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Original article

Assessment of metabolic and hormonal profiles and striatal dopamine D2 receptor expression following continuous or scheduled high-fat or high-sucrose diet in rats

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ABSTRACT

Background: Obesity has reached global epidemic proportions and is associated with serious medical comorbidities and economic consequences. In this preclinical study, we characterized how the palatable diet changed food intake pattern, caloric intake, metabolic profile and hormone levels. We also evaluated the expression of dopamine D2 receptors in the rat striatum.
Methods: Male Wistar rats were fed with either high-fat or high-sucrose diet for 5 weeks according to different feeding regimes: *ad libitum* access or scheduled for a 2-h period each day without caloric restriction during the remainder of the day.
Results: Both diets resulted in an enhancement in caloric intake and total body weight. Post-meal data showed that high-fat diet increased cholesterol, triglycerides and glucose concentrations. Animals fed on high sucrose diet were only hyperglycemic. High-fat diet schedules resulted in the enhancement of leptin concentrations, while increases in blood levels of ghrelin were noted after intermitted high-fat or continuous high-sucrose diet. Finally, we report that only *ad libitum* high-sucrose evoked a significant enhancement of the dopamine D2 receptor protein level and a reduction in the D2 mRNA and receptor affinity in the rat striatum. Independently of the diet type, a similar reduction in dopamine D2 receptor affinity (decrease in KD value) was found in the striatum of rats with intermittent food access.
Conclusion: The findings provide a better understanding of eating disorders and indicate that diet composition leading to obesity induces distinct changes in dopamine D2 receptor signaling in the striatum.

Introduction

Obesity has reached global epidemic proportions and is associated with serious medical comorbidities and economic consequences [1]. The main causes of overweight include an improper diet with excessive food intake, increased consumption of unhealthy food (rich in carbohydrates and fats), inappropriate food patterns or eating habits. The most common biologically-based subtype of obesity is associated with a form of irregular eating habits, like binge eating (BE) episodes, being defined as eating an excessive amount of food in a short time (*e.g.* 2 h daily), reflecting a form of hedonic eating that is not necessarily motivated by caloric need [2]. BE not only leads to unwanted weight gain, together with several peripheral metabolic or hormonal disturbances but is a trigger of a serious mental disease. Increased consumption of sweet and fatty food followed by dietary obesity could be driven by central mechanisms, in particular, the dopaminergic signaling in the midbrain and limbic brain regions. In fact, there is a strong association between consumption of palatable, energy-dense food, overeating and changes of dopamine neurons of the reward-associated pathways [3,4]. Clinical PET imaging studies have shown that obesity is associated with changes in striatal dopamine D2 receptors [4,5].

In the present study, we characterized how the type of the palatable diet and feeding schedule change the weekly food intake pattern, caloric intake and metabolic consequences (blood glucose,

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Group (abbreviation)	Week					
	1	2	3	4	5	6
Control (C)	Habituation	Continuous standard diet	Continuous standard diet	Continuous standard diet	Continuous standard diet	Continuous standard diet
High fat (HF)	Habituation	Continuous fat diet	Continuous fat diet	Continuous fat diet	Continuous fat diet	Continuous fat diet
High fat binge (HFB)	Habituation	Limited fat diet/standard diet	Limited fat diet/standard diet	Limited fat diet/standard diet	Limited fat diet/standard diet	Limited fat diet/standard diet
High sucrose (HS)	Habituation	Continuous sucrose diet	Continuous sucrose diet	Continuous sucrose diet	Continuous sucrose diet	Continuous sucrose diet
High sucrose binge (HSB)	Habituation	Limited sucrose diet/standard diet	Limited sucrose diet/standard diet	Limited sucrose diet/standard diet	Limited sucrose diet/standard diet	Limited sucrose diet/standard diet
		↓ Weight measurements (3 times/week) Serum analyses: lipids glucose	↓ Weight measurements (3 times/week) Serum analyses: lipids glucose	↓ Weight measurements (3 times/week) Serum analyses: lipids glucose	↓ Weight measurements (3 times/week) Serum analyses: lipids glucose	↓ Weight measurements (3 times/week) Serum analyses: hormones lipids glucose Dorsal striatum isolation D2 receptor mRNA D2 receptor binding D2 receptor protein

Fig. 1. Experimental design.

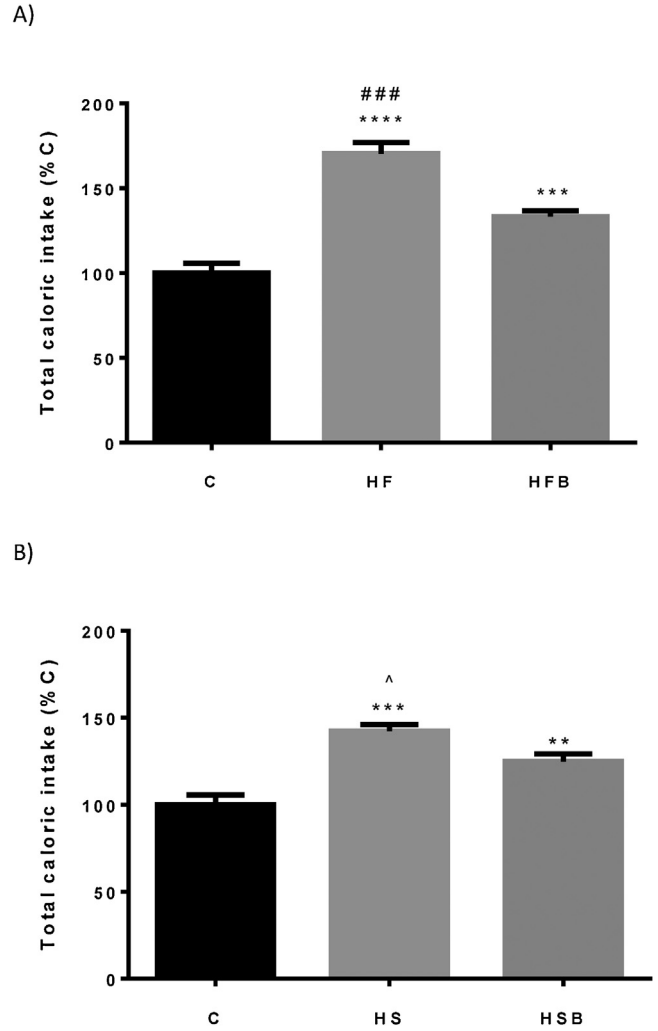

Fig. 2. Total caloric intake following diet schedules over 5 weeks in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM) and showed as % control. N = 8 rats/group. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs.* C; ### $p < 0.001$ *vs.* HFB; ^ $p < 0.05$ *vs.* HSB (Dunnett's test).

cholesterol and triglycerides). We also evaluated final postprandial leptin and ghrelin levels. Finally, we concentrated on the dopamine D2 receptor transcript and protein levels. In our experiments, rats were fed on high-fat (HF) or high-sucrose (HS) diet for 5 weeks either continuously or scheduled for a 2-h period each day without caloric restriction during the remainder of the day. These findings, by uncovering the factors that contribute to animal obesity and BE

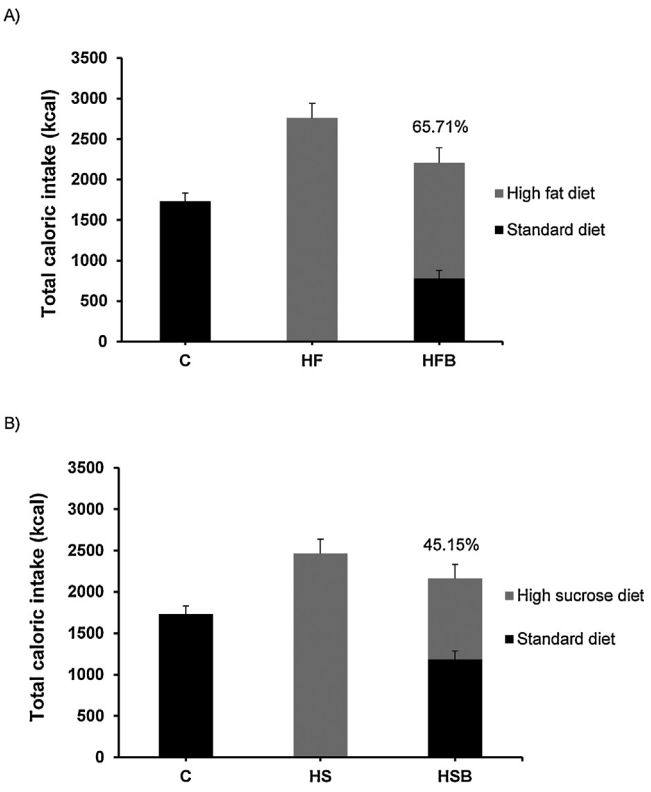

Fig. 3. Total caloric intake following diet schedules over 5 weeks in rats showing calories consumed from control diet and modified diets. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Black bars indicate calories derived from standard diet and grey bars indicate calories derived from modified diet. Percentages above bars refer to calories consumed from modified diet during scheduled feeding time. Data are presented as group means (±SEM). N = 8 rats/group.

