Brain, nutrition and metabolism
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Citation for published version (APA):

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Abstract

Recent studies showed that meal timing throughout the day contributes to maintaining or regaining weight after hypocaloric diets. Although brain serotonin and dopamine are well known to be involved in regulating feeding, it is unknown whether meal timing during energy restriction affects these neurotransmitter systems. We studied the effect of a 4-week hypocaloric diet with either 50% of daily calories consumed at breakfast (BF-group) or at dinner (D-group) on hypothalamic and thalamic serotonin transporter (SERT) binding and on striatal dopamine transporter (DAT) binding. The BF- and D-group lost a similar amount of weight. Striatal DAT and thalamic SERT binding increased in the BF-group, while decreasing in the D-group after the diet (delta DAT 0.37±0.63 vs -0.53±0.77 respectively, p=0.005; delta SERT 0.12±0.25 vs -0.13±0.26 respectively, p=0.032. Additional voxel-based analysis showed an increase in DAT binding in the ventral striatum in the BF-group and a decrease in the dorsal striatum in the D-group. During weight loss, striatal DAT and thalamic SERT binding increases weight-independently when 50% of daily calories are consumed at breakfast, while it decreases when caloric intake is highest at dinner. These findings might contribute to the earlier reported favorable effect of meal timing on weight maintenance after hypocaloric diets.
Introduction

Preventing weight regain after weight loss is a major challenge in the treatment of obesity since weight maintenance is difficult to achieve in the long-term (1, 2). Post-dieting weight regain is probably caused by a physiological adaptation in response to the weight loss and/or to persistent dysregulation of food intake associated with obesity. Following weight loss, long-term changes in circulating appetite-related signals such as leptin, ghrelin and peptide YY (PYY) promote food intake favoring subsequent weight regain (3). Besides total caloric intake, recent studies highlighted the importance of meal timing in modulating body weight and food intake. First, animal studies showed that feeding at an inappropriate time, i.e. during the light phase in nocturnal animals, resulted in more body weight gain (4). Second, in humans, several cross-sectional studies reported that eating most of the daily calories in the evening is associated with higher caloric intake and body weight (5, 6). Third, dietary intervention studies in obese humans showed that during energy restriction, eating most of the daily calories in the morning compared to the evening, resulted in more weight loss (7). Interestingly, Jakubowicz and colleagues also showed that subjects who consumed a larger breakfast were more successful in maintaining reduced body weight and even continued losing weight, whereas weight regain was observed in subjects who consumed a larger dinner (8). These data suggest that during caloric restriction, timing of food intake is an important determinant of long-term weight loss.

Body weight and food intake are under tight control of several brain pathways including hypothalamic neuronal circuits that orchestrate energy homeostasis upon endocrine and metabolic signals and brain reward circuitries involved in the hedonic aspects related to food (9, 10).

Within the hypothalamus, the neurotransmitter serotonin plays a central role in food intake by acting as an appetite suppressant. Serotonin signaling is in part regulated by the serotonin transporter (SERT) that controls available serotonin within the synaptic cleft. Several studies reported altered central SERT binding in obese humans (11-13). Moreover, short-term use of selective serotonin reuptake inhibitors (SSRIs) reduce food intake (14), while long-term use is associated with obesity (15). In addition, we recently showed lower diencephalic SERT binding in insulin resistant obese humans compared to lean controls (16) and we found a 30% reduction in SERT binding in response to a hypercaloric high fat high sucrose snacking diet in lean humans (17). Thus, lower SERT is associated with obesity and is affected by diet composition and meal pattern. At present, it is unknown whether reduced SERT is a cause or a consequence of obesity and whether reduced SERT is reversible after weight loss.

