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ORIGINAL ARTICLE

High fat-low protein diet induces metabolic alterations and cognitive dysfunction in female rats

Ravinder Naik Dharavath[1] · Shiyana Arora[1] · Mahendra Bishnoi[2] · Kanthi Kiran Kondepudi[2] · Kanwaljit Chopra[1]

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Abstract

Approximately one-third of the world population is suffering from MetS, and the same is expected to rise in the years to come. Worldwide, most of the staple diets contain high amounts of carbohydrates, fats and comparatively low quantities of proteins. The goal of this study was to evaluate the effect of high fat-low protein diet in the development of the metabolic syndrome and associated cognitive deficits in the female rats. The rats fed with high fat-low protein diet (HFLPD) and 15% oral fructose solution for 24 weeks. Body weight, food intake, water intake, fasting blood glucose, oral glucose tolerance, glycosylated hemoglobin (HbA_{1C}), and serum lipid profile were measured after every 4 weeks. Serum insulin, HOMA-IR index, rectal temperature, and systolic blood pressure were measured to confirm the manifestation of the hallmarks of metabolic syndrome. Behavioral tests for locomotion, anxiety, learning, and spatial memory were performed from the 12th week to till the end of the study. At the 24th week, oxidative stress assays and histopathology of liver, kidney, brain, and WAT were also performed. HFLPD significantly altered the physiologic and metabolic parameters which contributed to the manifestation of MetS. HFLPD also impaired the cognitive functions along with significant structural changes in the liver, kidney, WAT, and brain. The findings of this study reveal that HFLPD has the potential to induce the physiological, metabolic and histological alterations in rats, which eventually led to the development of MetS and also disrupted the cognitive functions in female rats.

Keywords Cognitive decline · Fructose feeding · High calorific diet · Hypertension · Insulin resistance · Metabolic syndrome

Abbreviations

BAT	Brown adipose tissue
BMI	Body mass index
CD	Cafeteria diet
HbA_{1C}	Glycosylated hemoglobin
HDL-c	High-density lipoprotein cholesterol
HFLPD	High Fat-Low Protein Diet
HOMA	Homeostatic model assessment
IR	Insulin resistance
LDL-c	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MWM	Morris water maze
OFM	Open field maze test
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
TSTQ	Time spent in target quadrant
WD	Western diet

Introduction

The metabolic syndrome (MetS) or Syndrome X or Insulin resistance syndrome is a cluster of metabolic abnormalities such as central obesity, dyslipidemia, hyperglycemia, insulin resistance and hypertension (Grundy et al. 2004; Alberti et al. 2005). MetS has become one of the significant public health challenges and contribute to around 17.5 million deaths worldwide (Report 2014; Diabetes Atlas 2017). According to the International Diabetic Federation (IDF), a quarter to

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✉ Kanwaljit Chopra
dr_chopra_k@yahoo.com

[1] Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh 160014, India

[2] Food and Nutritional Biotechnology Laboratory, National Agri-food Biotechnology Institute (NABI), SAS Nagar, Punjab 140306, India

one-third of the world's adult population suffers from metabolic syndrome. In accordance with the data available, the prevalence of MetS is increasing at an alarming rate among high, middle and low-income countries. and is expected to contribute to 80% of diabetes associated cardiovascular diseases (CVD) mortality cases in years to come (Kaur 2014; Report 2014). Apart from CVD, individuals with MetS are susceptible to other comorbid conditions, notably, cognitive deficits, polycystic ovary syndrome, non-alcoholic fatty liver, cholesterol gallstones, asthma, sleep disturbances and some forms of cancers (Grundy et al. 2004). Changes in lifestyle such as decreased physical activity, unbalanced and high calorific diet, stress, altered circadian rhythm, insomnia, smoking, alcohol consumption etc. are considered to be major risk factors for development of one or more hallmarks of MetS such as central obesity, dyslipidemia, hyperglycemia and hypertension (Grundy 2008; Shankar and Sundarka 2010).

Decline in cognitive functions have been reported in clinical studies conducted in patients suffering from metabolic disorders (Holloway et al. 2011; Francis and Stevenson 2013; Cordner and Tamashiro 2015) which is strongly supported by incredible preclinical pieces of evidence (White et al. 2009; Kanoski and Davidson 2011; Sachdeva et al. 2018). Feeding laboratory animals with hypercaloric diets such as high fat, high carbohydrate, high fat-high carbohydrate, high fructose corn syrup and cafeteria diet have resulted in increased anxiety, reduced locomotion (Kohsaka et al. 2007) and impaired learning and memory retention (Greenwood and Winocur 2005; Winocur and Greenwood 2005; Freeman et al. 2014).

According to reports available with WHO, the per capita consumption of carbonated soft drinks has increased by many folds worldwide (https://www.statista.com 2018). Increased soft drink consumption is also one of the primary sources for high amounts of refined sugar intake. In countries like India, where the majority of the population is vegetarian, most of the food items contain large quantities of carbohydrates (i.e., Wheat starch, corn starch, rice, and refined sugar) and fats (i.e., Butter, ghee, coconut oil, and vanaspati) but the protein content is shallow. Moreover, the reports on the impacts of low protein diet on health are very limited. Based on these facts and figures, the current study was designed to explore the implications of high fat-low protein diet in the development of MetS and associated cognitive deficits in female rats.

Material and methods

Chemicals

Only food grade ingredients were used to prepare the high fat-low protein (HFLPD). Casein purchased from Clarion Casein (Gujarat, India); crystalline fructose procured from the Galam group (Menashe, Israel); Cholesterol from MP Biomedicals (California, USA) and food grade Maltodextrin from Loba Chemie (Mumbai, India). Processed and packed whole wheat flour, butter, and coconut oil procured from the local market.

Kits & Reagents

Serum metabolic parameters such as glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) assessed by commercially available ERBA Diagnostic kits (Mannheim GmbH, Germany). Serum insulin commercial ELISA kit was obtained from Elabscience Biotechnology Inc. (Bethesda, USA). For spontaneous measurement of blood glucose for OGTT, Accu-Chek Activ glucometer purchased from Roche Diagnostics (Risch-Rotkreuz, Switzerland) was used. Percent glycosylated hemoglobin (HbA1c) was measured by the cation exchange resin method (Excel Diagnostics, Hyderabad, India).

High fat-low protein diet preparation and calorie calculation

Preparation of the HFLPD was primarily based on the ratio of macronutrients present in the staple diets consumed by the population of high, middle, and low-income countries. Although, the diet habits vary from one region to another. However, most of the food items contain enormous amounts of carbohydrates and fats, whereas these food items are also protein deficient. Based on this rationale, we prepared the HFLPD using 45% carbohydrate, 40% fat, and 15% protein sources. The finished HFLPD pellets are then kept at 2–8°C to prevent the microbial growth and used whenever needed. The energy content of normal pellet diet(NPD) and HFLPD assessed by bomb calorimeter (Parr 6200 Isoperibol Calorimeter, Parr Instrument Company, USA).

