



Cafeteria diet and caloric restriction affect metabolic but not behavioral characteristics in male Wistar rats

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ABSTRACT

This study aimed to evaluate the effects of a cafeteria diet and caloric restriction on behavioral and metabolic profiles of adult male Wistar rats. The rats were randomly divided into three groups ($n = 12/\text{group}$) and from 10 weeks of age fed either ad libitum standard rat chow (control group), ad libitum cafeteria diet in addition to standard chow (diet-induced obesity (DIO) group) or kept on caloric restriction (at 85% weight of controls; restricted group) for a period of 12 weeks. Body weight was assessed twice per week and glucose levels were measured at three times during the 12-week period. At week 11 the animals were behaviorally profiled using the multivariate concentric square field™ (MCSF) test. After 12 weeks of diet the animals were euthanized, blood collected, relative organ weights were assessed and plasma or serum levels of insulin, glucose, and lipid profile were measured. The DIO group gained 23% more weight than the control group ($p < 0.001$) and increased adipose tissue weight in comparison to the control ($p < 0.001$) and restricted ($p < 0.001$) groups. Glucose was significantly increased ($p < 0.001$) only during the second measurement at week 7 and insulin levels were elevated in the DIO group compared to controls and restricted groups ($p < 0.01$; $p < 0.001$, respectively). Plasma cholesterol levels were reduced for both DIO ($p < 0.01$) and restricted ($p < 0.001$) groups relative to controls. Adiponectin and leptin levels were higher for the DIO group in comparison to both the control ($p < 0.001$; $p < 0.05$) and restricted ($p < 0.001$; $p < 0.001$) groups. Thus, the two diets led to significant changes in body weight gain, adiposity, and metabolism. However, they did not alter the behavioral profiles in the MCSF test, suggesting that activity, exploration, risk assessment, risk taking or shelter seeking remained unaffected by the dietary interventions. The current findings suggest that an increase or reduction in energy intake resulted in no behavioral effects, despite the accompanying glycemic alterations potentially related to diabetes development.

1. Introduction

In recent decades, the escalating prevalence of obesity has become a global health crisis, affecting over 1 billion people worldwide with profound impact on health [1]. Recognizing the complex interplay between dietary habits, metabolic parameters, hormonal regulation, and the role of the brain is crucial for developing treatment strategies to address obesity. Experiments in animals enable studies of mechanisms behind the development of obesity, its complications, and identification of potential treatment targets.

Cafeteria-diet induced obesity in rodents represent a powerful and widely utilized tool, providing useful insights into the connections

between diet, metabolism and behavior [2]. The cafeteria-diet induced obesity model is designed to mimic human habits of consuming diets rich in energy-dense, palatable foods, often high in fats and sugars. This model goes beyond the simplicity of single-nutrient studies, highlighting the complexity of real-world dietary patterns and their impact on both physical and mental health [2].

Excess adiposity is associated with insulin resistance, where the cells become less responsive to insulin, resulting in elevated blood glucose and insulin levels [3]. Moreover, obesity is linked to dyslipidemia, marked by increased triglycerides and cholesterol levels. This so-called metabolic syndrome can lead to an increase risk of cardiovascular diseases and type 2 diabetes mellitus [4,5]. Adipose tissue produces factors

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that affect whole-body metabolism, such as the adipokines leptin and adiponectin, which are altered in obesity [6]. Leptin, a hormone crucial for regulating energy balance and suppressing appetite, becomes less effective due to leptin resistance, further contributing to weight gain [3]. Adiponectin, another hormone produced by adipose tissue, acts by sensitizing peripheral tissues such as the liver and muscle to insulin and its levels are often reduced in obesity, contributing to insulin resistance and the metabolic syndrome [7].

The link between obesity and mental health has been extensively investigated [8,9,10,11], extending beyond the physical aspects of weight management. Obese individuals often face multiple burdens, struggling not only with the challenges of weight but also with significant mental health effects [12], such as stress and anxiety.

Current animal behavioral tests often share a common feature of assessing a limited behavioral repertoire. To extend the assessed behavioral repertoire more than one test is often combined in a test battery. This approach poses challenges, as experiences from individual tests can influence the results of subsequent tests within a test battery, leading to carry-over effects [13,14]. To address this issue, the multivariate concentric square field™ (MCSF) test was developed. The MCSF enables animals to express a broad behavioral repertoire in a single trial to generate a behavioral profile [15,16,17].

Despite extensive previous research, there is still no consensus on how dietary interventions specifically impact physiology and behavior, particularly when comparing free-feeding controls to rats under caloric restriction. This study aimed to evaluate the impact of a cafeteria-diet on the behavioral and metabolic profiles of rats compared to animals on a standard diet (controls) and rats undergoing caloric restriction.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats (RccHan:WI, Envigo, Horst, the Netherlands, $n = 36$) were used. All animals were pair-housed in transparent cages of type IV ($59 \times 38 \times 20$ cm) with raised lids containing wood chip bedding (Tapvei Estonia OÜ, Estonia). A plastic tunnel (Scanbur AB, Sweden), two wooden sticks (Tapvei Estonia OÜ, Estonia) and Bed-r-Nest pucks (Scanbur AB, Sweden) were used for enrichment purposes. The cages were kept in an animal room with a reversed light/dark cycle (lights off from 7:00 to 19:00) and constant temperature (22 ± 1 °C) and humidity ($50 \pm 10\%$).

All animal experiments were approved by the Uppsala Animal Ethical Committee (permit number 5.8.18–12,996/2022) and followed the guidelines of the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS 2018:1192) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU).