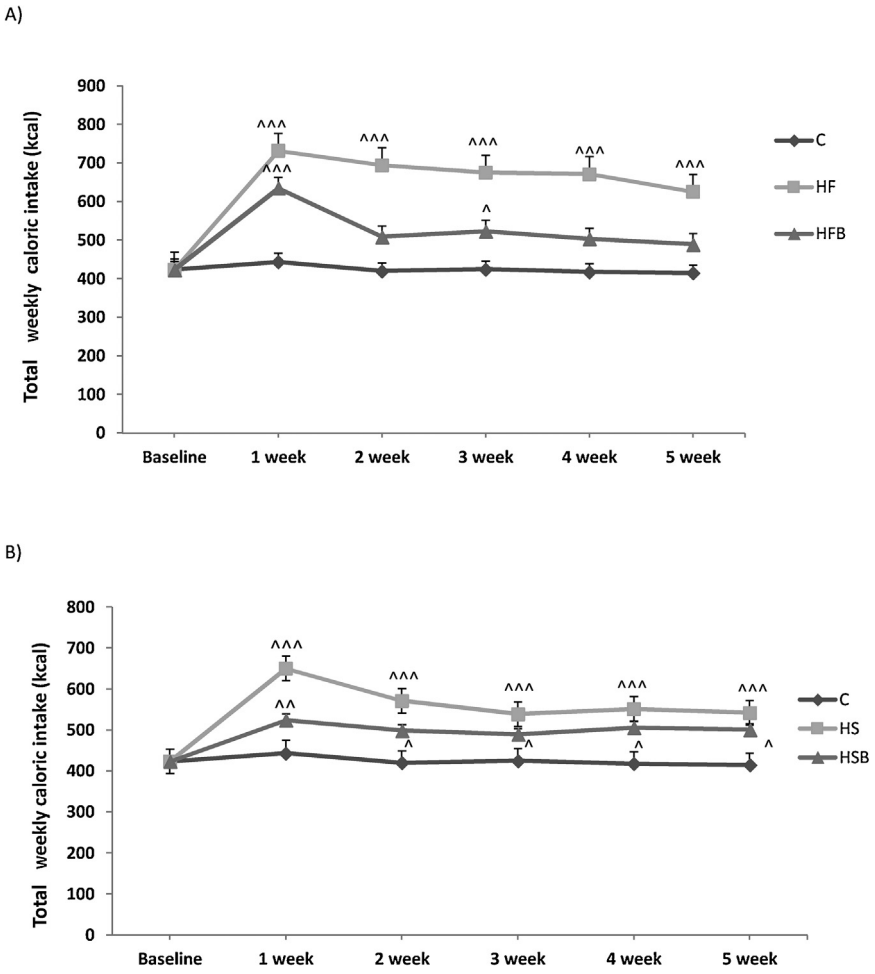

Fig. 4. Total weekly caloric intake during baseline and each diet in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$ *vs.* corresponding C (Newman-Keuls test).

evoked by the type and schedule of diets, provide a better understanding of eating disorders.

Materials and methods

Animals

Male Wistar rats (Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland) with an initial body weight of 290–310 g were housed individually at room temperature 22 °C (±1 °C) and humidity of 50% (±10%) under reversed 12 h:12 h light–dark cycle (light phase: 2.00 p.m.–2.00 a.m.). Before the start food intake schedules, the animals had *ad libitum* access to a standard pellet diet (fat: 3%, protein: 23.08%, carbohydrate: 73.92% composed of 19.5% simple carbohydrates and 54.77% complex carbohydrates; 2.86 kcal/g; Agropol, Poland). Water was freely available during the whole experiment. At the start of the study (week 0) control rats weighted 319 ± 13 g, while following 5-week feeding protocol −392 ± 13 g. All procedures were performed with accordance to the European Union Directive 2010/63/EU and approved by the I Local Commission of Ethics in Krakow.

Experimental design

As shown in Fig. 1, the dietary manipulation continued for 5 weeks during which rats were divided into 5 separate groups (n = 8 rats/group) with the following feeding regimes: (1) continuous access to standard diet for 24 h/day (control; C), (2) continuous access to HF diet (fat: 86.7% including 60% of saturated fatty acid, protein: 11.27%, carbohydrate: 2.03%; 6.16 kcal/g; Morawski TM, Poland), (3) limited access to HF diet for 2 h twice per day during dark phase from 10 a.m.–12 a.m. and 22-h access to standard diet (binge access; HFB), (4) continuous access to HS diet (fat: 3.1%, protein: 17.3%, carbohydrate: 79.6% composed of 58.4% simple carbohydrates and 21.2% complex carbohydrates; 3.65 kcal/g; Morawski TM, Poland), (5) limited access to HS sucrose diet for 2 h twice per day during dark phase from 8 a.m.–10 a.m. and 22-h access to standard diet (binge access; HSB).

During dietary manipulation body weight gain, food intake temporal pattern and biochemical markers (below) were examined at the end of every week for 5 weeks, while hormone levels were examined at the last experimental week; every measurement occurred at 2 p.m. On the last day of experiment, the animals were decapitated and their dorsal striata were collected on dry ice at 8 a.m. The brain samples were stored at -80 °C until use. The experimental procedure is depicted in the Fig. 1.

Body weight gain and food intake measurements

Every day, at defined time (2 p.m.) animals were weighted. In the last week of the acclimatization period (baseline week) and over the 5 weeks of the dietary schedule, food intake was recorded

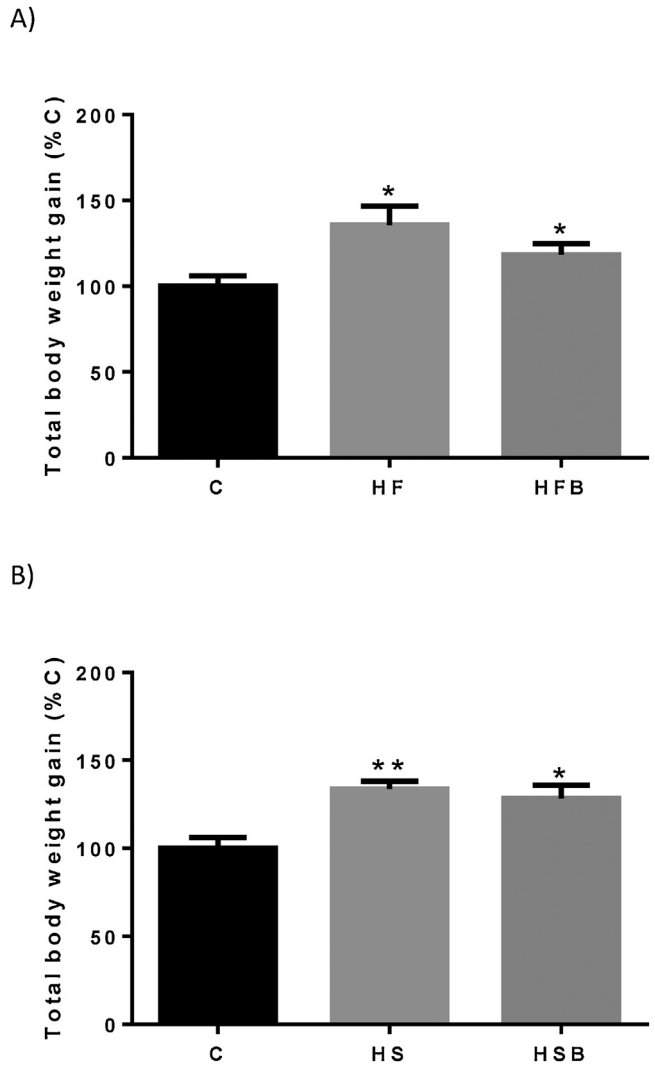

Fig. 5. Total body weight gain following diet schedules over 5 weeks in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. * $p < 0.05$, ** $p < 0.01$ *vs.* C (Dunnett's test).

daily at 10.00 a.m. (for continuous food intake) and twice daily at 8.00 a.m. and 10.00 a.m. (for binge access), and shown as weekly and total caloric consumption.

Blood collection and biochemical analysis

For glucose and lipid (cholesterol and triglycerides) profiles, blood samples were taken from rats' tails (through their incision) at 6 time points across (at 2 p.m.) the dietary manipulation, starting at baseline and at the end of each week. Blood was centrifuged (15 min, 3000 rpm, 4 °C) in order to obtain serum used to assess biochemical markers. Biochemical assays were performed by means of biochemical analyzer Alize with standard kits (Biomérieux, Poland) and control serum (Normal-Seronorm and Pathological-Pathonorm Serum; SERO, Norway). Serum ghrelin and leptin concentrations were determined using a rat/mouse ghrelin total assay (EZRGRT-91 K, Merck Millipore, USA) and rat leptin plate assay (EZRL-83 K, Merck Millipore, USA), respectively.