Animals

Female Wistar rats (155 ± 5 g) were acquired from the Central Animal House facility of Panjab University and accommodated under standard laboratory animal housing environment with 12:12 h light: dark cycle and ad libitum access to food (Ashirwad Industries, Mohali, India) and water. Animals were quarantined before initiating the experiment and familiarized to the laboratory. The use of laboratory animals was permitted by the Institutional Animal Ethics Committee (PU/IAEC/S/16/94; 2016) of Panjab University, and all experiments were performed as per the guidelines laid by Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. All the behavioral tests were carried out during 0900 and 1700 h of the day.

Animal experiments and physiological parameters

A total of 12, 8–10-week-old female Wistar rats were randomly divided into two groups, namely NPD and HFLPD with six rats in each group. Rats of the NPD group were provided with normal pellet diet and regular drinking water while the HFLPD group rats were fed with High fat-low protein diet and 15% oral fructose through drinking water for 24 weeks. Body weight of rats, feed intake and water intake were recorded at 0, 4th, 8th, 16th, 20th and 24th week, whereas the rectal temperature was monitored after 12th and 24th week of the study (Fig. 1). Other indices such as abdominal circumference, body length, and organ weights were also recorded on the day of study termination to compute some dependent parameters such as Lee index, body mass index, and organ to body weight ratio.

Blood sample collection and serum separation

All the animals were subjected to 8 h fasting (0600–1400 h) before the blood sampling. Blood was withdrawn (1 ml from each animal every 4 weeks) from the retro-orbital plexus of the animals under the influence of Thiopental Sodium (45 mg/kg; i.p.) induced anesthesia at 0, 4th, 8th week, whereas at 12th, 16th, 20th & 24th week the blood sampling was done after a day of behavioral experiments completion. The blood samples were kept at 4–8°C for 30 mins followed by centrifugation (1700×g/4°C/10 mins) to separate the serum after which the serum samples were utilized to perform various assays related to MetS hallmarks such as dyslipidemia, insulin resistance and hyperglycemia.

Serum biochemistry

Serum lipid profile

Serum total cholesterol, triglycerides, low-density lipoproteins, and high-density lipoproteins were estimated using commercial biochemical kits.

Hyperglycemia-related parameters

Fasting blood glucose Fasting blood glucose levels were measured in serum at 0, 4th, 8th, 16th, 20th & 24th week of the study by commercial kit. 0.005 ml of serum added to 0.5 ml of premixed reagent. The reaction mixture then incubated at 37°C for 15 mins, and the optical density was read at 505 nm.

Oral glucose tolerance test OGTT was performed at the 24th week of the study. Before OGTT, rats were kept on fasting from 0600 to 1400 h. of daytime. This test was performed according to the method reported elsewhere (Murtaza et al. 2014). Fasting blood glucose level was measured by tail snip method before the oral administration of glucose solution (2 g/kg) using Accu-Chek Activ glucometer. The blood glucose level was monitored by the same tail snip method after 30, 60, 90 & 120 mins of glucose administration. The area

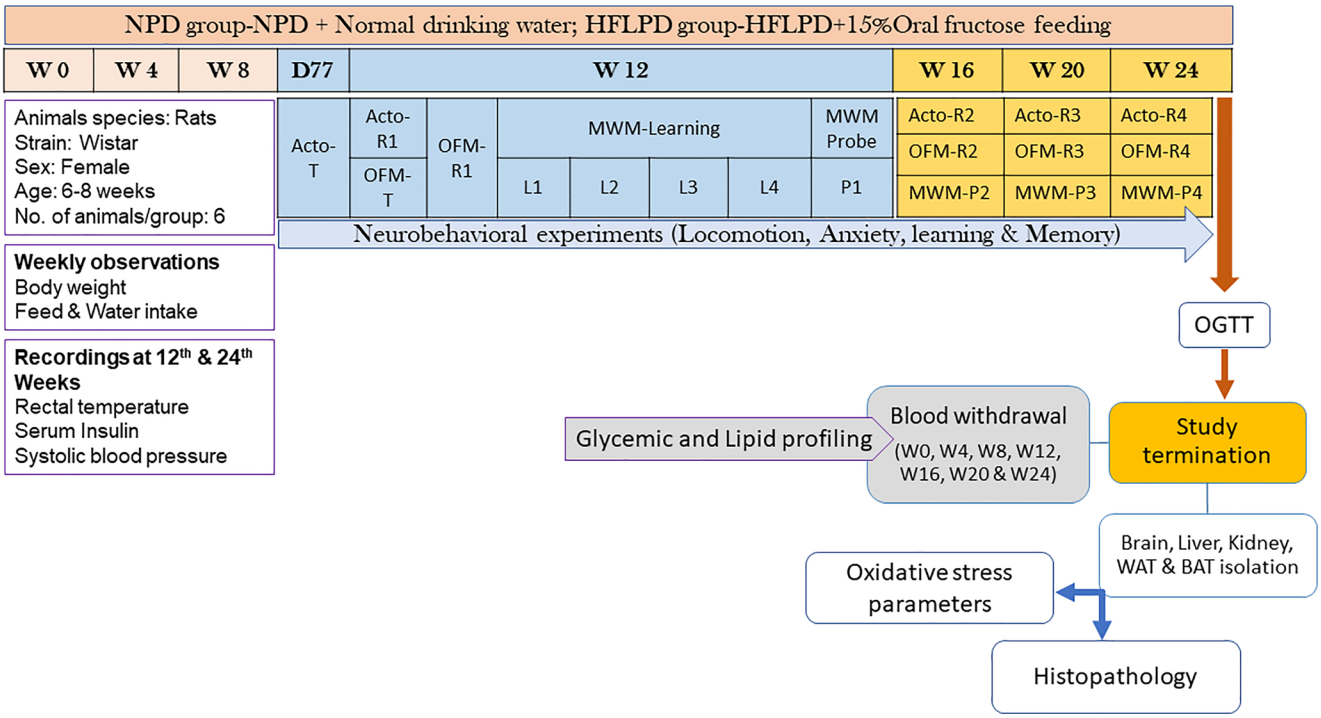

Fig. 1 Diagrammatic illustration of the experimental design. Acto-T (Actophotometer-trial); Acto-R (Actophotometer-recording); OFM-T (Open field maze test-trial); OFM-R (Open field maze test-recording); MWM-P (Morris water maze-Probe trial).

under the curve (AUC) was calculated to estimate tolerance to externally administered glucose.

Glycosylated hemoglobin (HbA_{1C}) The percent glycosylated hemoglobin was measured according to the user manual instructions. 0.05 ml of whole blood was mixed with 0.25 ml of lysing reagent and mixed well to prepare the hemolysate, which then used for the estimation of total hemoglobin (THb) and glycated hemoglobin (GHb).