2.2. Experimental design

The outline of the experiment is visualized in Fig. 1. Upon arrival, at eight weeks of age, the animals were left undisturbed for two weeks for acclimatization and adaptation to the reversed light/dark cycle [18]. Thereafter the animals were individually ear marked. All animals were randomized into three groups: control group, cafeteria diet-induced obesity (DIO) group, and the restricted group ($n = 12$ /group). The control group was given standard rat pellets (ssniff Spezialdiäten GmbH, Germany) ad libitum. The diet of the DIO group consisted of simultaneous ad libitum access to standard rat pellets, chocolate balls (Delicato, Sweden), cheese doodles (OLW, Sweden) and roasted, salted peanuts (Xtra Coop, Sweden) spread out in the cage. The cafeteria diet was changed daily to ensure that all food options remained available. The macro nutrient composition of the diets is shown in Table 1. The restricted group received an amount of standard rat pellets that was adjusted as needed to maintain the animals at 85% of the body weight of the control rats. They were fed at the beginning of the dark phase, and the chow was spread out in the cage to secure access for both individuals in a pair. In the event of a disturbance in the weight difference between the control and restricted groups, adjustments were made to the amount of food provided to the restricted group. All animals had access to water ad libitum. All animals were weighed twice per week and the cages were changed every Friday. The different diets were continued during behavioral testing to avoid any withdrawal effects that might have occurred following discontinuation [2].

2.3. Behavioral profiling in the MCSF test

The MCSF test has been described in detail elsewhere [15,16,17]. Briefly, it involves a square field (100×100 cm) with partitioning walls, creating distinct zones (Supplementary Fig. S1). Positioned in the center of the arena is a smaller square field referred to as the center (70×70 cm). For analysis, a central circle (CTRCI; 25 cm in diameter) is introduced in the center of the smaller squared field, for assessment of risk-taking versus thigmotaxic behavior. Surrounding the center are three accessible corridors leading to various zones: a sheltered area called the dark corner room (DCR) where the animals can seek shelter, an elevated platform with a hole board with two nose-poke holes

Table 1

The macro nutrient composition of the standard rat chow and cafeteria diet food items per 100 g of food.

Food	Energy (kJ)	kcal	Carbohydrates (g)	Fat (g)	Protein (g)
Rat chow	1300	310	34.5	3.4	19.1
Chocolate balls	2000	480	49.0	30.0	5.0
Cheese doodles	2200	520	53.0	30.0	10.0
Peanuts	2469	596	13.0	46.0	28.0

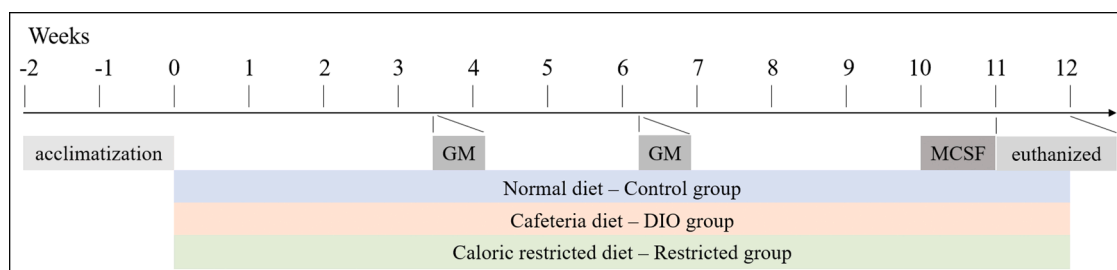


Fig. 1. Experimental outline. Body weight was measured twice per week except the last two weeks when body weight measures were taken once a week. Directly after euthanasia glucose was measured from the trunk blood, and blood and organs were sampled. Abbreviations: DIO, diet-induced obesity; GM, glucose measurement; MCSF, multivariate concentric square field™.

(hurdle) as explorative incentive, and an elevated brightly lit bridge construction as another risk area. The initial part of the bridge, the slope and the bridge entrance associated with risk-assessment behavior, is accessible from the corridor in between the hurdle and the slope. Lighting conditions (lux) within the arena are as follows: center and corridors < 30, DCR < 0.5, and bridge > 500.

The test was conducted by a female experimenter blind to dietary groups. To avoid first-in-line effects, a male rat of the same age and not included in the experiment, was allowed to explore the arena at the beginning of each test day. The experimental animal to be tested was started by being placed in the center facing the wall without a corridor entry and permitted to explore the arena for 20 min. Between animals, the arena was wiped with 10% ethanol and left to dry to spread olfactory cues from the previous animal.

EthoVision XT 15 (Noldus Information Technology, Wageningen, the Netherlands) was used to automatically record total distance (cm) moved and mean velocity (cm/s) in the arena and the specific zones, as well as latency (L, the time (s) to first visit a zone), frequency (F) of visits, duration (D, s) as well as duration per visit (D/F, s) in the specific zones. Manual scoring encompassed the frequency of climbing, rearing, grooming and stretched attend postures (SAPs). If the software encountered challenges in recognizing animal movements, manual recording of specific parameters was made. Furthermore, calculations were made to obtain the total activity (sum of all frequencies), and the frequency of visits, duration (D, s) as well as the duration per visit (D/F, s) in all corridors [16,17].

2.4. Euthanasia, trunk blood and tissue collection

At end of the study, rats from the different groups were alternated, subjected to a brief anesthesia (Isoflurane Baxter, 4%) until unconscious and euthanized by decapitation. Anesthesia was used to ensure secure handling of the DIO group due to size. Euthanasia took place between 1 and 10 h after the beginning of the dark phase. Trunk blood was collected and the brain, liver, adrenals, testis, epididymal white adipose tissue (eWAT) and the right soleus muscle were immediately removed, weighed and frozen in -20 °C isopentane (brains) or liquid nitrogen (remaining tissues). Samples were then stored in -80 °C until further analyses.