The dopaminergic system is involved in the reinforcing value of food and consumption of calorie dense food induces dopamine release in the striatum (18). There is a large body of evidence suggesting that downregulation of the dopamine-based reward circuitry in obese subjects contributes to compensatory overeating (for a review see (10)). In this regard, we previously showed lower striatal dopamine D2/3 receptor binding as well as blunted striatal dopamine
release in obese humans (19, 20), which may reflect a hypodopaminergic state. However, controversy exists about the role of the dopamine transporter (DAT), which plays an important role in the regulation of dopamine levels, in food intake and obesity. Some studies reported no correlation (12, 21), whereas other studies showed a negative correlation between striatal DAT availability and body mass index (BMI) (22). In rodents on a high fat diet, striatal DAT binding is reduced (23-25) and dopamine receptor agonists reduce food intake which was associated with an increase in DAT binding in the nucleus accumbens shell (26). In addition, the dopamine and norepinephrine reuptake inhibitor methylphenidate has an anorexigenic effect in both animals (27) and humans (28), which might be mediated via increased availability of synaptic dopamine levels.

In summary, weight regain after diet-induced weight loss is a frequently observed phenomenon and reduces the long-term efficacy of dietary interventions for obesity. The determinants of weight regain are only partly understood and include long-term changes in appetite-related metabolic signals favoring food intake as well as meal timing during the hypocaloric diet phase (29, 30). Whether meal timing during weight loss can influence cerebral serotonergic and dopaminergic pathways is unknown. We therefore investigated the effect of consuming 50% of daily calories at breakfast versus at dinner on SERT and DAT binding during a weight loss intervention study in obese insulin-resistant subjects.

**Materials and methods**

**Subjects**

We included 25 obese (BMI ≥ 30kg/m², age 50-80 years) men with a stable weight three months prior to inclusion. All subjects had impaired fasting glucose defined as fasting plasma glucose levels ≥ 5.6 mmol/l and/or were insulin resistant defined as fasting insulin levels ≥ 73 pmol/l (31). Exclusion criteria were use of any medication except for anticoagulants, 5-alpha reductase inhibitors and drugs related to treatment of components of the metabolic syndrome (excluding insulin, oral glucose lowering drugs and beta-blockers), history of any psychiatric disorder, shift work, irregular sleep pattern, regular vigorous exercise, restrained eaters, history of eating disorder, alcohol/drug abuse and smoking. Subjects were recruited via local advertisements. The trial was performed between November 2014 and January 2016 and registered at the national trial register as NTR-5399. All subjects provided written informed consent. The study was approved by the medical ethics committee of the Academic Medical Centre, Amsterdam and conducted according to the Declaration of Helsinki of October 2013.

**Diet intervention and randomization**

We performed a 4-week randomized intervention trial. At inclusion, the Munich ChronoType Questionnaire (MCTQ) was used to determine subjects’ chronotype and the Eating Disorder Examination-Questionnaire (EDE-Q) to assess restrained eating behavior. After inclusion, subjects started a one-week run-in phase and consumed a eucaloric weight maintaining diet based on measured resting
energy expenditure (REE). REE was measured using indirect calorimetry (Vmax Encore 29, Carefusion, San Diego, CA, USA) at the beginning and end of the run-in week and was used to calculate the caloric need (50% of caloric need calculated as 1.3 x REE x 0.5) during the hypocaloric diet intervention. After the run-in week, subjects were randomized into one of the two diet intervention groups. The breakfast (BF) group consumed 50% of the daily calories at breakfast, 35% at lunch and 15% at dinner. The dinner (D) group consumed 15% of the daily calories at breakfast, 35% at lunch and 50% at dinner. Subjects followed the hypocaloric diet for four weeks. Breakfast and dinner consisted of a protein enriched liquid drink (Fresubin protein energy drink; Fresenius Kabi, Bad Homburg, Germany) with a nutritive value of 1.5 kcal/ml; 27 energy% protein, 32.3 energy% carbohydrates, and 40 energy% fat (9% saturated fatty acids, 73% monounsaturated fatty acids, 18% polyunsaturated fatty acids) and by one bar (Modifast ProtiPlus; Nutrition & Santé Benelux, Breda, the Netherlands) with a nutritive value of 94 kcal; 36 energy% protein, 36 energy% carbohydrates, and 28 energy% fat (36% saturated fatty acids). The amount of liquid drink per serving was calculated based on daily caloric need. Protein intake was 0.82 ± 0.1 g/kg. For lunch, subjects chose from different sandwiches consisting of sandwiches with savory fillings. All lunch options contained the same amount of kcal and macronutrient compositions and the amount of sandwiches to be consumed was calculated based on daily caloric need. Subjects consumed their breakfast between 7:00-9:00 AM, lunch between 12:00 AM-2:00 PM and dinner between 6:00-8:00 PM. They visited the research unit weekly for dietary monitoring and body weight control. REE was performed weekly. To increase dietary compliance, subjects reported their food intake and meal timing on a diet diary and online using www.mijnvoedingscentrum.nl/eetmeter. An overview of the study design is presented in Figure 1.