Insulin resistance (IR)

Fasting serum insulin

Serum insulin levels were measured at two different time points (12th & 24th week) of the study according to the user manual of rat insulin ELISA kit. Briefly, 0.1 ml of serum sample was added to the precoated plate followed by the addition of 0.1 ml of biotinylated insulin antibodies and 0.1 ml HRP conjugate solution and 0.09 ml of substrate solution with intermittent washes and incubations at 37°C as described in the manual. The optical density was measured at 450 nm after adding the stop solution.

HOMA-IR index

Insulin resistance (HOMA-IR) index was calculated at two-time points (12th & 24th week) by using the following formula (Sachdeva et al. 2018).

$$HOMA = \frac{\text{fasting insulin (mUI/ml) x fasting glucose (mmol/L)}}{22.5}$$

Hypertension

Systolic blood pressure of the rats was monitored at two different time points (12th & 24th week) of the study by non-invasive tail-cuff method (NIBP Apparatus, AD Instruments, Australia). Rats were restrained for 30 mins a day for 1 week before the actual blood pressure recording. Acclimation was done to minimize the animal movement in the restrainer while measuring the blood pressure.

Neurobehavioral assessment

Various behavioral paradigms were chosen to assess different cognitive functions of rats fed with HFLPD. The behavioral experiments were carried out from the 12th week of the study until 24th week with an interval of the 4 weeks (Fig. 1).

Locomotor activity

The locomotor activity was assessed by actophotometer. A method reported by Kumar with minor changes was followed (Kumar et al. 2008). The equipment is a 40 cm × 40 cm × 25 cm chamber with five built-in photosensors in each of two rows at two different heights (5 cm & 15 cm). The photosensors located on the lower row records horizontal activity (locomotion) whereas the sensors on top row record the vertical movement (rearing). At the 12th week, each animal was allowed for 5 mins to explore the environment followed by 5 mins assessment on the next day. At 16th, 20th, and 24th week, the locomotor behavior of the animals assessed for 5 mins without any prior exposure. The arena was wiped with 70% ethyl alcohol after each trial.

Anxiety-like behavior

Open field maze (OFM); a method reported by (Souza et al. 2007) with modifications was followed. An open field maze is a circular chamber with a diameter of 90 cm and protected by the 30 cm height wall. The maze divided into the central area and peripheral area. The animals were released into the central region and observed. The total distance traveled, time spent in the central part, time spent in the periphery, immobility time, and the number of rearings recorded for each animal. At the 12th week, each animal was allowed for 5 mins to explore the environment followed by 5 mins assessment on the next day. At 16th, 20th and 24th week, the locomotor behavior of the animals directly scored for 5 mins without any prior exposure. The arena was wiped with 70% ethyl alcohol after each trial and left to dry before the introduction of the next animal. The tests were recorded and scored with the help of the computer-assisted tracking system (EthoVision 3.1 version, Noldus Information Technology, Wageningen, Netherlands).

Learning & Memory retention

MWM was used to evaluate the learning capability and spatial memory retention, as described in one of our previous report (Sachdeva et al. 2018). Various brightly colored cues placed around the maze, which is noticeable by rats in the maze, these cues will help the rats in spatial coordination. The locations of the platform and cues continued to be the same throughout the study. The experimental environment was spacious, noise-free, and maintained at 28.5 ± 2 °C. The MWM task was performed for five consecutive days, out of which rats have given training trials for initial 4 days; four trials a day with each trial of a maximum time of 90s with a 30s interval between each trial. One after another, rats released into the water maze at one of four quadrants with their heads facing the wall. The rat left for the 90s to find the invisible platform. After reaching onto the platform, the animal was left there

for the 20s to observe the surroundings before proceeding to the next trial. If the rat failed to reach the platform within the maximum allowed time of the 90s, it was guided with the help of a rod/stick and allowed to remain on the platform for 20s. Each rat was given four trials a day, each from all the four quadrants and for four consecutive days. The time to reach the platform (escape latency in seconds) and total distance traveled to reach the hidden platform (path length in cm) were measured by using the EthoVision 3.1 version software.

Memory consolidation test: Probe trial

At the 12th week, a probe trial (P1) was performed on day five to evaluate the degree of memory consolidation after 4 days of training. The hidden platform removed from the maze, and the particular quadrant then considered as the target quadrant. Each rat released into the water maze for a total duration of the 90s. Subsequent probe trails P2, P3, and P4 were performed on 16th, 20th and 24th week respectively, without repeating the 4-days trial. Total time spent in the target quadrant and frequency or number of entries into the target quadrant were calculated with the computer-assisted EthoVision software.

Tissue homogenate preparation

After feeding the rats with HFLPD for 24 weeks, animals fasted for 8 h (0600–1400 h.) before the sacrifice. The brain, liver, and kidney from each animal were carefully dissected out and washed with chilled saline (0.9% sodium chloride) to clear off the blood and homogenized in ice-cold phosphate buffer (pH 7.4). The tissue homogenates were first centrifuged (800×g/5 min/4°C) to remove the nuclear debris followed by another step of centrifugation (10,500×g/20 min/4°C) to obtain post-mitochondrial supernatant which stored at −80°C until further analyses.

Oxidative stress and antioxidants estimation

Malondialdehyde quantification

Thiobarbituric acid-reactive substances (TBARS) assay was performed by (Wills 1966) method. 0.5 ml of post-mitochondrial supernatant of each tissue (cortex, hippocampus, liver, and kidney) were mixed with an equal volume of Tris–hydrochloride and incubated at 37°C for 2 h. 1 ml of 10% trichloroacetic acid (TCA) was added to the reaction mixture and centrifuged (1000×g/10 min/room temperature) to separate the supernatant. Equal volumes of supernatant and 0.67% thiobarbituric acid (TBA) were taken into the tubes and kept in boiling water for 10 min. After cooling, an equal volume of double distilled water added to the supernatant and absorbance was measured at 532 nm. TBARS level was measured using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ and expressed as nmol of malondialdehyde per mg protein.

Quantification of reduced glutathione

The reduced glutathione was estimated by (Jollow et al. 1974) method. The assay protocol briefly, 1.0 ml of post-mitochondrial supernatant was added to an equal volume of sulphosalicylic acid (4%) to precipitate the samples by incubation at 4 °C for 60 mins. Precipitated samples, then subjected to centrifugation (1200×g/15 min/4°C) to obtain a clear supernatant. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml Ellman's reagent (0.1 mM, pH 8.0) in a total volume of 3.0 ml. The developed yellow color was read immediately at 412 nm. GSH levels represented as μmoles/mg protein. The same method was followed for other types of tissues.

Estimation of endogenous catalase

Endogenous catalase levels were estimated as per the method described by (Bansal et al. 2018). 0.05 ml of post-mitochondrial supernatant, 1.95 ml phosphate buffer (0.05 M, pH 7.0) and 1.0 ml hydrogen peroxide (0.019 M) in a total volume of 3.0 ml. Variations in optical density were noted at a wavelength of 240 nm, and the enzyme activity expressed as μmol of H_2O_2 decomposed/mg protein. The same method followed for other types of tissues.