2.5. Measurement of whole blood glucose and plasma insulin, leptin and adiponectin

Glucose levels were measured by a CONTOUR®XT glucometer (Ascensia Diabetes Care Holdings AG, Basel, Switzerland) at weeks 4 and 7 after the start of the dietary intervention from whole blood harvested from the hind leg lateral saphenous vein, and from trunk blood after euthanasia. Blood samples at weeks 4 and 7 were collected between 1 and 6 h after the beginning of the dark phase.

Quantification of total adiponectin in plasma was performed by the enzyme-linked immunosorbent assay (ELISA) Quantikine® Rat Total Adiponectin/Acrp30 immunoassay (RRP300, R&D Systems, Minneapolis, MN, USA) kit in accordance with the manufacturer's protocol, with a sensitivity limit of 0.004 ng/mL.

Plasma insulin and leptin levels were simultaneously quantified using the Milliplex® MAP Rat Metabolic expanded magnetic bead panel (#RMHE-120 K; EMD Millipore, Billerica, MA, United States). The assay was performed according to the manufacturer's instructions in a 96-well plate, and all analyses were run in duplicates. Standard curves were generated using standards containing known concentrations of the analytes, as provided by the manufacturer. Also, two Quality Control samples with known concentrations of the analytes were included to ensure accuracy. The Median Fluorescent Intensity (MFI) for all standards, controls and unknowns was measured on the Bio-Plex 200 (Bio-Rad, CA, USA) instrument. The standard curve for each analyte was constructed using the 5-parameter logistic (5-PL) curve fitting method.

Unknown samples were then plotted against the respective standard curve to determine their concentrations. The minimum limit of detection for insulin was 28.6 pg/mL and for leptin 9.7 pg/mL, both with an intra-assay of less than 10% and an inter-assay of less than 20%.

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the formula $HOMA-IR = (\text{Insulin } (\mu\text{U/mL}) \times \text{Glucose (mmol/L)})/22.5$. This calculation provides an estimate of insulin resistance, with higher HOMA-IR values indicating greater insulin resistance and potential metabolic dysfunction. This method was used to evaluate differences in insulin sensitivity among different diet groups. Insulin and glucose levels were measured from blood samples collected after euthanasia.

2.6. Measurement of serum cholesterol, triglycerides and fructosamine

Serum cholesterol, triglycerides and fructosamine levels were analyzed with Beckman Coulter DxC 700AU automatic biochemistry analyzer (Beckman Coulter, Brea, CA, US) with reagents for cholesterol and triglycerides from Beckman Coulter, and fructosamine reagents from Sentinel Diagnostics (Milano, Italy). The measurement ranges were as follows: cholesterol 0.5–18 mmol/L, triglycerides 0.1–11.3 mmol/L and fructosamine 11–1000 μmol/L. All analyses were run in duplicates and conducted at the Clinical Chemical Laboratory at the SLU University Animal Hospital (UDS).

2.7. Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics version 28 software. GraphPad Prism 10 was utilized for both statistical analyses and figure creation. Data were considered statistically significant at $p < 0.05$. Normality was assessed by using the Shapiro-Wilk W test.

Most of the descriptive parameters from the MCSF test were not normally distributed; hence, non-parametric statistics were used. The Kruskal-Wallis test by rank was used for comparing the dietary groups, with multiple comparisons used for pairwise comparisons. Data are expressed as median, lower and upper quartiles. Activity over time was assessed using the Friedman test followed by the Wilcoxon matched pairs test when appropriate.

The data from the MCSF were also analyzed using a rank-order procedure, i.e. the trend analysis [16]. The trend analysis groups parameters into the functional categories general activity (total activity, number of visits to the corridors, duration per visit to the corridors [reversed], number of visits to the center and the total distance moved in the arena), exploratory activity (duration in the corridors [reversed], duration in the center [reversed], duration in the hurdle, number of rearings and nose-pokes in the hole board holes), risk assessment (number of SAPs, number of visits to, duration in, and duration per visit in the slope, and number of visits to, duration in, and duration per visit in the bridge entrance), risk-taking behavior (number of visits to, duration in and duration per visit on the bridge and number of visits to, duration in, and duration per visit to the CTROI) and shelter-seeking behavior (number of visits to, duration in, and duration per visit to the DCR). The dietary groups were compared using the Kruskal-Wallis test by rank with multiple comparisons used for pairwise comparisons.

All morphometric and metabolic parameters were log-transformed to achieve normality, and then analyzed using one-way analysis of variance (ANOVA) with multiple comparisons, corrected by the Benjamini, Krieger and Yekutieli test. Data are expressed as mean ± standard deviation (SD). Repeated measures ANOVA was used to analyze changes in body weight over time within each group. Pearson correlation was used to examine the relationships between body weight, eWAT and metabolic variables.

To explore differences between the dietary groups and identify key variables contributing to these differences, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA (version 17.0.2, Sartorius Stedim Data Analytics AB, Umeå, Sweden).

OPLS-DA is a multivariate method that visualizes the separation between predefined groups (dietary groups) while identifying the variables that contribute most to this separation. Metabolic parameters were used as input variables. OPLS-DA was then applied to model the relationships between the variables and the dietary groups. The first two components were used for visualization.

3. Results

3.1. Body weight

Body weight is shown in Fig. 2A-B. There were no differences in body weight between the groups at the start of the experiment. The DIO group had gained significantly more weight ($p < 0.05$) than the controls already after one week on the diet, while the restricted group had gained significantly less weight compared to both the control ($p < 0.05$) and the DIO ($p < 0.001$) group. The significant weight differences between the groups were maintained throughout the experiment until final sampling [F (2, 33) = 67.67, $p < 0.001$]. At the end of the experiment, the DIO group had a mean body weight of 574.8 ± 43.1 g and had gained 23% more weight ($p < 0.001$) than the controls (485.0 ± 27.5 g), while the restricted rats (424.2 ± 21.0 g) had gained 17% less weight than the controls ($p < 0.001$).