Nucleic acids and protein extraction

Total RNA and protein from frozen dorsal striatum (18–22 mg) were extracted using Gene MATRIX Universal DNA/RNA/Protein Purification Kit (EURx, Ltd., Poland) following the manufacturer`s protocol. Briefly, brain samples with a ceramic beads (2.8 mm) and 300 μl of lysis buffer were homogenized using the Bioprep-24 Homogenizer (Allsheng, China). The quantity and the quality of RNA was determined by gel electrophoresis as well as spectrometric determinations (NanoDrop ND-1000, Thermo Scientific, USA). RNA was stored at −70 °C until use. Protein samples were stored at −20 °C for later analysis.

Real time-qPCR

The first cDNA strand synthesis was carried out using 500 ng of total RNA and hexamer primers with Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) following to the manufacture`s recommendations. The level of mRNA transcript of dopamine D2 receptor (*Drd2*) was analyzed by real-time PCR and normalize to an endogenous control (*Hprt1*). The 10 μl of reaction mixture consisted of 4.5 μl of cDNA (diluted in 2 times), 5 μl of TaqMan Gene Expression Master Mix (Applied Biosystems, USA) and 0.5 μl of TaqMan assay (Applied Biosystems, USA). Each PCR reaction was run in duplicate on 96-well plate using the Bio-Rad CFX96 PCR system, and standard thermal cycling conditions (50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 and 60 °C for 1 min). The data were analyzed using CFX Manager Software 2.1. To assess the relative expression of *Drd2* gene the comparative delta delta CT method ($2^{-\Delta\Delta Ct}$) was used. Fold changes of >2 or <0.5 were set as cut off values.

Western blot

The protein samples, obtained from the rat dorsal striata during RNA isolation, were homogenized in 2% sodium dodecyl sulphate (SDS) (Sigma Aldrich, USA). The homogenates were boiled at 95 °C for 10 min, and then were centrifuged at 10,000 rpm for 10 min at 4 °C in order to collect supernatants. For the assessment of the protein concentrations in the supernatant, a bicinchoninic acid assay (BSA) was used. The samples were mixed with the sample loading buffer (125 mM Tris−HCl, pH 6.8; 4% SDS, 20% glycerol, 10% 14.3 M mercaptoethanol, 2 mM EDTA, bromophenol blue) in a ratio of 1:1 and subsequently boiled for 5 min. Then, the protein samples were electrophoresed on 12% sodium dodecyl sulfate-polyacrylamide gel (at 100 V for 90 min). Proteins were transferred onto polyvinylidene difluoride (PVDF; Bio-Rad) membranes using a MiniTrans-Blot Cell (Bio-Rad) and transfer buffer (0.30% Tris-Base, 1.44% glycine, 20% absolute methanol) at 110 V at low temperature (with ice) for 60 min. The protein standard of molecular weights was used (Bio-Rad Laboratories, Precision Plus Protein – Dual Xtra Standards). The PVDF membrane was blocked for 1 h at room temperature with a blocking solution. The blocking buffer for the membranes contained 5% milk in tris-buffered saline, TBS (20 mM/l of Tris−HCl, pH 7.5, 150 mM/l NaCl) with 0.1% Tween 20 (200 μl/100 ml TBS). Then the membranes were incubated overnight with a primary polyclonal rabbit dopamine D2 antibody (1:500 dilution in 1% milk, Santa Cruz Biotechnology; sc-9113) and then incubated with 1:6000 dilution of a horseradish-peroxidase-conjugated anti-rabbit secondary antibody (Santa Cruz Biotechnology Inc; sc-2030). The protein bands were visualized using an enhanced chemiluminescence reagent (WesternBright™ Quantum Chemiluminescent HRP Substrate Kit; Advansta corporation). The quantitative analysis of specific bands was performed with the G-Box Syngene using Genesys densitometry software (GeneTools

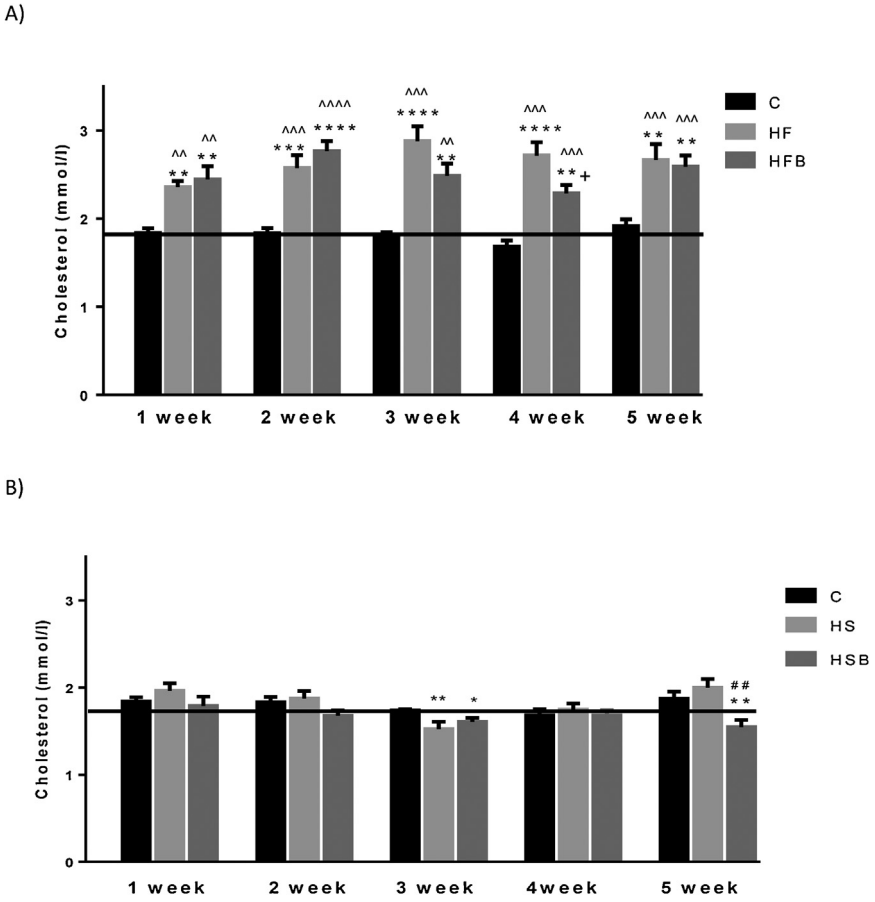

Fig. 6. Blood cholesterol profile following diet schedules over 5 weeks in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. ^^ $p < 0.01$, ^^^ $p < 0.001$, ^^^^ $p < 0.0001$ *vs.* C (Newman-Keuls test); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs.* C; $^{+}$ $p < 0.05$ *vs.* HF; $^{\#\#}$ $p < 0.01$ *vs.* HS (Dunnett's test).

version 4.03; Synoptic Ltd; Cambrigde, England). The expression of dopamine D2 protein was evaluated relative to that of β-actin HRP antibody (1:1000 dilution, Santa Cruz Biotechnology Inc; sc-47778. The quantitative analysis of specific bands was performed with the G-Box Syngene using Genesys densitometry software (GeneTools version 4.03; Synoptic Ltd; Cambrigde, England).

Radioligand binding

The dopamine D2 receptor density and affinity were assessed in the frozen rat dorsal striatal membranes by saturation binding experiments, as previously reported [6] with some modifications. Frozen rat striatum was homogenized in 50 mM Tris−HCl, pH 7.4, 7 mM $MgCl_2$, 1 mM EDTA, and a cocktail of protease inhibitors (Roche Diagnostics, Mannheim, Germany). The membranes were precipitated by centrifugation at 4 °C for 50 min at 50,000×g (3K3 centrifuge, Sigma Laboraxentrifugen, Germany) and washed through rehomogenization in the same buffer once more. The protein concentration was determined for the membrane homogenates by means of BCA Protein Assay (Pierce, Sweden) using as a standard bovine serum albumin (BSA). Pelleted membranes were resuspended to a concentration of 20 mg/ml, immediately used or stored at −80 °C until required. Resuspended membranes were incubated with [^{3}H]spiperone (final concentration 0.25–8.0 nM) in 50 mM Tris buffer (pH 7.4, 37 °C) for 60 min. Nonspecific binding was defined by radioligand binding in the presence of 50 nM (+)-butaclamol (Sigma-Aldrich, USA). The reaction was terminated by rapid filtration through UniFilter 96 GF/B filter microplate and ten rapid washes with 200 μl 50 mM Tris buffer (pH 7.4) were performed using automated Harvester 96 (Tomtec, USA). The UniFilter microplates were dried overnight at 37 °C in dry incubator. The UniFilter bottoms were sealed and liquid scintillator Betaplate Scint (PerkinElmer) was added to each well. The plates were allowed to equilibrate for 1 h and then radioactivity was counted in MicroBeta TriLux 1450 scintillation counter (PerkinElmer) at approximately 30% efficiency.