Figure 1. Study design

Endpoints and sample size determination
The primary endpoints were serotonin and dopamine transporter binding. Secondary endpoints were food intake-related hormones and hunger scores. The study was powered to detect a 15% difference in the specific binding ratio of $^{123}$I-FP-CIT to SERT. This is based on a previous study where we found a 30% reduction in this binding ratio of $^{123}$I-FP-CIT to SERT in the diencephalon after a hypercaloric snacking diet for 6 weeks (0.65 ± 0.15 vs 0.46 ± 0.13, mean difference 0.19 ± 0.07) (17). Based on this, a sample size calculation was performed with a significant level of 0.05, power of 90%, a standard deviation of 0.07 and a difference in effect size of 15%. Power analysis was performed using nQuery Advisor 7.0.
**SPECT imaging**

Before and after the diet and after an overnight fast, subjects underwent single photon emission computed tomography (SPECT) imaging 2 and 3h after intravenous administration of approximately 100 MBq $^{123}$I-FP-CIT (specific activity > 750 MBq/nmol; radiochemical purity > 98%, produced according to GMP criteria at GE Healthcare, Eindhoven, The Netherlands). This radiotracer binds predominantly to DAT in the striatum, and to SERT in extrastriatal brain areas, as validated earlier (32). Extrastriatal SERT and striatal DAT binding can be assessed optimally at 2 and 3h after bolus injection, respectively, as described previously (33). Participants were scanned at 10:30 AM (i.e. 2h after bolus injection of the radiotracer) and at 11:30 AM (3h post-injection). Each participant was pre-treated with potassium iodide to block thyroid uptake of free radioactive iodide. SPECT imaging was performed using the Inspira HD system, a brain-dedicated tomographic SPECT scanner (Neurologica, Boston, USA) with the following parameters: acquisition time per slice = 180 s; slice thickness = 4 mm; slices were acquired from the level of the cerebellum up to the striatum; energy window = 159 keV ± 20%. An adult head CT template was manually aligned in a rigid transformation and used for attenuation correction. An Iterative Expectation Maximization Algorithm tailored to the unique method of sampling across the field-of-view with a point spread function correction was used to reconstruct the data into 3D images.

**Magnetic Resonance Imaging**

A T1-weighted (T1w) MRI scan of the brain was obtained for anatomical reference on a 3.0 T Philips Ingenia scanner (Philips Healthcare, Best, The Netherlands) with a 32-channel receive-only head coil and the following scan parameters: TR/TE= 7/3.18 ms; flip angle = 9°; 1 mm isotropic resolution. The individual SPECT scan was co-registered to each individual MRI scan using 6-parameter rigid body registration in SPM (SPM 8, Wellcome Trust Centre for Neuroimaging, London, UK). To optimize registration, a high intensity striatal mask was superimposed on the T1w scan to resemble the contrast on the SPECT scan (Figure 2 depicts