3.2. Behavioral profiling

No differences between the groups were observed for any descriptive parameter in the 20-min MCSF test (Supplementary Table S1). Neither did the trend analysis reveal differences between the groups for the functional categories general activity ($H = 1.44$, $p = 0.486$), exploratory activity ($H = 1.4$, $p = 0.497$), risk assessment ($H = 0.66$, $p = 0.720$), risk taking ($H = 0.15$, $p = 0.930$) or shelter seeking ($H = 0.58$, $p = 0.750$; Fig. 3). Finally, no differences between the groups in activity over time were revealed (Supplementary Fig. S2). A high degree of variability was observed within each group, indicating substantial individual differences in behavioral responses. However, no association between behavioral profiles and metabolic parameters were observed (data not shown).

3.3. Tissue weights

Tissue weights are shown in Table 2. No group differences were found for brain weight nor the weight of the soleus muscle. However, the liver weights were significantly different between the groups [F (2, 31) = 12.35, $p < 0.001$] with post-hoc analysis revealing that the restricted group had a lower liver weight in comparison to both the control ($p < 0.001$) and the DIO group ($p < 0.001$). The eWAT weight was also significantly different between the groups [F (2, 31) = 63.71, $p < 0.001$], with the DIO group having a higher eWAT weight in comparison to both the control ($p < 0.001$) and restricted groups ($p < 0.001$).

3.4. Metabolic measurements

The cafeteria diet induced significant effects on serum glucose levels (Table 3) after seven weeks of diet [F (2, 33) = 8.92, $p < 0.001$]. Post-hoc analysis revealed that the DIO group had 12% and 17% higher glucose compared to the control ($p < 0.001$) and restricted ($p < 0.001$) groups, respectively. No significant difference was found for fructosamine levels (Table 3) between the groups. Plasma insulin levels (Table 3) were significantly different between groups [F (2, 33) = 8.92, $p < 0.001$]. Post-hoc analysis showed that the DIO group had around 40% and 65% higher plasma insulin compared to the control ($p < 0.01$) and restricted ($p < 0.001$) groups, respectively, while there was no difference between the control and restricted group. HOMA-IR differed between the groups [F (2, 32) = 9.50, $p < 0.001$] and the post-hoc analysis showed that the DIO group had a significantly higher HOMA-IR level in comparison to the control ($p < 0.01$) and restricted ($p < 0.001$) groups (Table 3).

Plasma leptin and adiponectin (Table 3) levels differed between groups [leptin: F (2, 33) = 17.89, $p < 0.001$; adiponectin: F (2, 33) = 33.08, $p < 0.001$]. Post-hoc analysis showed that the DIO group had higher levels of leptin and adiponectin in comparison to both controls and restricted rats (leptin: $p < 0.05$; $p < 0.001$, adiponectin: $p < 0.001$; $p < 0.001$, respectively). Moreover, the restricted group had significantly lower leptin levels in comparison to controls ($p < 0.01$). Adiponectin to leptin (AL) ratio (Table 3) showed differences between the groups [F (2, 33) = 965, $p < 0.001$]. Post-hoc analysis showed that both the DIO and the restricted groups had significantly higher AL ratio than the controls

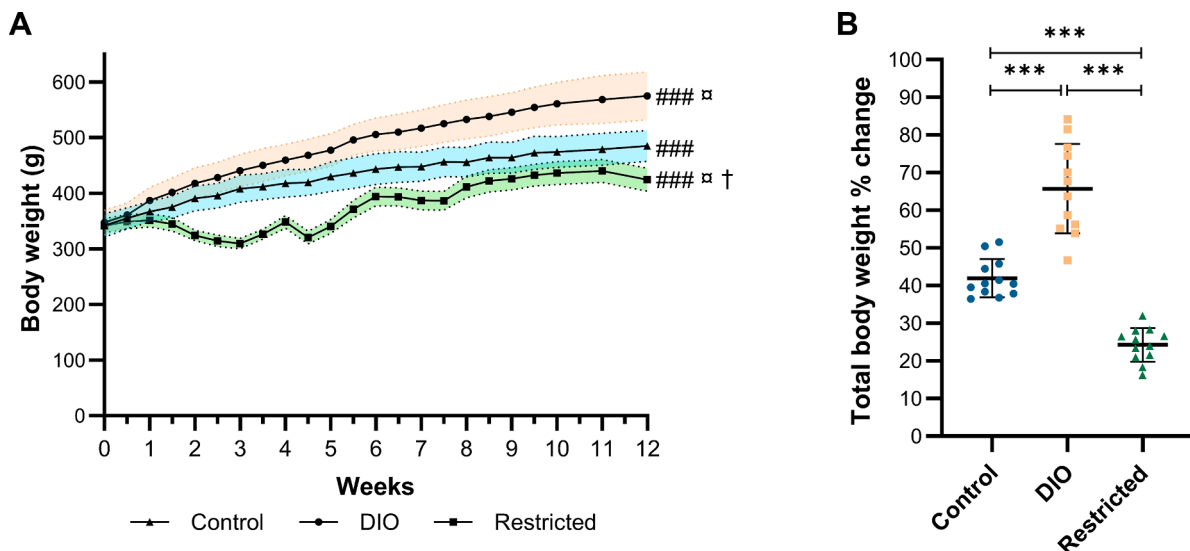


Fig. 2. Body weight (g; A) and total body weight change (%; B) during the 12 weeks of diet for the control, diet-induced obesity (DIO) and restricted groups ($n = 12$ /group). Body weight was measured twice per week except the last two weeks when body weight measures were taken once a week. Data represent mean \pm SD. A significance difference between groups was observed at week one and remained for the rest of the experiment. \square $p < 0.05$ compared to the DIO group (one-way ANOVA with multiple comparisons); $###$ $p < 0.001$ within group differences relative to the start (repeated measures ANOVA); $***$ $p < 0.001$ (one-way ANOVA with multiple comparisons).