Statistical analysis

All data were expressed as group mean (± SEM). Biochemical markers (glucose, lipids, hormones) and total weekly caloric intake were analyzed with repeated measures (RM) two-way analysis of variance (ANOVA) for factors diet, week and their interactions or by an one-way ANOVA, followed by *post-hoc* Newman Keuls or Dunnett's test, respectively. Body weight gain and leptin contents were studied with using Student *t*-test. For binding analyses, Bmax and Kd values were calculated from samples reactivity (ccpm) by non-linear regression analysis. Calculations were performed by GraphPad Prism 6.0 software. The criterion for statistically relevance for all groups was established at $p < 0.05$.

Results

Food intake measurements and body weight

The 5-week dietary manipulation changed total caloric intake in rats fed with HF (one-way ANOVA; $F(2,21) = 41.82$, $p < 0.0001$) and HS diet (one-way ANOVA; $F(2,21) = 20.87$, $p < 0.0001$). *Ad*

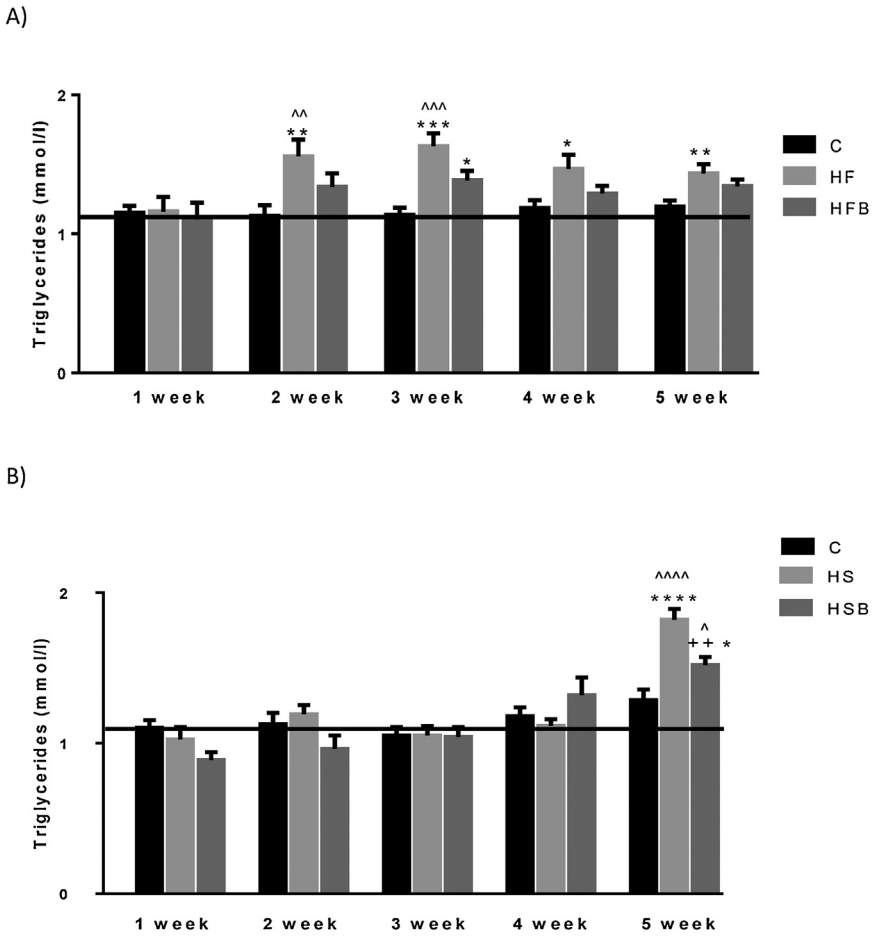

Fig. 7. Blood triglycerides profile following diet schedules over 5 weeks in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$, ^^^^ $p < 0.0001$ *vs.* C (Newman-Keuls test); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs.* C; ++ $p < 0.01$ *vs.* HS (Dunnett's test).

libitum HF diet animals consumed >70% ($p < 0.0001$) more calories than control animals (mean total caloric intake 1732±97.92 kcal), while those on HFB schedule had *ca.* 33% elevated total caloric intake ($p < 0.001$) (Fig. 2, upper panel).

Ad libitum HS diet animals consumed >42% ($p < 0.0001$) more calories than control animals (mean total caloric intake 1732 ± 97.92 kcal), while those on HSB schedule had *ca.* 24% elevated total caloric intake ($p < 0.001$) (Fig. 2, bottom panel).

At the completion of the 6-week experiments, total food intake structure showed differences in schedule-fed rats. HFB-fed rats consumed 65.71% of daily calories from HF diet what displaced calories consumed from control diet from total daily intake, but compensation was incomplete (Fig. 3, upper panel). HSB-fed rats consumed 45.15% of daily calories from HS and to complete total daily intake the rest consumed calories came from control diet (Fig. 3, bottom panel).

As shown in Fig. 4 rats rapidly adapted their feeding behavior to diet schedules. RM two-way ANOVA indicated a significant diet x week interaction effect for animals with both-diet groups (HF diet - $F(5,105) = 5.353$, $p < 0.001$; HS diet - $F(5,105) = 7.836$, $p < 0.001$). *Ad libitum* HF rats had the highest caloric intake in week 1 and this increase lasted to week 6 ($p < 0.001$), whereas HFB rats had the highest caloric intake only in week 1 ($p < 0.001$) *vs.* weeks 2–5 and *vs.* control rats (Fig. 4, upper panel).

For HS diet, only rats with *ad libitum* access to food showed significant enhancement in caloric intake in week 1 (by 24%, $p < 0.001$) and in weeks 4 and 5 (8–13%, at least $p < 0.05$) (Fig. 4, bottom panel).

At the end of the experiment HF- ($F(2,21) = 5.059$, $p = 0.016$) and HS- ($F(2,21) = 8.55$, $p = 0.0019$) diet induced increases in rat body weight gain as compared to control animals (Fig. 5). *Ad libitum* HF rats showed the highest body weight gain (92.97 ± 7.5 g) and they gained 35.7% more than control animals ($t = 2.848$ df = 14, $p < 0.05$). HFB rats had also a significant (>22%) weight gain increase ($t = 2.611$, df=14, $p < 0.05$).

Within animals fed with HS diet, *ad libitum* rats showed the highest body weight gain (91.53 ± 4.14 g) and they gained 33.6% more than control animals ($t = 4.456$, df = 14, $p < 0.001$). In HSB-fed rats body weight gain increased up to 88 (±11) g and significantly differed from control animals ($t = 2.901$, df = 14, $p < 0.05$).

Lipid and glucose profiles

Blood cholesterol, triglycerides and glucose concentrations were measured at baseline, and after each week of dietary manipulation. As shown by RM two-way ANOVA, 5-week feeding regimes resulted in a significant interaction between diet and time on cholesterol profile for HF and HS diets (Fig. 6). In the rats fed with HF or HFB, cholesterol concentration significantly raised following 1 st week food and this increase was left significantly (at least $p < 0.01$) till the 5th week (Fig. 6, upper panel).

In the rats fed with HS or HSB diet, small but significant reduction in cholesterol concentration was apparent at week 3 and week 5 as detected by an one-way ANOVA (Fig. 6, bottom panel).