![Figure 2. Co-registered SPECT to individual MRI](image-url)
ROI analysis

ROI analysis was performed to determine DAT binding in the striatum, and SERT binding in the thalamus and hypothalamus (Figure 3A). Striatal and thalamic masks were extracted from individual T1w MRI scans using Freesurfer (version 5.3.0., (34, 35)). Hypothalamic masks were manually drawn on the individual T1w MRI using ITK-SNAP (version 3.4.0, PICSL, University of Pennsylvania) using anatomical landmarks as previously described (36) (Figure 2A). We used these manually drawn ROIs because it is not possible to identify binding in the hypothalamus on SPECT images due to limited spatial resolution and manually drawing has been shown to be a reliable method (37). The cerebellum was used as a reference region for non-displaceable binding (nonspecific binding and free radioactivity). To obtain individual cerebellar masks in T1w space, the cerebellum (without vermis) from the Harvard-Oxford subcortical atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) was warped to the individual T1w MRI using FSL (FMRIB Software Library, version 5.0.6, Oxford, UK). Subsequently, all region-of-interest (ROI)s were resliced to the SPECT scan. Specific to non-specific binding ratios were calculated as follows: (mean ROI binding - mean non-specific binding assessed in the cerebellum)/mean non-specific binding cerebellum, which represents the binding potential (SNS-BR).

Voxel-based analysis

To confirm the findings of the ROI analysis in the striatum, as well as to evaluate DAT binding in subdivisions of the striatum, we also assessed changes in DAT binding over time within groups at a voxel level, using FMRIB Software (38), as different parts may represents different functions (39, 40). First, individual binding ratios were determined by subtracting cerebellar mean count (as specified above) from all voxels, and then dividing all voxels by cerebellar mean count. Second, these individual images were registered to standard 2 mm MNI space (MN152), using a 9 degrees of freedom (DOF) translation. Next, a permutation-based nonparametric test (5000 permutations) (41) was performed, implementing threshold-free cluster enhancement at p < 0.01 (42). This was performed solely within the striatum, as defined by the Harvard-Oxford subcortical atlas.

Hunger scores

Hunger and appetite scores were assessed using 10-cm visual analog scales (VAS) after an overnight fast on the same day as the SPECT scans were performed.

Laboratory analysis

Plasma glucose levels were determined using the glucose oxidation method with the Biosen glucose analyzer (EKF Diagnostics, Barleben, Germany). Insulin levels were determined by immunoassay on an IMMULITE 2000 system (Diagnostic Products, Los Angeles, CA) and leptin and ghrelin were measured with an 125I radioimmunoassay (Millipore) as previously described (43, 44).
**Statistical analysis**

Normally distributed variables are presented as mean ± standard deviation and non-normally distributed variables as median with interquartile range [IQR]. Baseline characteristics between the BF- and D-group were compared by a two-sided non-paired samples Student’s T-test. Within subject changes before and after the diet were analyzed using a two-sided paired samples Student’s T-test for normally distributed variables or using a Related-Samples Wilcoxon Signed Rank test for non-normally distributed variables. We used a two-sided non-paired Student’s Test to compare differences in change over time between the BF- and D-group for normally distributed variables and a Mann-Whitney test for non-normally distributed variables. All data were analyzed using SPSS for Windows, version 23 (SPSS Inc. Chicago, Illinois, USA).

**Results**

**Participants and baseline characteristics**

Forty-five subjects were screened of which 25 subjects were included. Two subjects dropped out after the run-in week due to 1) claustrophobia precluding the MRI scan and 2) a busy work schedule precluding to adapt to the study protocol. Twenty-three subjects were randomized into either the BF-group (n=12) or D-group (n=11) and all subjects completed the study. Baseline characteristics of the participants are presented in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>BF-group</th>
<th>D-group</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>11</td>
<td></td>
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<tr>
<td>Age (yrs)</td>
<td>60.7 ± 7.7</td>
<td>59.0 ± 8.5</td>
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<td>Body weight (kg)</td>
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<td>111 ± 16.6</td>
<td>0.64</td>
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<td>BMI (kg/m²)</td>
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<td>Fasting glucose (mmol/L)</td>
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<td>5.3 ± 0.8</td>
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<tr>
<td>Fasting insulin (pmol/L)</td>
<td>121 ± 72.4</td>
<td>110 ± 35.6</td>
<td>0.67</td>
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</table>

Data are expressed as mean ± SD.
Weight loss and caloric intake

Body weight was significantly reduced in the BF-group (108±13.0 kg vs 101±13.1 kg, p=<0.001) and in the D-group (111±16.6 kg vs 104±16.3 kg, p=<0.001) after the diet. Both, absolute and percentage weight loss did not differ between the BF- and D-group (-7.0±1.4 kg vs -6.8±2.0 kg, p=0.86 and -6.5±1.5 % vs -6.2±1.9 %, p=0.70, respectively). Total average caloric intake per day was similar between the BF- and D-group (1257±134 kcal vs 1298±140 kcal, p=0.48) as well as macronutrient composition. In addition, the time at which breakfast (8:20±0:36 h vs 8:27±0:23 h, p=0.57), lunch (13:08±0:35 vs 12:58±0:16 h, p=0.41), and dinner (18:44±0:44 vs 18:33±0:22 h, p=0.29) were consumed did not differ between groups.