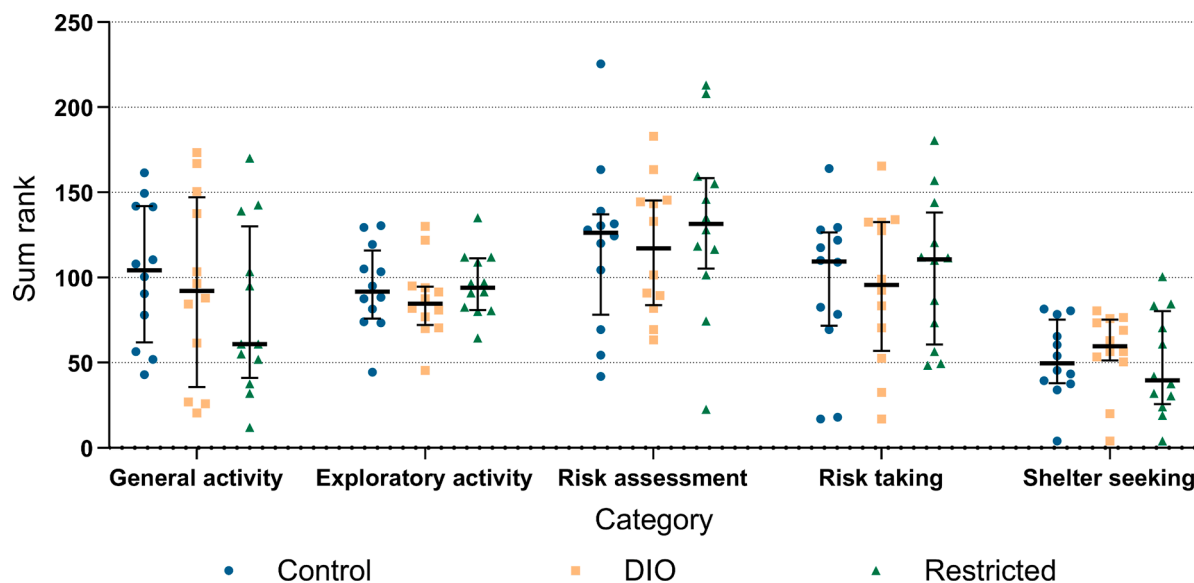


Fig. 3. The sum rank of the functional categories general activity, exploratory activity, risk assessment, risk taking and shelter seeking in the MCSF trend analysis for the control, diet-induced obesity (DIO) and restricted groups ($n = 12/\text{group}$). Data represent individual rats, median and interquartile range (IQR). No significant differences between the groups were observed (Kruskal Wallis test with multiple comparisons).

Table 2

Tissue weight (g) and weight as % of total body weight of the brain, liver, epididymal white adipose tissue (eWAT) and soleus muscle in the control, diet-induced obesity (DIO) and restricted groups ($n = 10\text{--}12/\text{group}$).

Tissue	Control	DIO	Restricted
Brain (g)	2.12 ± 0.14	2.12 ± 0.09	2.08 ± 0.09
Brain (% of total body weight)	0.44 ± 0.03	0.37 ± 0.03 α	0.49 ± 0.02 $\alpha\alpha\alpha$
Liver (g)	13.99 ± 0.94	14.40 ± 0.86	11.70 ± 1.99 $\alpha\alpha\alpha$
Liver (% of total body weight)	2.86 ± 0.19	2.52 ± 0.26 α	2.75 ± 0.41
eWAT (g)	8.30 ± 2.92	23.70 ± 5.21 $\alpha\alpha\alpha$	7.85 ± 1.97 $\dagger\dagger$
eWAT (% of total body weight)	1.68 ± 0.54	4.1 ± 0.73 $\alpha\alpha$	1.85 ± 0.44 $\dagger\dagger$
Soleus muscle (g)	0.21 ± 0.04	0.26 ± 0.08	0.23 ± 0.06
Soleus muscle (% of total body weight)	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02

Data represent mean ± SD. $\alpha p < 0.05$, $\alpha\alpha\alpha p < 0.001$ compared to the control group; $\dagger\dagger p < 0.001$ compared to the DIO group (one-way ANOVA with multiple comparisons).

($p < 0.001$).

Serum total cholesterol (Table 3) differed significantly between the groups [$F(2, 30) = 8.74, p = 0.001$] with the post-hoc analysis showing significantly lower total cholesterol levels both for the DIO ($p < 0.01$) and restricted ($p < 0.01$) groups in comparison to the control group. Moreover, serum triglyceride (Table 3) levels were found to be significantly different between the groups [$F(2, 26) = 12.25, p = 0.0002$] with the DIO group having higher levels in comparison to both the control and restricted groups ($p < 0.001$; $p < 0.001$, respectively).

Correlations between metabolic measurements, body weight and eWAT weight are shown in Table 4. When the groups were collapsed, and all rats were analyzed there were positive correlations between all parameters except adiponectin and HOMA-IR (Table 4A). When the groups were analyzed separately, the control rats had significant positive associations between leptin levels and HOMA-IR and body weight, HOMA-IR and body weight as well as HOMA-IR and eWAT weight. Moreover, there was a trend for a positive correlation between body

Table 3

Metabolic parameters in the control, diet-induced obesity (DIO) and restricted groups ($n = 7\text{--}12/\text{group}$).