Quantification of superoxide dismutase (SOD)

Cytosolic SOD quantified by (Kono 1978) method. 2 ml of assay reagent mix (0.1 mM EDTA +50 mM sodium carbonate +96 mM of nitro blue tetrazolium (NBT)) dispensed into a glass cuvette, a mixture of post-mitochondrial supernatant (0.05 ml) and hydroxylamine hydrochloride (0.05 ml; pH 6.0) was added to the cuvette. The levels of SOD were measured in terms of auto-oxidation of hydroxylamine and expressed as SOD units/mg protein. Changes in optical density were recorded at 560 nm for 2 min at 30/60 s intervals.

Histopathology

Two days after performing OGTT at the 24th week (on 168th day), the animals were subjected to transcardial perfusion under Thiopental anesthesia. The brain, liver, kidneys, and white adipose tissues were dissected out of the animal and stored in 4% paraformaldehyde for histopathology purpose. The various steps of histopathology were carried out according to a modified method reported by Singh et al. (Singh et al. 2017). Briefly, tissues were treated with a series of water, ethyl

alcohol (50%, 70%, 90%, 95% & 100%), absolute ethyl alcohol: xylene (75:25, 50:50 & 25:75), xylene: paraffin (75:25, 50:50 & 25:75) and with 100% paraffin before preparing the tissue paraffin blocks. 5 μm thin sections were obtained by using a regular microtome and the sections were subjected to hematoxylin & eosin staining to observe the anatomical changes at the microscopic level. The microphotographs further analyzed with Image J, an open source image analyzing software (Image J 1.52a, National Institute of Health, USA).

Statistical analysis

The GraphPad Prism 5.0. software was used to analyze the data. Two-way ANOVA followed by Bonferroni's post-hoc test was applied to analyze the time point data, whereas student's t test was applied to analyze a few of the physiological parameters. A p value of less than 0.05 was considered significant. Significance levels and the labels shown in the results are @ $p < 0.05$, # $p < 0.01$, * $p < 0.001$ vs. NPD group at the respective time points.

Results

Energy calculations

This experimental diet (HFLPD) was found to be hypercaloric (19.41 kJ/g) in nature as compared to NPD (14.36 kJ/g).

Table 1 The composition of NPD and HFLPD with calorie calculation

Ingredients per 100 g		
Ingredient	NPD	HFLPD
Carbohydrates	**63.7**	**45**
Whole wheat starch	–	35
Maltodextrin	3.5	10
Corn starch	45.5	–
Cellulose	4.7	–
Dextrin	10	–
Fats	**6.3**	**40**
Soybean oil	6.3	-
Butter	–	30
Coconut oil	–	9
Cholesterol	–	1
Proteins	**25**	**15**
Casein	25	15
Vit & Mineral mix (AIN-93 M premix)	4.5	–
DL-Methionine	0.5	0.5
Energy (kJ/100 g)	1436	1941

HFLPD contains almost 35% more energy as compared to the NPD (Table 1).

Effect of HFLPD on physiological parameters

HFLPD led to a significant rise in the body weight from the 4th week onwards till 24th week as compared to the NPD rats (Fig. 2a). The feed intake of HFLPD rats was significantly increased from the 8th week, and it continued until the end of the study (Fig. 2b). There wasn't any significant difference in water intake in the two groups throughout the study period (Fig. 2c). The rectal temperature of HFLPD rats was found to be marginally increased at W12 but was significantly higher at the 24th week as compared to the NPD group (Fig. 2d). No significant difference was noticed in indices such as body length (nostril to anus) and abdominal circumference of HFLPD rats. However, at W24, the lee index and BMI of HFLPD fed rats were significantly high as compared to NPD group (Table 2). HFLPD feeding caused significant enlargement of the liver, whereas no difference observed in kidney size of HFLPD fed rats (Table 2). Significantly higher amount of white adipose tissue collected from HFLPD rats (7.45 ± 1.0 g) as compared to NPD group (34.37 ± 7.97 g) and the WAT to body weight ratio found to significantly higher in HFLPD group (Table 2). Moreover, rats fed with HFLPD contained substantially fewer amounts of brown adipose tissue compared to NPD fed rats (Table 2).

Effect of HFLPD on serum lipid profile

HFLPD feeding led to an undesirable alteration in serum lipid profile. A significant rise in the serum triglycerides and low-density lipoproteins were evident from the 8th week, whereas a substantial increase in total serum cholesterol seen from a 12th week till the end of the study (Fig. 3a–d). The decrease in serum high-density lipoprotein levels was first evident at 4th week, and the further reduction in HDL levels was observed to be time-dependent (Fig. 3c) whereas the lipid profile of NPD animals remained unchanged throughout the study (Fig. 3a–d).

Effect of HFLPD on insulin resistance

The serum insulin levels of HFLPD fed rats at 12th and the 24th week was significantly higher as compared to the NPD group (Fig. 4a). Elevated serum glucose levels and insensitive glucose uptake in muscle cells led to the secretion of high amounts of insulin to improve glucose utilization, which otherwise led to an insulin resistant state in HFLPD fed rats. HOMA-IR index of HFLPD rats was significantly higher at 12th and 24th of the study as compared to the NPD group (Fig. 4b).