ROI analysis

Striatal DAT binding

SPECT scans could not be analyzed in three subjects in the BF-group because of technical issues with the scanner during imaging. Overall, striatal DAT binding was not significantly different after weight loss, but between group comparisons show that striatal DAT binding ratios increased in the BF-group and decreased in the D-group, resulting in a significant change over time between both diet groups (table 2 and Figure 3B and 3C).

(Hypo)thalamic SERT binding

Hypothalamic SERT binding was not affected after weight loss and did not differ significantly between the BF- and D-group (table 2 and Figure 3D and 3E). Within subjects analysis yielded no difference in thalamic SERT binding after weight loss, but between group comparisons showed that subjects in the BF-group showed an increase in thalamic SERT binding, while subjects in the D-group showed a decrease after the diet, resulting in a significant change over time between both diet groups (table 2 and Figure 3F and 3G).
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Figure 3. Striatal DAT and (hypo)thalamic SERT binding. ROI mask of striatum (blue), thalamus (green) and hypothalamus (red) (A). Overall striatal DAT binding ratios (B) and hypothalamic (D) and thalamic (F) SERT binding ratios did not differ significantly before and after the hypocaloric diet. Timed caloric intake significantly altered striatal DAT (C) and thalamic SERT (G) binding ratios between the BF-group and the D-group, but not hypothalamic SERT binding ratio (E). Data are expressed as mean ± SEM. *= p<0.05, **= p<0.01.
Exploratory voxel-based analysis

We performed a voxel-based analysis to explore whether the observed differential change in striatal DAT binding over time in the BF- and D-group could be attributed to a specific change in either the ventral or the dorsal part of the striatum. A within group comparison of the BF- and D-group showed a cluster of voxels within the ventral part of the striatum representing the increase in DAT binding in the BF-group after the diet and a cluster of voxels within the dorsal part of the striatum, representing the decrease in DAT binding in the D-group (p=<0.01)(Figure 4). These findings suggest a differential effect of both diets on DAT in the ventral vs dorsal striatum during energy restriction. However, statistical significance was not maintained after correction for multiple corrections.

![Figure 4. Voxel-based analysis.](image)

Food intake-related hormones and hunger scores

Overall, ghrelin concentrations were significantly increased after weight loss, but did not differ significantly between the BF-and D-group (table 2 and Figure 5A and 5B). Leptin concentrations were significantly decreased after weight loss, but were not different between the BF- and D-group (table 2 and Figure 5C and 5D). In addition, overall hunger and appetite scores were not different after weight loss or between the diet groups (table 2 and Figure 5E-5H).
<table>
<thead>
<tr>
<th>Table 2. Intervention data before and after a 4-week hypocaloric diet</th>
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<tr>
<td>All subjects</td>
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<td>----------------</td>
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<td></td>
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<tr>
<td>Striatal DAT binding (SNR-BR)</td>
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<td>Hypothalamic SERT binding (SNR-BR)</td>
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<td>Thalamic SERT binding (SNR-BR)</td>
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<td>Ghrelin (pg/mL)</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
</tr>
<tr>
<td>Hunger scores (cm)</td>
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<tr>
<td>Appetite scores (cm)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or median (IQR). SNR-BR: specific-to-nonspecific binding ratio. * change over time between the BF- and D-group.
Figure 5. Food intake-related hormones and hunger scores. Plasma ghrelin concentrations significantly increased (A) and plasma leptin concentrations significantly decreased (C) after weight loss. Timed caloric intake did not affect the change over time on both hormones (B and D). Hunger and satiety scores did not change after weight loss (E and G) and were not affected by timed caloric intake (F and H). Data are expressed as mean ± SEM or median (IQR). *** = p<0.001.
Discussion