	Control	DIO	Restricted
Glucose metabolism			
Blood glucose mmol/L (week 4)	5.91 ± 0.48	6.49 ± 0.45	5.88 ± 0.69
Blood glucose mmol/L (week 7)	5.74 ± 0.41	6.43 ± 0.46 α	5.56 ± 0.39 $\dagger\dagger$
Blood glucose mmol/L (week 12)	7.88 ± 0.99	8.47 ± 0.62	7.96 ± 0.84
Serum fructosamine $\mu\text{mol/L}$	216.3 ± 9.3	218.5 ± 10.6	218.7 ± 11.6
Plasma insulin $\mu\text{U/mL}$	71.8 ± 22.2	100.7 ± 24.7	61.1 ± 23.2 $\dagger\dagger$
HOMA-IR	24.95 ± 7.05	36.90 ± 9.47 $\alpha\alpha$	21.55 ± 8.69 $\dagger\dagger$
Lipids			
Serum total cholesterol mmol/L	2.23 ± 0.23	1.85 ± 0.26 α	1.80 ± 0.27 $\alpha\alpha\alpha$
Serum triglycerides mmol/L	1.52 ± 0.40	2.34 ± 0.51 α	1.40 ± 0.42 $\dagger\dagger$
Adipokines			
Plasma leptin ng/mL	3.85 ± 0.87	4.87 ± 1.12 α	2.83 ± 0.52 $\alpha\alpha$
Plasma adiponectin $\mu\text{g/mL}$	4.80 ± 0.89	9.43 ± 1.74 α	5.55 ± 1.61 $\dagger\dagger$
Adiponectin/leptin ratio	1.31 ± 0.42	1.99 ± 0.39 α	2.02 ± 0.62 $\alpha\alpha$

Data represent mean ± SD. $\alpha p < 0.05$, $\alpha\alpha < 0.01$, $\alpha\alpha\alpha p < 0.001$ compared to the control group; $\dagger\dagger p < 0.001$ compared to the DIO group (one-way ANOVA with multiple comparisons).

weight and eWAT weight ($p = 0.06$, Table 4B). In the DIO group there were significant positive correlations between adiponectin levels and eWAT weight as well as body weight and eWAT weight (Table 4C). Finally, in the restricted group there was a significant positive correlation between leptin levels and body weight. Moreover, there was a trend for a positive correlation between leptin levels and eWAT weight ($p = 0.05$, Table 4D).

3.5. Multivariate data analysis

The OPLS-DA score plot (Fig. 4A) provides a visual representation of

Table 4

Correlations between metabolic measurements, body weight (g, BW) and epididymal white adipose tissue (g, eWAT) in all rats as well as in the control, diet-induced obesity (DIO) and restricted groups ($n = 12/\text{group}$).

A. All rats					
	Leptin	Adiponectin	BW	HOMA-IR	eWAT
Leptin					
Adiponectin	0.57***				
BW	0.80***	0.66***			
HOMA-IR	0.68***	0.39	0.69***		
eWAT	0.69***	0.84***	0.87***	0.67***	
B. Control					
	Leptin	Adiponectin	BW	HOMA-IR	eWAT
Leptin					
Adiponectin	0.04		0.66*	0.61*	0.33
BW	0.66*	-0.32		0.20	0.54
HOMA-IR	0.61*	0.20	0.64*		0.61
eWAT	0.33	0.54	0.61	0.64*	
C. DIO					
	Leptin	Adiponectin	BW	HOMA-IR	eWAT
Leptin					
Adiponectin	0.52		0.48	0.47	0.49
BW	0.48	0.53		-0.05	0.74**
HOMA-IR	0.47	-0.05	0.34		0.23
eWAT	0.49	0.74**	0.72**	0.23	
D. Restricted					
	Leptin	Adiponectin	BW	HOMA-IR	eWAT
Leptin					
Adiponectin	0.11		0.11	0.47	0.57
BW	0.68*	-0.30		0.52	-0.35
HOMA-IR	0.47	-0.52	0.27		0.40
eWAT	0.57	-0.35	0.40	0.44	

Values represent Pearson correlation coefficient. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Pearson correlation). HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

the individual rats in the control, DIO and restricted groups, respectively. The horizontal component captures the variation between the dietary groups, while the vertical component captures variation within the groups. The DIO rats were clearly separated from the control and restricted individuals, while a slight overlap between restricted and control rats was found. The loading plot (Fig. 4B) identified the variables that contributed most to the separation between the groups, highlighting the key metabolic changes associated with the different diets. Variables close to the origin, i.e. fructosamine and the terminal glucose measure (Fig. 4B), had little contribution to the model, which is in line with the conventional statistical analysis (Table 3). Cholesterol, which was lower in the DIO and restricted groups (Table 3), loaded closer to the control group, while the remaining analytes loaded in association to the DIO group (Fig. 4B).

4. Discussion

The present study aimed to investigate the impact of a cafeteria diet and caloric restriction on behavioral and metabolic profiles of adult male rats. Cafeteria diet for 12 weeks induced obesity, insulin resistance and a mild metabolic dysregulation in the DIO rats, while caloric restriction resulted in a somewhat healthier metabolic profile compared to both DIO and the ad libitum fed control rats. Correlations between metabolic measurements, body weight and eWAT weight revealed an expected positive association between body weight and eWAT weight in all groups. Despite these effects, neither of the diets affected activity, exploration, risk assessment, risk taking or shelter seeking as assessed by the MCSF test.