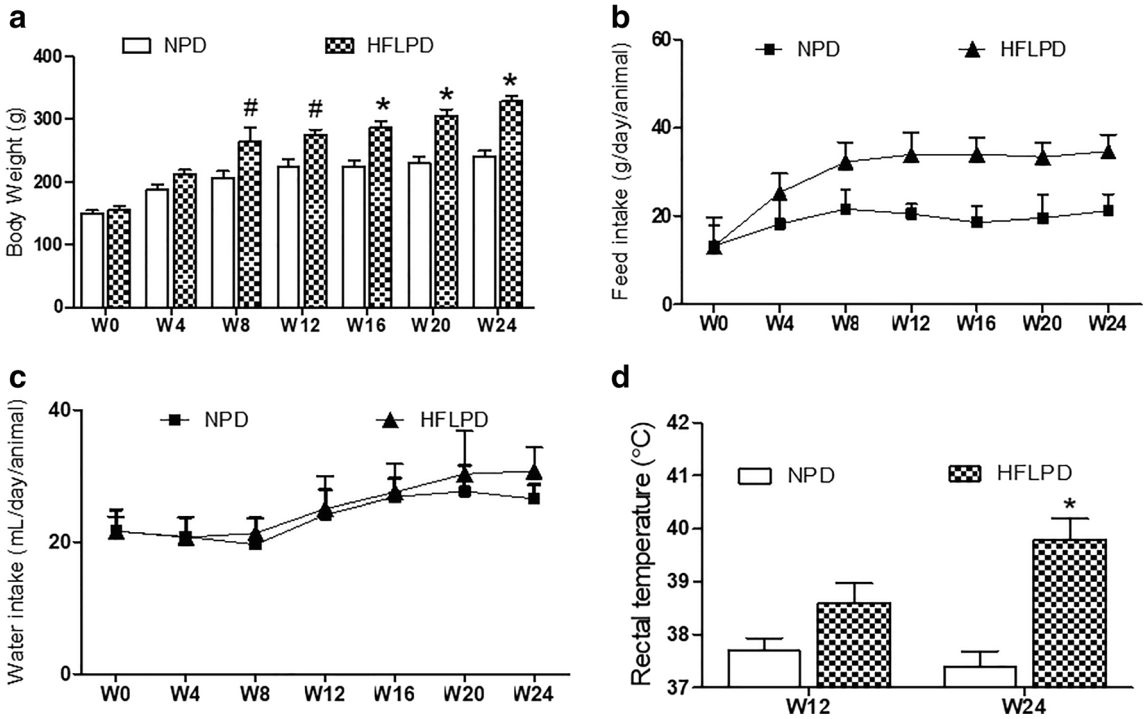

Fig. 2 Effect of HFLPD feeding at 0, 4th, 8th, 12th, 16th, 20th and 24th week on Body weight (**a**), Feed intake (**b**), and Water intake (**c**). Effect of HFLPD on the rectal temperature of rats at 12th and 24th week of study (**d**). Values expressed as mean ± SEM. 0 W = 0 week; W4 = 4th week; W8 = 8th week; W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

Effect of HFLPD on hyperglycemia parameters

Hyperglycemic state was assessed by fasting serum glucose levels, glycosylated Hb, and oral glucose tolerance test. Fasting serum glucose levels and the percent glycosylated Hb were found to be significantly elevated after feeding the animals with HFLPD for 4 weeks, and a similar trend observed till the 24th week of the study (Fig. 5a and b). During the glucose tolerance test, after the 24th week, NPD and HFLPD groups showed peak systemic glucose levels after 30 mins of oral glucose administration. NPD animals managed to clear the excess glucose from the system within 30 mins after reaching the peak levels whereas, in case of HFLPD group, the glucose levels at 120 mins after oral glucose administration remained higher than the serum glucose before oral administration of groups (Fig. 5c). HFLPD fed rats have displayed more elevated glucose AUC as compared to NPD animals (Fig. 5d), which is an indicator of glucose intolerance.

Effect of HFLPD on systolic blood pressure

Systolic blood pressure (SBP) of the HFLPD fed rats was significantly higher at the 12th week (132 ± 1.18 mmHg) and the 24th week (147 ± 2.24 mmHg) as compared to the NPD group (115 ± 1.47 & 112 ± 1.76 mmHg) (Fig. 4c).

Cognitive functions interpretations

HFLPD feeding reduces locomotion

In the actophotometer, the locomotor activity of HFLPD fed animals declined. Horizontal beam cuts by HFLPD fed

Table 2 Physiological parameters and relative organ weights at 24th week

	Body length (cm)	Abd. Circum. (cm)	BMI	Lee Index	Relative organ weights (%)				
					Brain	Liver	Kidney	WAT	BAT
NPD	22.08 ± 0.45	17.50 ± 0.48	0.51 ± 0.02	0.28 ± 0.004	0.72 ± 0.02	3.20 ± 0.15	0.34 ± 0.01	3.10 ± 0.42	0.19 ± 0.01
HFLPD	22.83 ± 0.56	20.58 ± 1.04	0.65 ± 0.02#	0.30 ± 0.003@	0.55 ± 0.01*	3.57 ± 0.13	0.27 ± 0.01#	12.98 ± 1.36*	0.20 ± 0.01

Values are mean ± standard error of mean ($n = 6$). $P < 0.05$ vs NPD group was considered statistically significant. Lee index = cube root of body weight (g) /body length (cm); Body mass index (BMI) = body weight (g)/length^2 (cm^2); Relative organ weight = Organ weight (g)/Animal body weight (g) × 100. @ $p < 0.05$, # $p < 0.01$, * $p < 0.001$ vs. NPD group

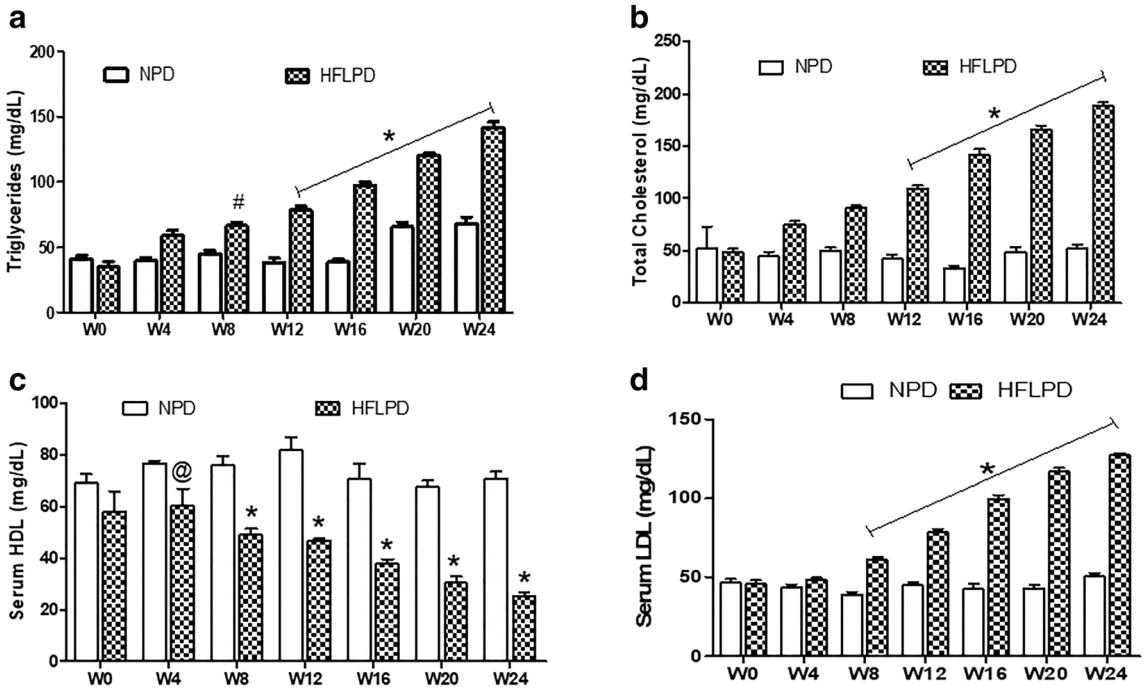

Fig. 3 Effect of HFLPD feeding at 0, 4th, 8th, 12th, 16th, 20th and 24th week on Serum triglycerides (**a**), Serum total cholesterol (**b**), Serum HDL-c (**c**) and Serum LDL-c (**d**). Values expressed as mean ± SEM. 0 W = 0 week; W4 = 4th week; W8 = 8th week; W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

animals were significantly lower at 12th, 16th, 20th, and 24th week of the study, as compared to NPD fed animals (Fig. 6a). Furthermore, there was a dip in the total distance traveled (Fig. 7a) and a significant increase in the immobility time of HFLPD animals in the OFM arena (Fig. 7b).