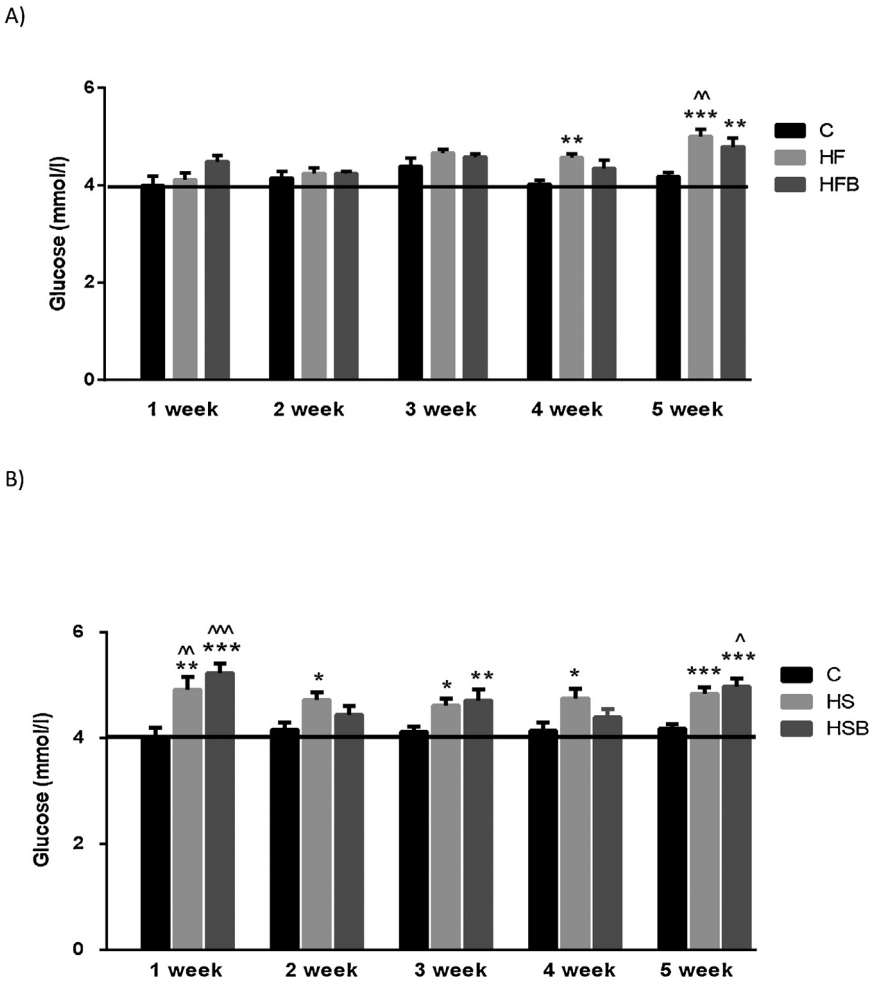

Fig. 8. Blood glucose profile following diet schedules over 5 weeks in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (± SEM). N = 8 rats/group. ^ $p<0.05$, ^^ $p<0.01$, ^^^ $p<0.001$ *vs.* C (Newman-Keuls test); * $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* C (Dunnett's test).

As shown by RM two-way ANOVA, 5-week feeding regime resulted in a significant interaction between diet and time on triglycerides profile for HF and HS diets (Fig. 7). In the rats fed with HF or HFB, triglycerides concentration significantly increased following 2nd and 3rd week (at least $p<0.01$) while an one-way ANOVA showed also changes following 4rd and 5th week in animals fed with HF diet (Fig. 7 upper panel). *Post-hoc* Newman-Keuls test indicated significant rises (at least $p<0.001$) after 4rd and 5th week of consumption of this diet.

In the rats fed with HS or HSB, the significant increases in triglyceride concentration were noted following 5th week of diet exposure (Fig. 7, bottom panel).

RM two-way ANOVA showed a significant week x diet interaction on glucose profile for HF and HS diets (Fig. 8). In the rats fed with HF, glucose level significantly raised following 5th week ($p<0.01$) while an one-way ANOVA showed also increases following 4rd and 5th week in animals fed with HF or following 5th week in rats scheduled for HFB (Fig. 8, upper panel).

In the rats fed with HS or HSB, the significant increases in glucose concentration were observed following 1 st and 5th week of both feeding schedules, while an one-way ANOVA detected also significant rises for all time points of the experiment with HS and after 3rd week with HSB (Fig. 8, bottom panel).

Hormone levels

The leptin and ghrelin were measured at the end of the experiment, following 5-week of dietary manipulation. The leptin levels in control rats reached 13.05 ± 0.16 ng/ml. An one-way ANOVA revealed significant effect on leptin in rats fed with HF or HFB ($F(2,21)=4.443$, $p<0.05$), but not in rats fed with HS diet ($F(2,21)=1.1$) (Fig. 9). The rats consumed HF or HFB showed significant ($p<0.05$, Student t-test) enhancement (by 30% ($t=2.618$, $df=14$) and 27% ($t=2.756$, $df=14$), respectively) of serum leptin levels (Fig. 9, upper panel).

The ghrelin levels in control rats reached 0.33 ± 0.02 ng/ml. An one-way ANOVA revealed significant effect on ghrelin in rats fed either with HF ($F(2,18)=3.08$, $p<0.05$) or HS diet ($F(2,18)=12.45$, $p<0.001$) (Fig. 10). The rats consumed HFB and those exposure to HS diet showed a significant increase (at least $p<0.01$) in terminal ghrelin concentration (Fig. 10).

Dopamine D2 receptor transcript

An one-way ANOVA revealed significant effect on dopamine D2 receptor mRNA in rats fed with HS/HSB diet ($F(2,23)=9.555$, $p<0.001$), but not in rats fed with HF/HFB diet ($F(2,23)$ = 1.213,

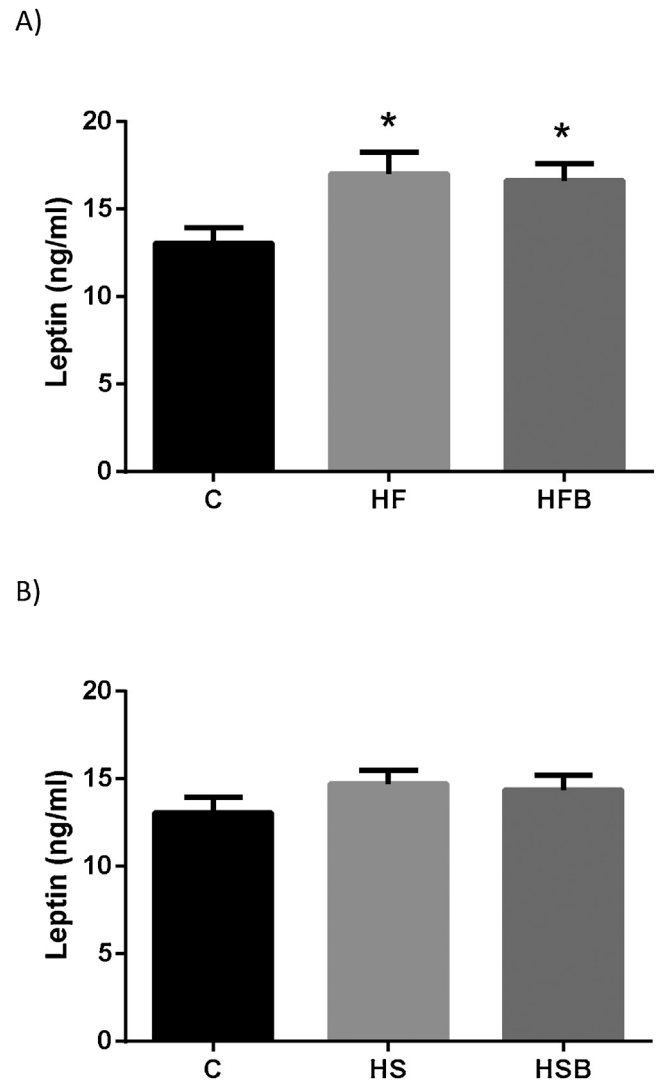

Fig. 9. Terminal blood leptin level following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. * $p < 0.05$ *vs.* C (Student *t*-test).

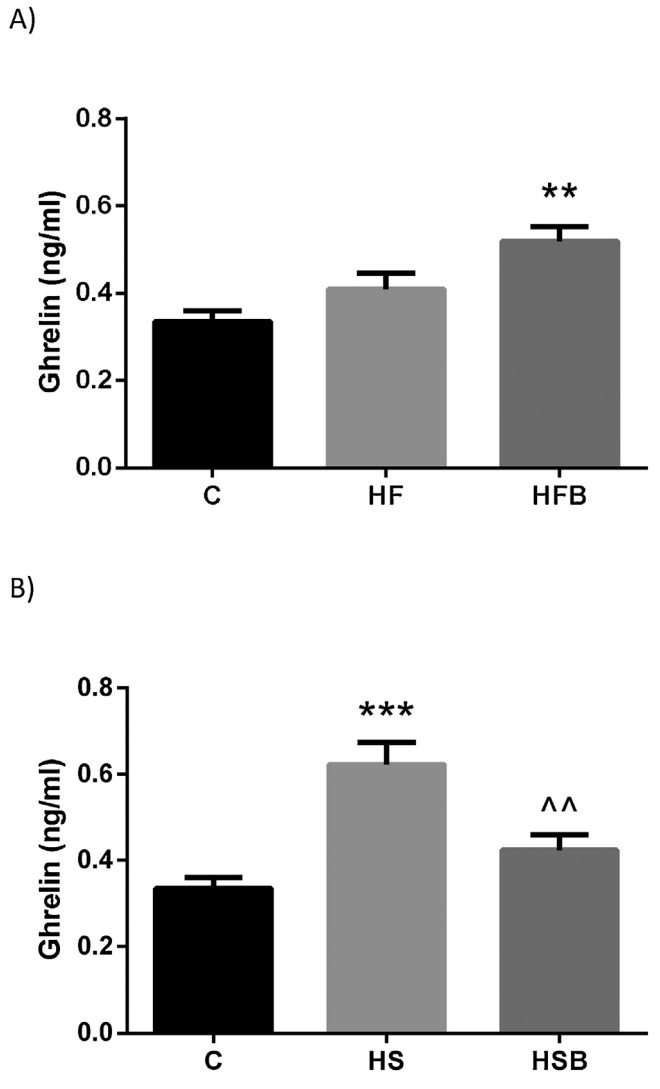

Fig. 10. Terminal blood ghrelin level following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Data are presented as group means (±SEM). N = 8 rats/group. ** $p < 0.01$, *** $p < 0.001$ *vs.* C; ^^ $p < 0.01$ *vs.* HS (Dunnett's test).