We show a differential effect of timing of daily calorie intake on brain DAT and SERT during diet-induced weight loss in obese men. Consuming 50% of daily calories at breakfast increased DAT binding in the striatum and SERT binding in the thalamus, while consuming the majority of daily calories at dinner reduced DAT binding in the striatum as well as SERT binding in the thalamus. In addition, we show in an exploratory analysis that eating most of the calories in the morning increased DAT binding particularly in the ventral striatum, while eating most of the calories in the evening decreased DAT binding in the dorsal striatum. These differential effects were independent of weight loss or macronutrient intake per se, as these did not differ between the diet groups.

There is an increasing number of studies suggesting beneficial effects of higher energy intake in the morning compared to the evening on body weight and weight loss maintenance (7, 8). However, so far, the mechanism linking meal timing and weight loss maintenance is unclear. An increase in DAT binding as shown in the BF-group might contribute to the beneficial effects, since lower striatal DAT is associated with increased food intake (23, 24). Moreover, in rats with diet-induced obesity, increased striatal DAT binding is accompanied by reduced hyperphagia (26), however the data about hyperphagia should be interpreted with caution as rats were pair-fed. At present, it is still debated whether higher DAT binding reflects higher or lower extracellular and/or synaptic dopamine levels and this makes the interpretation and functional consequences of the differences in DAT binding between the BF- and the D-group difficult. Extracellular and synaptic dopamine levels depend on tonic and phasic dopamine release and are regulated by the DAT, pre-synaptic dopamine D2-like autoreceptors, postsynaptic dopamine D2-like receptors as well as dopamine synthesis (45). Studies in rats demonstrated reduced striatal DAT after a high fat diet (23, 24) and a DAT gene polymorphism, associated with decreased DAT expression, was accompanied with binge eating (46). In addition, obesity prone rats showed lower striatal DAT expression, reduced postsynaptic dopamine D2 receptor density and exhibited higher motivation for food compared to obesity resistant rats, suggesting that lower DAT favors food intake (25). On the other hand, DAT inhibitors reduce food intake in humans (28). These seemingly contradictory results might be explained by a difference between acute and chronic DAT inhibition and differences in synaptic dopamine levels. The increased striatal DAT availability after weight loss in the BF-group might be an upregulation in response to increased extracellular dopamine levels. From our data, it could be hypothesized that during weight loss, consuming most of the calories in the morning precludes overconsumption and weight regain by positively affecting striatal dopamine signaling and reducing the reinforcement value of omitted food (47). This hypothesis fits with the dopamine reward deficiency hypothesis in obesity (10).

It remains speculative how meal timing per se affects striatal DAT availability. The increase in striatal DAT binding in de BF-group could reflect a direct metabolic feedback of nutrients consumed at a certain time, since DAT expression fluctuates
across the light/dark cycle and dictates the diurnal variation in extracellular dopamine levels and dopamine release (48, 49). Jakubowitz et al. suggested changes in food-intake related hormones as a possible mechanism to explain meal timing effects on body weight as they reported lower overall daily hunger scores and higher satiety scores paralleled by higher overall ghrelin suppression following meal challenges in humans who consumed most of the calories in the morning compared to the evening during their weight loss intervention (7, 8). However, these data could be biased by the difference in caloric content of these test meals, as the meal challenge was performed in subjects who consumed either a large or a small breakfast (8). In addition, we did not observe differences in fasting ghrelin, leptin or hunger scores. Finally, since the dopaminergic system also plays a role in glucose metabolism and is responsive to insulin (50-52), differences in insulin sensitivity might have led to a possible confounding effect, however, we did not find a difference in hepatic or peripheral insulin sensitivity between the BF- and D-group after weight loss (unpublished observation).