HFLPD feeding causes anxiety-like behavior

Rearing frequency of HFLPD fed animals in OFM significantly and time-dependently reduced from the 12th week to 24th week of the study as compared to the NPD group (Fig. 6c), whereas in the actophotometer, the reduction in rearing frequency observed at 20th and 24th week (Fig. 6b). Moreover, the time spent in the center of the OFM arena by HFLPD animals was significantly lowered after the 16th week to 24th week as compared to NPD animals (Fig. 7c), and on the other side, the HFLPD feeding increased the time spent in the periphery of the OFM arena (Fig. 7d).

Effect of HFLPD on learning and memory retention in MWM

During the initial 4-days trial of MWM, the escape latency (Fig. 8a) and pathlength (Fig. 8b) to reach the platform was slightly low in HFLPD rats but not significant as compared to NPD fed rats. However, the HFLPD group showed a substantial increase in the time spent in the target quadrant (TSTQ) and frequency of entries into the TQ during the probe trials P2 and P3, respectively on the 16th and 20th week (Fig. 8c). While the TSTQ and frequency of entries into TQ of

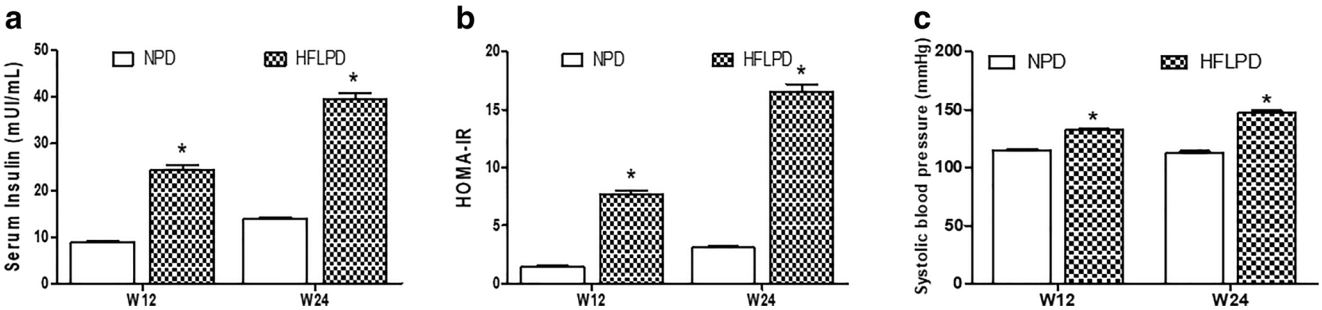

Fig. 4 Effect of HFLPD feeding at 12th and 24th week on Serum insulin (**a**), HOMA-IR index (**b**), and Systolic blood pressure (**c**). Values expressed as mean ± SEM. W12 = 12th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

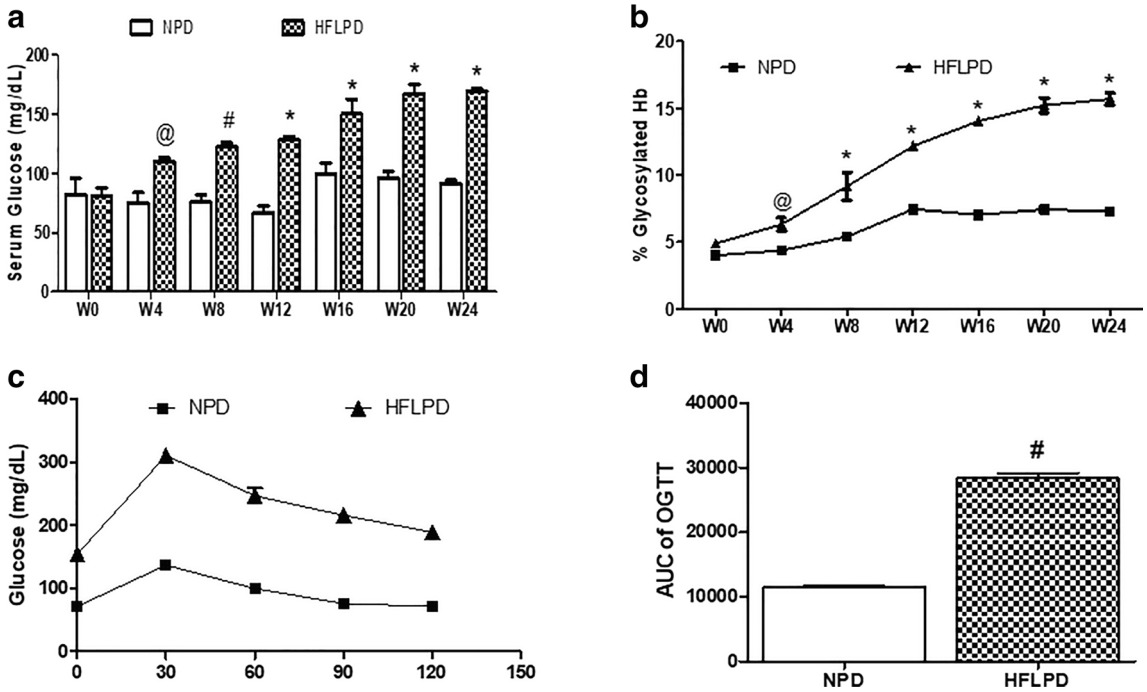

Fig. 5 Effect of HFLPD feeding at 0, 4th, 8th, 12th, 16th, 20th and 24th week on Serum glucose (**a**), Glycosylated Hb (**b**), Oral glucose tolerance (**c**) and AuC of OGTT (**d**). Values expressed as mean ± SEM. 0 W = 0 week; W4 = 4th week; W8 = 8th week; W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