$p = 0.317$) (Fig. 11). *Post-hoc* Dunnett's test revealed significant decrease ($p < 0.001$) in dopamine D2 receptor transcript level only in animals with HF diet as compared to control group (Fig. 11, bottom panel). However, the latter changes were below FC cut-off value (Table 1).

Dopamine D2 receptor binding

The dopamine D2 receptor density in control rats reached 102 ± 12 fmol/mg protein. An one-way ANOVA indicated significant changes in the dopamine D2 receptor density in rats fed with HS diet ($F(2,15) = 39.86$, $p < 0.0001$), but not with HF diet ($F(2,15) = 0.2143$, $p = 0.809$). *Post-hoc* analyses revealed that rats consumed HS diet showed significant rise (*ca.* 38%) in dopamine D2 receptor density (Fig. 12, bottom panel).

A significant change in dopamine D2 receptor affinity was demonstrated for animals fed with HF/HFB ($F(2,15) = 10.78$, $p = 0.0013$) and HS/HSB ($F(2,15) = 69.89$, $p < 0.0001$) diets. *Post-hoc* analyses revealed that rats scheduled for HFB or HSB showed significant fall (*ca.* 35 and 50%, respectively), while those with HF diet displayed a 38% rise in dopamine D2 receptor affinity (Fig. 13).

Dopamine D2 receptor protein

As shown on Fig. 13, a significant change in dopamine D2 receptor protein expression affinity was demonstrated for animals fed with HS/HSB diet ($F(2,20) = 25.52$, $p < 0.001$), but not with HF/HFB diet ($F(2,19) = 2.788$, $p = 0.087$). *Post hoc* analyses revealed that rats exposure to HS or HSB diet schedule demonstrated a significant ($p < 0.001$) enhancement in dopamine D2 receptor expression (Fig. 14).

Discussion

We report here that 5-week consumption of HF or HS diet resulted in a significant enhancement (by *ca.* 40–60%) in caloric intake and 20–30% increase in total body weight. Furthermore, weekly analysis of food caloric intake indicates that consumption of both diets reached maximum following a one-week exposure, probably due to

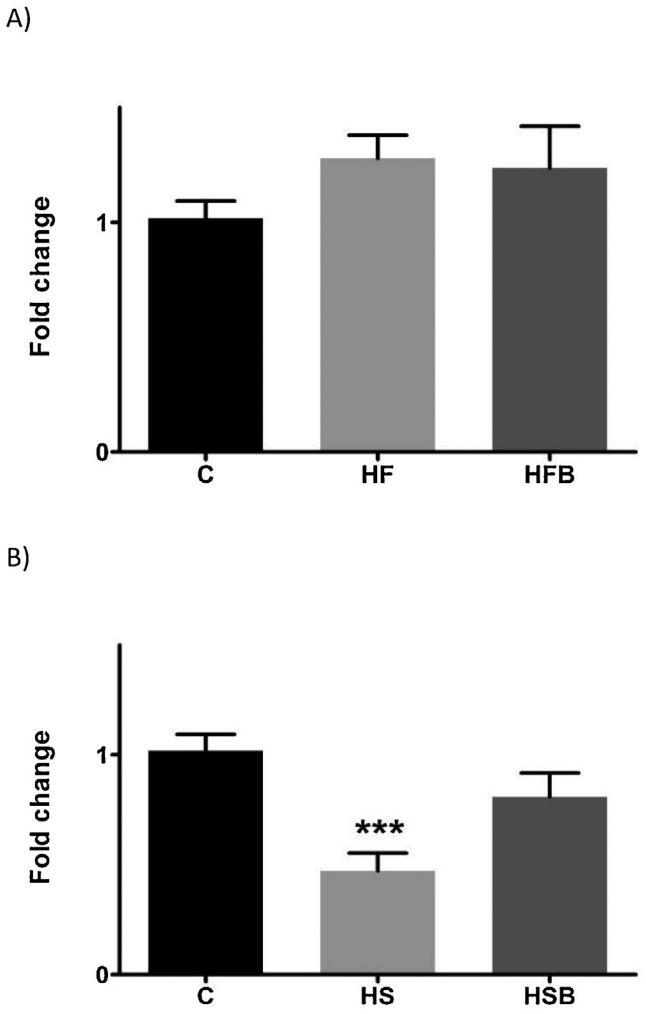

Fig. 11. The level of dopamine D_2 receptor mRNA following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). mRNA level was measured by real-time PCR. Data are presented as group means (±SEM). N = 8 rats/group. *** $p < 0.001$ *vs.* C (Dunnett's test).

novelty and increased palatability of food compared to the control diet, which is in line with previous observations [7,8].

At the same time, rats fed with HFB or HSB had also an increased caloric intake and higher weight gain than control animals. Thus, rats having HSB diet showed almost stable weekly caloric intake from week 1 till week 5, while those consuming HF diet reached maximal caloric intake after the first week of exposure and later displayed a 20% rise in food intake as compared to control animals. The HFB feeding schedule without caloric restriction seems to be effective in promoting hyperphagia, since animals rapidly adapted to their feeding schedules, binged on the palatable diet when it was offered and consumed more calories from the modified diets. On the other hand, the animals with HSB diet consumed over the course of the experiment *ca.* 45% of their average baseline caloric intake. The differences in caloric intake between the diet types consumed intermittently maybe linked to variable animal preferences for nutrients during circadian feeding pattern. As shown recently, rodents prefer carbohydrate-rich meals at dark onset, and protein and fat during the late hours of the dark phase [9]. We also observed that the palatable food intake did not the escalate over the course of experiment, which means that the chosen feeding protocol does not meet the binge eating criterion outlined by Corwin et al. [10].

Table 1
Effects of diet type and schedule on the expression of dopamine D2 receptor mRNA level.

Group	Fold change	*p* value
Control	1.02 ± 0.07	
HF	1.28 ± 0.10	0.0552
HFB	1.24 ± 0.18	0.2828
HS	0.47 ± 0.08	0.0002
HSB	0.80 ± 0.11	0.1334

Abbreviations: HF – high fat; HFB – high fat binge; HS – high sucrose; HSB – high sucrose binge.

Depending on the diet type and feeding schedule, rats showed different metabolic adaptations as determined based on post-meal data. Thus, all animals fed with HF or HFB showed increases in cholesterol concentrations that appeared already following 1 week of diet consumption and lasted till the end of the experiment. The consistent increases in postprandial cholesterol levels may suggest the acute nature of the effect. At the same time, the triglyceride and glucose profiles in rats exposed to HF or HFB diet reflected rather metabolic alteration as they appeared in the 2nd and 5th week of the experiment, respectively.

Post-meal blood of animals fed with HS or HSB was hyperglycemic relative to control rats, and this metabolic outcome

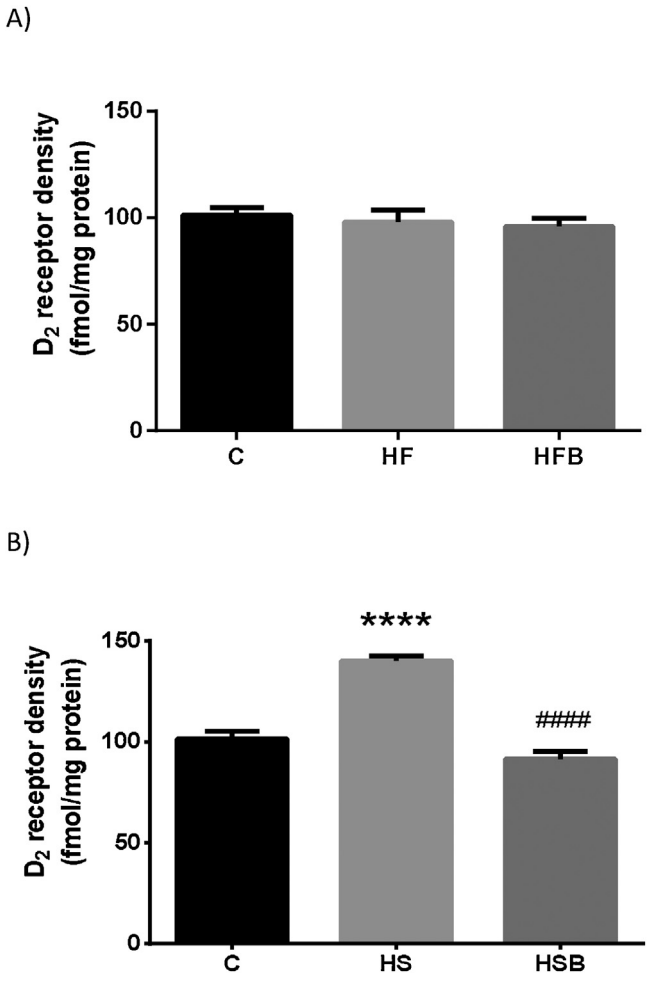

Fig. 12. The dopamine D_2 receptor density (Bmax) in the dorsal striatum following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Density was determined in autoradiographic analysis. Data are presented as group means (±SEM). N = 8 rats/group. **** $p < 0.0001$ *vs.* C; #### $p < 0.0001$ *vs.* HS (Dunnett's test).