The lack of group differences in the MCSF test was an unexpected finding since the test previously has been useful in revealing decreased risk-taking behavior in rats preferring a high-fat diet [19]. However,

Wistar rats from different vendors display marked differences in behavioral profiles in the MCSF [20], as well as in body weight gain when fed standard chow [21]. It is also evident that the literature on behavioral effects following obesogenic diets is far from concordant [22, 2]. What explains these discrepancies in study outcomes is not completely known, but factors such as different protocols for inducing obesity, age of exposure to obesogenic diets and behavioral studies, and sex [22,2], as well as experience in behavioral studies [23] and choice of behavioral test [24] may impact. The most commonly used tests are the open field and elevated plus maze tests, and interpretations are based on a single or a few descriptive parameters [22,2]. For example, when reviewing previous studies that have investigated the effects of an adult exposure to cafeteria diet on behavior in male Wistar or Sprague-Dawley rats, the results are indeed contrasting. In the elevated plus maze, studies have revealed increased [25,26], decreased [27] or no difference [28, 29] in open arm activity. A more consistent finding seems to be that obesogenic diets are without effect on locomotor activity [22,2], in agreement with the present study. Fewer studies have been devoted to the effects of caloric restriction on behavior. Here a recent meta-analysis revealed that caloric restriction seemed to be without effects on behavior [22], in line with the present findings. Thus, the results from the MCSF test expands on previous data, indicating that both weight gain and weight reduction may be without effects on general activity, exploration, risk assessment, risk taking and shelter seeking when a more comprehensive test is used for evaluation.

Our results demonstrated that the cafeteria diet successfully induced an obese phenotype with greater body weight gain and elevated eWAT weight, along with early manifestations of metabolic impairments, characterized by insulin resistance and elevated circulating triglycerides and leptin levels compared to both the control and the calorie-restricted groups. Differences in body weight and glucose levels could be seen already after one and four weeks of diet, respectively, in the DIO rats. After 12 weeks of diet the multivariate analysis revealed a distinct separation of the DIO group from the control and restricted groups. Thus, the results herein align with previous studies [30,31,2,32] indicating that high-energy, palatable foods in rodents lead to a rapid and substantial increase in body weight, adiposity and metabolic dysfunction, the latter however being mild in our study.

To address pathological changes associated with obesity, it is crucial to compare these effects to healthy controls. *Ad libitum* chow feeding in rodents, as used for the control group, is widely used as the control condition both for behavioral and metabolic research. However, ad libitum feeding has been shown to decrease overall health, cause excessive fat accumulation and induce chronic diseases, and its use as a control group has therefore been questioned [33]. Caloric restriction has been shown to lead to a healthier metabolic profile in rats [31] and mice [34,35] when the animals had a 15–20% lower body weight compared to controls. Therefore, a calorie restricted group, maintained at approximately 15% lower body weight than controls, was included herein. The restricted group weighed less than controls already after one week of diet, and this was maintained throughout the study period. They also had lower circulating leptin and cholesterol levels at the end of the study, in agreement with previous studies [36,37]. However, neither total nor relative eWAT amount, HOMA-IR nor glucose or insulin levels were lower in the restricted animals compared to controls. In agreement, Jantsch et al. [31] did not find differences in visceral fat weight, glucose and triglyceride levels or HOMA-IR when comparing control rats with animals on caloric restriction, despite a 30% reduction in chow fed to the calorie restricted rats.

Typically, obesity is associated with decreased adiponectin levels, which may contribute to the metabolic disturbances [38]. However, herein the DIO rats had higher circulating adiponectin levels. A recent systematic review indicate similar findings in the literature [38], and high adiponectin levels after cafeteria diet may be a compensatory mechanism to facilitate carbohydrate utilization. [39]. Furthermore, while adiponectin levels are typically associated with insulin sensitivity,