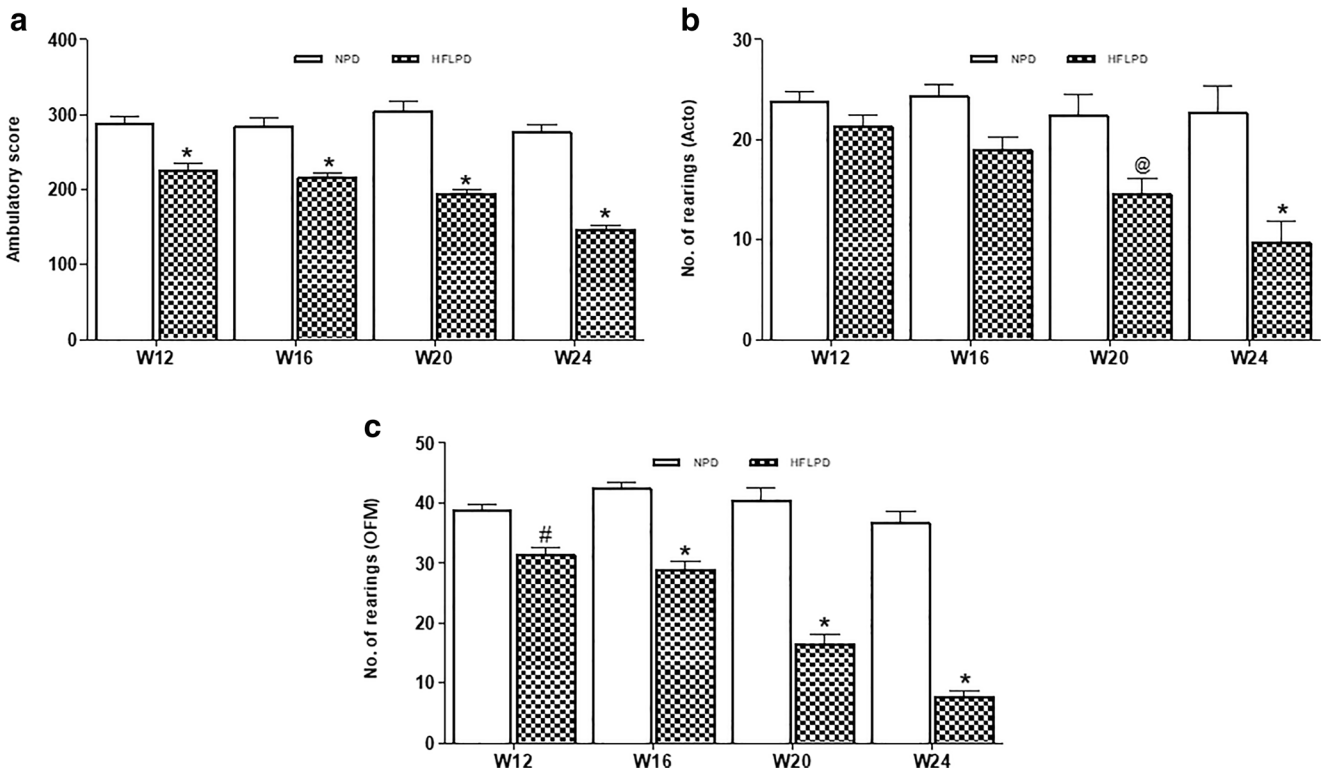

Fig. 6 Effect of HFLPD feeding at 12th, 16th, 20th and 24th week on locomotor activity in actophotometer (**a**), rearing frequency in actophotometer (**b**) and rearing frequency in OFM (**c**). W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

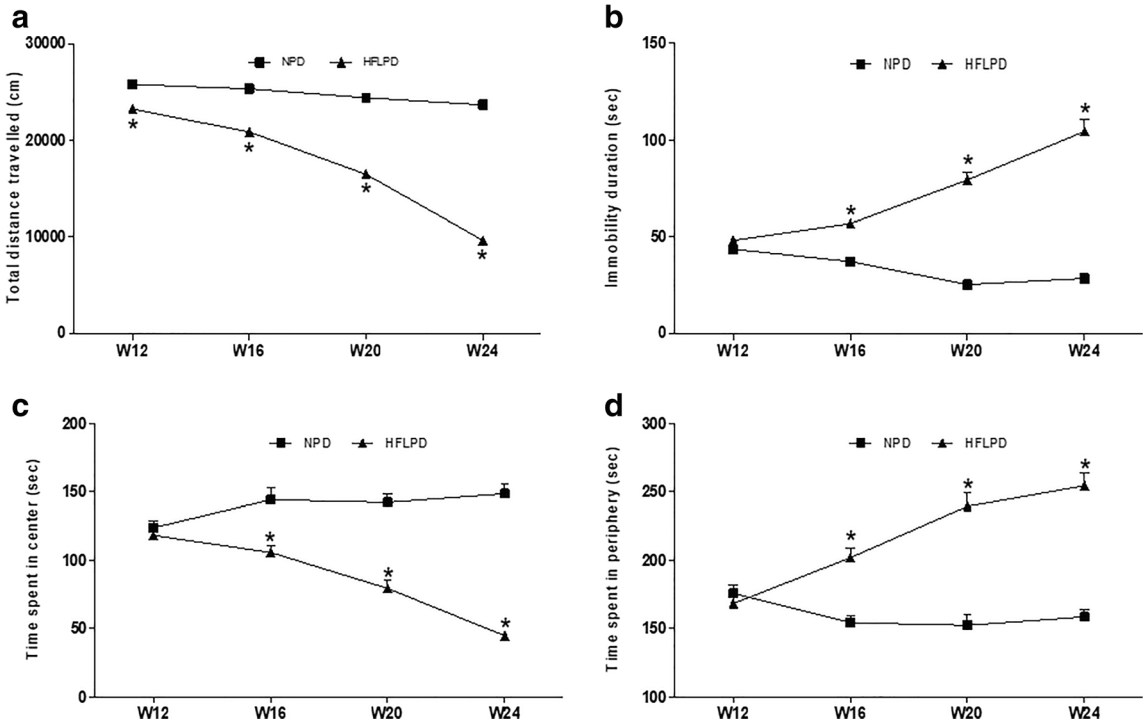

Fig. 7 Effect of HFLPD feeding at 12th, 16th, 20th, and 24th week on anxiety levels in OFM. Total distance traveled in OFM (**a**), immobility duration (**b**), time spent in center (**c**), and time spent in the periphery (**d**). W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