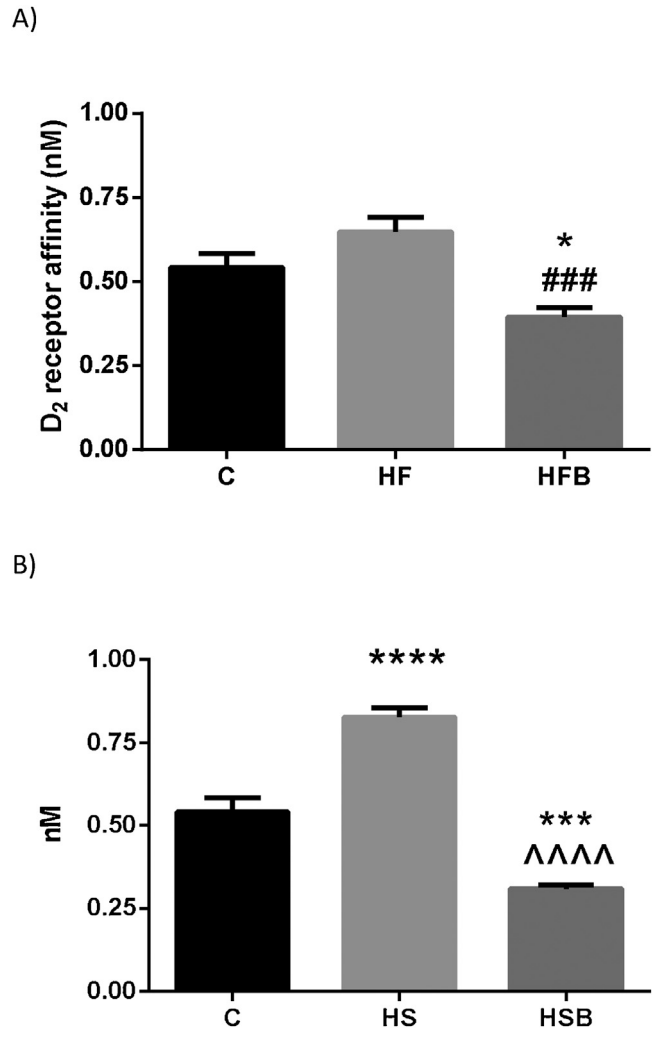

Fig. 13. The dopamine D_2 receptor affinity (Kd) in the dorsal striatum following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Receptor affinity was determined in autoradiographic analysis. Data are presented as group means (±SEM). N = 8 rats/group. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ *vs.* C; ### $p < 0.001$ *vs.* HF; ^^^^ $p < 0.0001$ *vs.* HS (Dunnett's test).

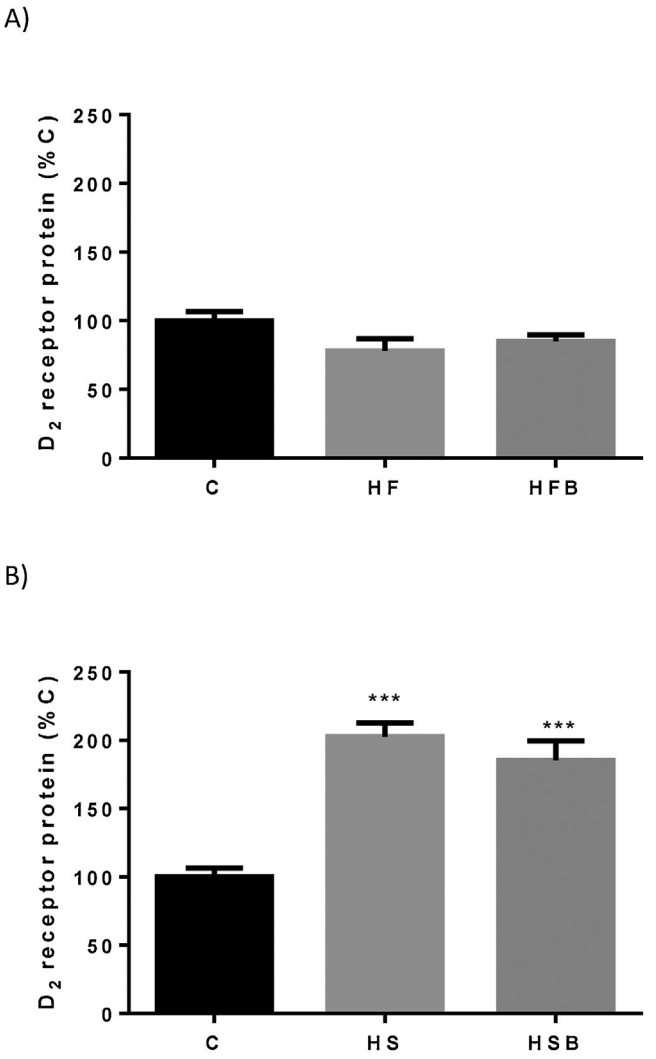

Fig. 14. The D_2 receptor level in the dorsal striatum following diet schedules in rats. Animals were given either standard diet (control; C), continuous fat diet (HF), 2-h daily limited fat diet (HFB), continuous sucrose diet (HS) or 2-h daily limited sucrose diet (HSB). Receptor expression was measured by Western Blot analysis. Data are presented as group means (±SEM). N = 8 rats/group. *** $p < 0.001$ *vs.* C (Dunnett's test).

indicates potentiation of the glucose response to previous meal intake. The highest glucose concentrations in rats were observed following a 1-week dietary manipulation and were linked with a highest caloric intake which might be related to novelty and/or food palatability. Whereas glucose profiles appeared to be mainly influenced by HS diet, the changes in cholesterol and triglyceride concentrations became significant following 3 or 5 weeks of treatment with particular feeding schedule, however, this metabolic adaptation was not parallel to weekly caloric intake, body weight gain or body fat mass.

Food intake, appetite, hunger signals and body weight are regulated by metabolic hormones, including leptin and ghrelin, among other factors. Leptin is produced mainly in fat cells, however, the stomach, heart, placenta and skeletal muscle secrete this hormone, as well. It acts as a satiety hormone and/or as a response to starvation. As found in this study, 5-week HF or HFB diet schedules resulted in the highest concentrations of leptin which reached the same levels in these groups. It should be underlined that despite similar increase in total body weight in rats fed on HF and HS diets, the leptin levels in animals consuming HS diet did not differ from controls was failed to support the putative relationship between obesity and leptin levels in rats fed on this type of diet. In the recently published excellent review by Stanhope [11], the author described metabolic consequences of a simple carbohydrate diet (with high-fructose content), which included dyslipidemia and increased body weight gain without increased post-meal leptin secretion. The latter alterations were found also in the present study at the end of 5-week period of rat feeding with HS diet. It was hypothesized that a simple carbohydrate, especially high-fructose diet consumption promoted weight gain because it did not stimulate leptin secretion which serves as an appetite suppressor [12,13] and is a key regulator of energy homeostasis [14]. In support of the above hypothesis, leptin-deficient subjects exhibited increased hunger and impaired satiety [15].