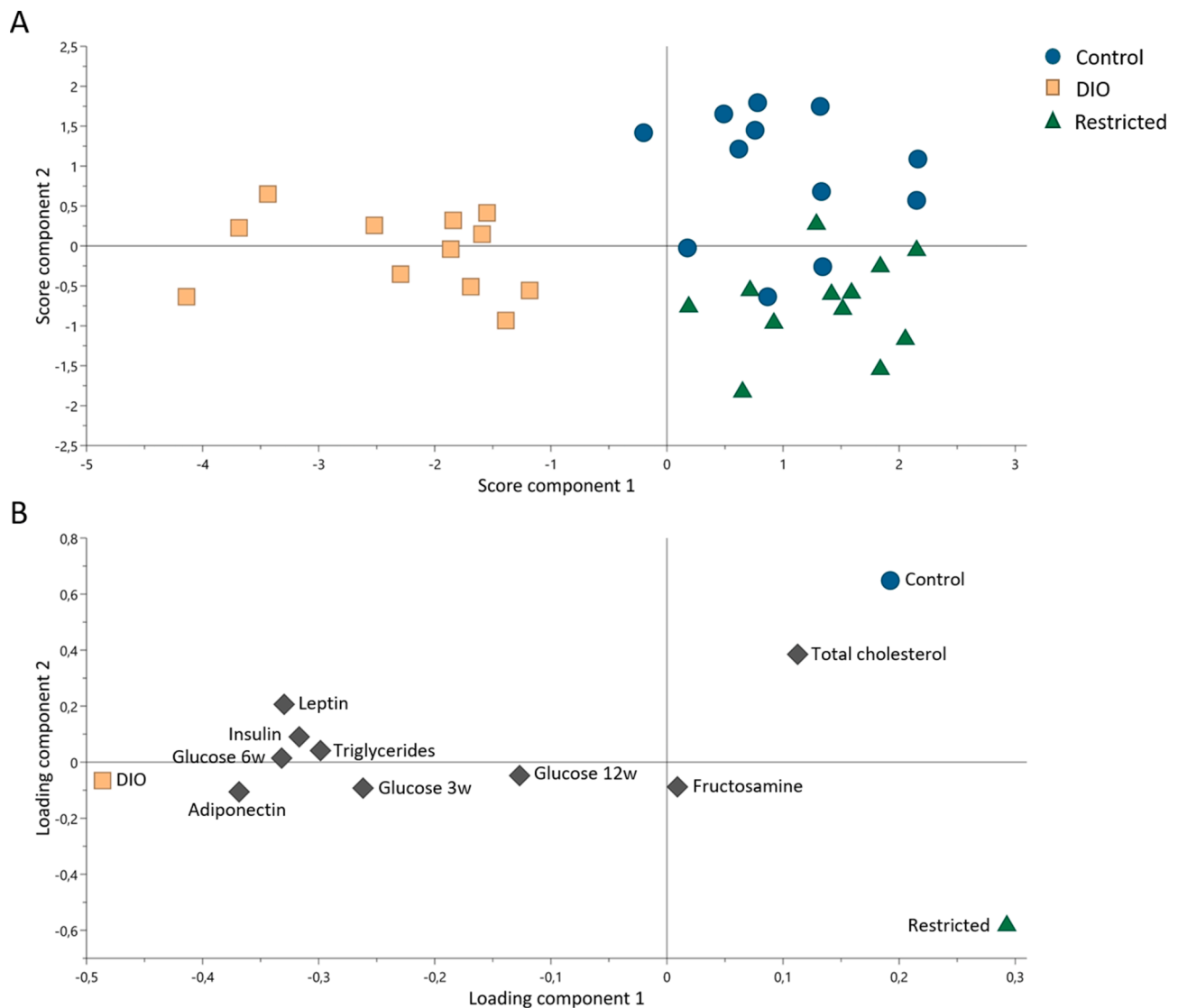


Fig. 4. OPLS-DA score plot showing the individual rats (A) and the variable loading plot of the metabolic parameters (B) after 12 weeks of diet for the control, diet-induced obesity (DIO) and restricted groups ($n = 12/\text{group}$; two significant components, $R2X(\text{cum}) = 0.642$, $R2(\text{cum}) = 0.679$, $Q2(\text{cum}) = 0.546$, $R2Y(\text{cum}) = 1.000$).

they also reflect adipose tissue amount and function and can be elevated in the early stages of adiposity development, before significant metabolic dysfunction occurs [40,41]. In our study, the diet combining chocolate balls and cheese doodles, together with the moderate adiposity may explain the higher adiponectin levels in the DIO group despite the insulin resistant state of the animals. Similarly, in a high-sucrose diet model, increased adiponectin was not protective against the development of metabolic syndrome or insulin resistance [42].

The adiponectin/leptin ratio, is typically reduced in human obesity [43] but was also increased in both the DIO and restricted groups. The higher adiponectin levels in the DIO group may also explain the lower cholesterol levels, as adiponectin has a role in cholesterol clearance [44], whereas the lower cholesterol levels in the restricted group likely link directly to the lower fat and energy intake compared to controls. Similar results have been observed in another study on effects of cafeteria diet or caloric restriction [45]. Given the bidirectional adiponectin changes in literature, the relevance of the adiponectin/leptin ratio in studies of diet-induced obesity has been questioned [38].

4.1. Strengths and limitations

One of the strengths of this study is the comprehensive assessment of both behavioral and metabolic measures in rats subjected to cafeteria diet as well as caloric restriction. The use of the MCSF test provided a broad behavioral profiling, yet surprisingly, no differences in exploration, risk assessment, risk taking or shelter seeking were found between the groups. However, there are also important behavioral aspects, e.g. cognitive processes, that were not assessed in the present work that should be investigated in future studies.

Metabolic measurements offered insight into the dietary impact on peripheral physiology. The DIO rats displayed an adverse metabolic phenotype having measures such as hyperglycemia and insulin resistance modestly elevated compared to the other groups. However, cholesterol and adiponectin levels, as well as the adiponectin/leptin ratio displayed a pattern that would typically be seen in metabolically healthy individuals. Thus, there are clear differences to the characteristics of typical human obesity, and this is a limitation of the study.

Notably, since the rats were pair-housed, the individual food/diet intake could not be assessed, and hence the quantitative intake of both energy and specific nutrients was uncertain. However, pair or group

housed animals are recommended over single housed animals in this kind of studies [2,46]. An additional limitation is that the blood samplings were not conducted under standardized conditions, e.g. fasting and at a specific time of day, which may influence the results of the metabolic parameters measured. However, throughout the 12 weeks of diet the restricted rats were fed in the mornings in order to coincide with the onset of their active period and it was hard to foresee the consequences of a change in that routine only on the days of blood and tissue sampling. Optimally, all groups should have been euthanized and sampled under standardized conditions, but that was not feasible from a practical perspective. Moreover, tissue weights of inguinal white adipose tissue (iWAT) and brown adipose tissue were not collected. The massive amount of iWAT in the DIO group made tissue collection for weight hard to standardize. Due to time constraints during tissue collection, brown adipose tissue was not prioritized.

Moreover, only male rats were investigated and given the prevalence of obesity and comorbid complications in women [1], and the fact that female animals are understudied [2], future studies should be conducted in both sexes.

5. Conclusion

In agreement with previous studies, the two diets led to significant changes in body weight gain, adiposity, and modest changes in metabolism. However, they did not alter the behavioral profiles in the MCSF test, suggesting that activity, exploration, risk assessment, risk taking or shelter seeking remained unaffected by the dietary interventions. The current findings suggest that an increase or reduction in energy intake resulted in no behavioral effects, despite the accompanying glycemic alterations potentially related to diabetes development.

Data availability

Data will be made available on request.

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CRedit authorship contribution statement

Christakis Kagijs: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Susanne Hetty:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Alfild Grönbladh:** Writing – review & editing, Investigation. **Maria J Pereira:** Writing – review & editing, Supervision. **Jan W Eriksson:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Erika Roman:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that there are no commercial or financial relationships that could be seen as potential conflicts of interest in conducting this research.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.physbeh.2024.114731](https://doi.org/10.1016/j.physbeh.2024.114731).

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