Since several studies imply functional differences between the ventral and dorsal striatum in relation to food reward, we performed an additional exploratory voxel-wise analysis to examine whether there was a difference in DAT binding within sub-regions of the striatum. Interestingly, in the BF-group, we found an increase in DAT binding mainly in the ventral striatum, while in the D-group, DAT binding was in particular decreased in the dorsal striatum. This exploratory finding seems interesting since the ventral striatum mediates the liking/hedonic properties of food intake and motivation to eat, whereas the dorsal striatum is predominantly associated with eating habits and cognition (39, 40). However, the role of the ventral versus dorsal striatum in food intake might be less separated than suggested, since it was shown earlier that extracellular dopamine levels increase in the dorsal, but not in the ventral striatum in response to the display of food (47) and consumption of palatable food in humans (53). These similarities in function might be explained by a significant overlap of the ventral and dorsal striatum networks in obesity (54). On the one hand, our findings about the differences in striatal DAT binding in ventral vs dorsal striatal parts should be interpreted with caution because the voxel-based analysis did not survive corrections for multiple comparisons, and detailed follow up studies with a larger sample size are needed to confirm this finding. On the other hand, the voxel-based analysis confirmed the results of the ROI analysis which was performed for DAT binding within the whole striatum. This increases the likelihood that our DAT results are not a false positive finding. Although the overall change in striatal DAT binding in the ROI analysis was 11-15% (see Table 2), this is larger than the test-retest reproducibility of striatal DAT imaging in healthy controls which is approximately 8% (55).

Since we recently showed a trend towards lower hypothalamic SERT binding in obese compared to lean subjects and a reduction in diencephalic SERT binding after a hypercaloric high-fat high-sugar snacking diet (16, 17), we hypothesized that the meal timing effects on weight loss maintenance were through increasing hypothalamic SERT. Surprisingly, we did not find a significant difference in hypothalamic SERT binding between the BF- and the D-group, whereas the change
in thalamic SERT binding differed significantly between the diet groups. Although
the literature about the role of the thalamus in feeding behavior is sparse, several
studies suggested an essential role for the thalamus in the integration of feeding
related input (56). In addition, the thalamus mediates the communication between
the hypothalamus and reward areas (56). The paraventricular thalamic nucleus
(PVT) receives neuropeptide Y (NPY) and orexin input from the hypothalamus
and projects these neurons to the nucleus accumbens (57-59).
In addition, the raphe directly projects to the thalamus and is responsive to
nutrient intake (60). Therefore, meal timing during weight loss diets could
affect the metabolic feedback to the raphe and its serotonergic input to the
thalamus. Based on the effects of the diets on striatal DAT and thalamic, but not
hypothalamic, SERT binding, we conclude that daily timed caloric intake has a
more pronounced effect on brain circuits involved in reward than on the circuits
related to the homeostatic component of food intake.

Our study has some limitations. First, although all subjects were scanned after an
overnight fast, due to the study design, the D- group consumed more calories the
night before the scan compared to the BF-group. Whether this difference in semi-
fasting conditions affected our results is difficult to establish. However, it seems
unlikely that a change in DAT and SERT binding in the opposite direction could
be explained by a difference in calorie consumption at least 12 hours prior to the
scan. Moreover, fasting concentrations of ghrelin and leptin, as well as hunger and
satiety scores did not differ between the BF- and D-group, suggesting a similar
physiological fasting response. Second, all studies were performed in men and
results may differ in women and in different age groups. Third, $^{123}$I-FP-CIT is not a
selective radiotracer as it binds to both SERT and DAT. Consequently, our results
are in need of replication using a selective SERT tracer and positron emission
tomography. Finally, future studies are required to examine the long-term effect
of meal timing on weight loss and brain DAT and SERT binding.

In conclusion, weight loss in obese men who consumed 50% of the daily calories
at breakfast was accompanied by a statistically significant increase in striatal DAT
binding and thalamic SERT binding, while consuming 50% of the daily calories
at dinner showed the opposite. These findings were independent of weight loss,
plasma ghrelin or leptin concentrations, or hunger scores. The increase in striatal
DAT binding might explain the earlier reported favorable effect of meal timing
on weight maintenance after hypocaloric diets (8). Therefore, providing most
calories in the morning during hypocaloric diets might be a promising strategy to
prevent weight regain in obese subjects.
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