In this paper, we also demonstrate the changes in the final ghrelin levels in rats fed on HF, HFB, HS or HSB diets. Ghrelin is produced and released mainly in the stomach with small amounts also released by the proximal intestine, pancreas and brain. Blood ghrelin levels are regulated by food intake and they increase before eating and when fasting (in line with increased hunger) while eating reduces concentrations of the hormone. Furthermore, it is postulated that ghrelin could be involved in the long-term regulation of body weight

as a higher body weight in obese subjects is linked with lower fasting ghrelin levels, and significantly reduced postprandial ghrelin suppression compared to normal weight individuals. In the light of the above, it was unexpected to find in this study that final blood levels of ghrelin were not correlated with the weight of rats given continuous access to HF diet as the highest hormone increase was noted in animals with intermittent exposure to the diet. These findings indicate that ghrelin signaling may play a role in increasing consumption of the palatable food when access is restricted. Elevated ghrelin levels were also found in rats that developed binge-like eating following a 2-h daily palatable food exposure (the present paper; [16]). In human studies, the postprandial suppression of ghrelin is also attenuated in obese-BE populations [17–19]. On the other hand, the highest increase (by 85%) in the final ghrelin level was reported in rats with HS diet. It was shown that elevated levels of ghrelin also resulted in an increased weight gain (the present paper, [20]) and greater accumulation of body fat [20]. Moreover, together with an enhancement of the final ghrelin levels, we observed significant weekly increases in blood glucose. This result mimics data on uncontrolled diabetic rats showing hyperphagia and significantly higher plasma glucose and ghrelin concentration than control animals [21,22]. Based on the present findings, we may conclude that blood ghrelin levels are regulated by type of the diet and feeding schedule but not by free access to palatable food. It should be added that we collected post-meal data, so a different pattern of blood hormones before a scheduled diet is plausible.

Human and animal studies revealed the impairment in dopamine signaling in the striatum of obese subjects in whom the increase in the neuronal activity upon eating a palatable meal, observed in healthy subjects, was blunted [4,23]. Hence, with low dopamine release, obese subjects may initiate eating palatable food to compensate for this deficit [24,25]. However, due to the inconsistencies in the literature [26–28], the relationship between dopamine D2 receptor expression and obesity is still unknown. Our current data extend previous observations (above) and show the diet- and feeding schedule-induced alteration in the dopamine D2 receptor signaling. Thus, rats fed with HF did not demonstrate any significant change in striatal dopamine D2 receptor mRNA or protein what supports earlier data on mice with 12-week consumption of HF food as well as on rats with the long-term, calorie-restricted HF diet [1, for review see: 29] in which obesity was not associated with dopamine D2 receptor protein expression in the striatal areas. In contrast to our present and the above cited authors' findings, quantitative autoradiography revealed that 3-week exposure to HF diet increased by *ca.* 30% dopamine D2 receptor binding density in the dorsal and ventral parts of the mouse striatum as well as in the nucleus accumbens shell [30]. The difference between the latter and present data may be linked to the composition and energy density of the HF diets and biochemical methodology used ([^{3}H]raclopride with autoradiography on striatal sections *vs.* [^{3}H]spiperone binding in the whole striatum).

In contrast to the HF diet, 5-week HS diet evoked significant enhancement of the dopamine D2 receptor density, as well as with reduction in the receptor mRNA expression and affinity. Interestingly, independently of the diet type, a similar reduction in dopamine D2 receptor affinity (decrease in KD value) was found in the striatum of rats with intermittent food access. The lower dopamine D2 receptor affinity in the striatum associated with a habit-forming learning may suggest (i) an increased pre-meal endogenous striatal dopamine concentration due to food expectancy (see Methods), or (ii) changes in the receptor protein structure, however, further analyses are needed to define whether reduction in dopamine D2 receptor affinity has implications for the BE. A paper showed a reduction in the dopamine D2 receptor mRNA levels after a chronic intermittent bingeing on a sucrose solution in rats [28]. Of note, the latter authors noted a greater decrease was seen in the rat nucleus accumbens than in the striatum; the former brain area was not studied in the present paper. The recent paper by Adams et al. [26] showed that rats maintained on the long-term, calorie-restricted HF or HS diets, having the same body weight and hormone levels, differed in behavioral and molecular responses. For example, the HF-fed animals, in contrast to those consuming HS diet, were more impulsive, parallel to dopamine D2 receptor reduction in the ventral, but not dorsal, striatum. Our present and Adams et al. [26] studies indicated that diet composition leading to obesity induced distinct changes in dopamine D2 receptor signaling.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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References

[1] Shamseddeen H., Getty JZ, Hamdallah IN, Ali MR. Epidemiology and economic impact of obesity and type 2 diabetes. Surg Clin North Am 2011;91:1163–72.
[2] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.
[3] Stice E, Yokum S, Blum K, Bohon C. Weight gain is associated with reduced striatal response to palatable food. J Neurosci 2016;30:13105–9.
[4] Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cognit Sci (Regul Ed) 2011;5:37–46.
[5] Dunn JP, Kessler RM, Feurer ID, Volkow ND, Patterson BW, Ansari MS, et al. Relationship of dopamine type 2 receptor binding potential with fasting neuroendocrine hormones and insulin sensitivity in human obesity. Diabetes Care 2012;35:1105–11.
[6] Frankowska M, Marcellino D, Adamczyk P, Filip M, Fuxe K. Effects of cocaine self-administration and extinction on D2 -like and A2A receptor recognition and D2 -like/Gi protein coupling in rat striatum. Addict Biol 2003;18:455–66.
[7] Bake T, Morgan DG, Mercer JG. Feeding and metabolic consequences of scheduled consumption of large, binge-type meals of high fat diet in the Sprague-Dawley rat. Physiol Behav 2014;128:70–9.
[8] Boggiano MM, Artiga AI, Pritchett CE. High intake of palatable food predicts binge-eating independent of susceptibility to obesity: an animal model of lean vs obese binge-eating and obesity with and without binge-eating. Int J Obes 2007;31:1357–67.
[9] Tempel DL, Shor-Postner G, Dwyer D, Leibowitz SF. Nocturnal patterns of macronutrient intake in freely feeding and food-deprived rats. Am J Physiol 1989;256: R541-8.
[10] Corwin RL, Avena NM, Boggiano MM. Feeding and reward: perspectives from three rat models of binge eating. Physiol Behav 2011;25:87–97.
[11] Stanhope KL. Sugar consumption, metabolic disease and obesity: the state of the controversy. Crit Rev Clin Lab Sci 2016;53:52–67.
[12] Rezvani R, Cianflone K, McGahan JP, Berglund L, Bremer AA, Keim NL, et al. Effects of sugar-sweetened beverages on plasma acylation stimulating protein, leptin and adiponectin: relationships with metabolic outcomes. Obesity 2013;21:2471–80.
[13] Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. J Clin Endocrinol Metab 2004;89:2963–72.
[14] Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr 2009;89:980S–4S.
[15] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–95.
[16] Cottone P, Sabino V, Steardo L, Zorrilla EP. Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. Neuropsychopharmacology 2008;33:524–35.
[17] Geliebter A, Gluck ME, Hashim SA. Plasma ghrelin concentrations are lower in binge-eating disorder. J Nutr 2005;135:1326–30.
[18] Monteleone P, Fabrazzo M, Tortorella A, Martiadis V, Serritella C, Maj M. Circulating ghrelin is decreased in non-obese and obese women with binge eating disorder as well as in obese non-binge eating women, but not in patients with bulimia nervosa. Psychoneuroendocrinology 2005;30:243–50.
[19] Troisi A, Di Lorenzo G, Lega I, Tesauro M, Bertoli A, Leo R, et al. Plasma ghrelin in anorexia, bulimia, and binge-eating disorder: relations with eating patterns

and circulating concentrations of cortisol and thyroid hormones. Neuroendocrinology 2005;81:259–66.
[20] Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908–13.
[21] Dong J, Peeters TL, De Smet B, Moechars D, Delporte C, Vanden Berghe P, et al. Role of endogenous ghrelin in the hyperphagia of mice with streptozotocin-induced diabetes. Endocrinology 2006;147:2634–42.
[22] Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H, Oikawa S. Role of ghrelin in streptozotocin-induced diabetic hyperphagia. Endocrinology 2002;143:4934–7.
[23] Volkow ND, Wang GJ, Tomasi D, Baler RD. The addictive dimensionality of obesity. Biol Psychiatry 2013;73:811–8.
[24] Davis C, Levitan RD, Reid C, Carter JC, Kaplan AS, Patte KA, et al. Dopamine for "wanting" and opioids "for liking": a comparison of obese adults with and without binge eating. Obesity 2009;16:1220–5.
[25] Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience 2009;159:1193–9.
[26] Adams WK, Sussman JL, Kaur S, D'Souza AM, Kieffer TJ, Winstanley CA. Long-term, calorie-restricted intake of a high-fat diet in rats reduces impulsive control and ventral striatal D2 receptor signaling – two markers of addiction vulnerability. Eur J Neurosci 2015;42:3095–104.
[27] Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci 2010;13:341–635.
[28] Spangler R, Wittkowski KM, Goddard NL, Avena NM, Hoebel BG, Leibowitz SF. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. Brain Res – Mol Brain Res 2004;124:134–42.
[29] Rospond B, Szpigiel J, Sadakierska-Chudy A, Filip M. Binge eating in pre-clinical models. Pharmacol Rep 2015;67:504–12.
[30] South T, Huang XF. High-fat diet exposure increases dopamine D2 receptor and decreases dopamine transporter receptor binding density in the nucleus accumbens and caudate putamen of mice. Neurochem Res 2008;33:598–605.