over time in the HFHS-hf group is based on lower intake of fat and chow.

Data are mean ± s.e.m. * marks significantly different energy intake for HFHS-hf and HFHS-lf groups (post-hoc, p < 0.05).

Figure S1. Energy intake from chow (A), fat (B), and sugar (C) over time for HFHS-hf and HFHS-lf rats.
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