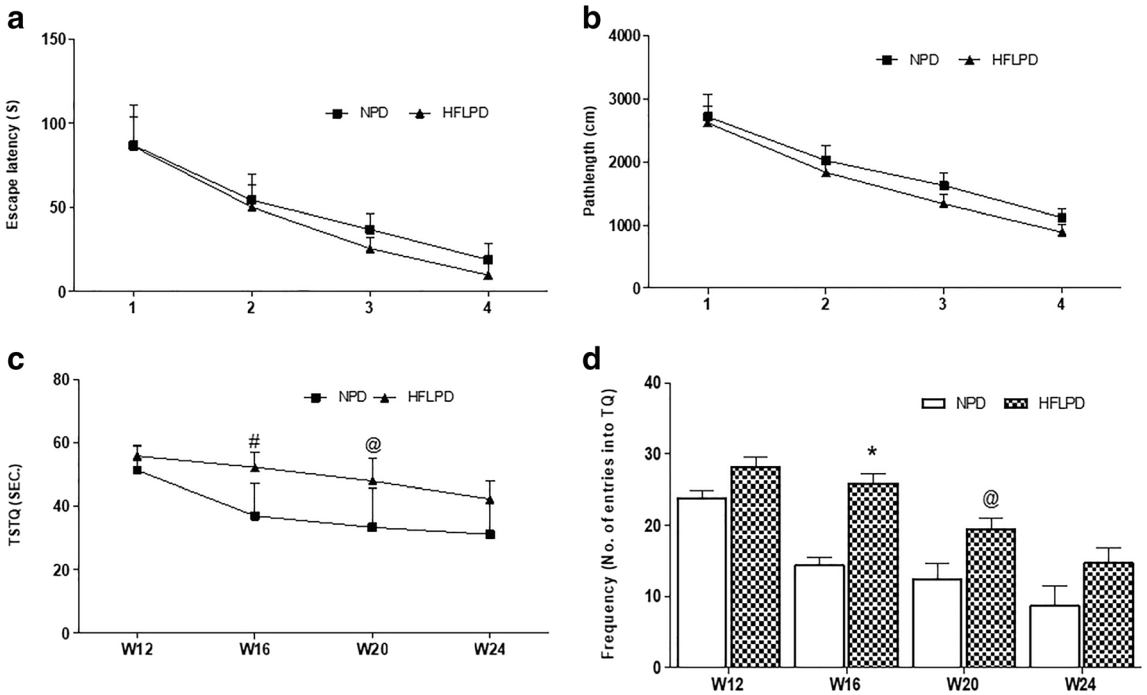

Fig. 8 Effects of HFLPD feeding at 12th, 16th, 20th, and 24th week on task learning and spatial memory retention. Escape latency (**a**), pathlength (**b**), time spent in the target quadrant (**c**), and frequency of entries into target quadrant (**d**). W12 = 12th week; W16 = 16th week; W20 = 20th week; W24 = 24th week. *P* values less than 0.05 were considered significant. @, # and * represents $p < 0.05$, $p < 0.01$, and $p < 0.001$ correspondingly vs. NPD group at the respective time points

HFLPD group not significantly altered from NPD during P1 and P4 at W12 and W24 (Fig. 8d).

Chronic HFLPD feeding elevates oxidative stress

Feeding young female rats fed with HFLPD for 24 weeks, significantly increased the oxidative stress in tissues of various vital organs. The lipid peroxidation in cortex, hippocampus, liver, and kidney tissues was elevated by many folds as compared to the NPD group animals. Moreover, HFLPD feeding also caused a reduction in the endogenous antioxidant enzymes in all the above-tested tissues (Table 3).

Effect of HFLPD on anatomical structures of brain, liver, kidney & WAT

The sections of the liver revealed that the architecture of the liver got manipulated by feeding the animals with HFLPD feeding. Enlarged hepatic parenchymal cells with fat accumulation which infers to the occurrence of fatty liver, whereas the tightly packed parenchymal cells seen in the NPD group. HFLPD disrupted the endothelium of central and hepatic portal veins. A large number of fatty liver cells observed in HFLPD as compared NPD (Fig. 9a and b). Chronic HFLPD feeding resulted in the enlargement of glomeruli and disrupted the epithelial lining of proximal convoluted tubules. An increased inflammatory response was seen in glomeruli (Fig. 9c and d). HFLPD feeding led to adipocyte hypertrophy and fat accumulation in WAT (Fig. 9e and f). The microphotographs of the brain sections of HFLPD fed rats showed a significant neuronal cell loss in cortex and hippocampal regions. The cortical areas consisted of neurons with swollen cell bodies (hypertrophic neurons) and vacuoles within the cell bodies (Fig. 10b). Moreover, the hippocampal pyramidal layer distorted with a vast neuronal loss (Fig. 10d) (See: Supplementary figures file).

Discussion

Several preclinical and clinical reports positively correlate that the consumption of various high calorific diets with the development of one or more hallmarks of metabolic syndrome include obesity, insulin resistance, diabetes, and associated cardiovascular complications (Poppitt et al. 2002; Hariri and Thibault 2010; Panchal et al. 2011; Buettner and Buettner 2016). The MetS associated cognitive dysfunctions in the male population is well recorded. There is very few evidence available on the involvement of diet composition/ratio in the development of metabolic disorders and associated cognitive decline in the female population (Underwood and Thompson 2016). Thus, the main aim of this study was to show how the High fat-low protein diet is involved in the development of metabolic alterations, finally leading to MetS and associated cognitive deficits.

The constituents used in HFLPD and their ratio were decided based on their rate of consumption by the people. The composition of HFLPD is very different from other widely used high calorific diets. HFLPD is composed of high amounts of carbohydrates and fats and a moderate amount of proteins. The reason for choosing female Wistar rats for this specific study was the high prevalence of MetS in female (Kaur 2014; Ranasinghe et al. 2017).

In our study, most of the physiological and serum metabolic indices started to alter from the 4th week and advanced until the 24th week of the study. HFLPD is a highly palatable diet with a decent odor of butter and other components of the food, which we believe, would have also contributed to the significantly increased feed intake of HFLPD fed animals. The weight gain can be explained by increased energy intake and disrupted energy expenditure in animals (Kulkarni et al. 2015). No significant change in the water intake upon HFLPD feeding observed. Increased brown fat directly correlated to the thermogenesis in various studies, but in our study, we found significantly elevated thermogenesis despite the

Table 3 Impact of HFLPD on oxidative stress and antioxidant indices in brain, liver and kidney

		LPO	GSH	Catalase	SOD
Cortex	NPD	1.42 ± 0.12	1.52 ± 0.02	2.75 ± 0.09	3.29 ± 0.18
	HFLPD	15.56 ± 2.21*	0.07 ± 0.01*	0.49 ± 0.05*	2.39 ± 0.27
Hippocampus	NPD	1.88 ± 0.31	0.27 ± 0.02	1.97 ± 0.11	4.90 ± 0.39
	HFLPD	20.54 ± 3.61*	0.07 ± 0.01*	0.47 ± 0.07#	2.16 ± 0.22*
Liver	NPD	4.78 ± 0.47	0.84 ± 0.05	10.46 ± 0.75	5.95 ± 0.25
	HFLPD	22.68 ± 3.07*	0.23 ± 0.02*	3.75 ± 0.27*	2.97 ± 0.20*
Kidney	NPD	2.00 ± 0.31	0.50 ± 0.03	3.27 ± 0.18	4.82 ± 0.24
	HFLPD	18.83 ± 2.54*	0.14 ± 0.01*	0.63 ± 0.08*	2.25 ± 0.46*

HFLPD feeding elevates oxidative stress and reduces antioxidant enzyme levels in cortex, hippocampus, liver and kidney at 24th week. *P* values less than 0.05 were considered as significant. Units of expression: LPO – nmoles of MDA/mg protein; GSH - μmoles/mg protein; Catalase - μmoles of H_2O_2 decomposed/mg protein; SOD – SOD units/mg protein. @ $p < 0.05$, # $p < 0.01$, * $p < 0.001$ vs. NPD group

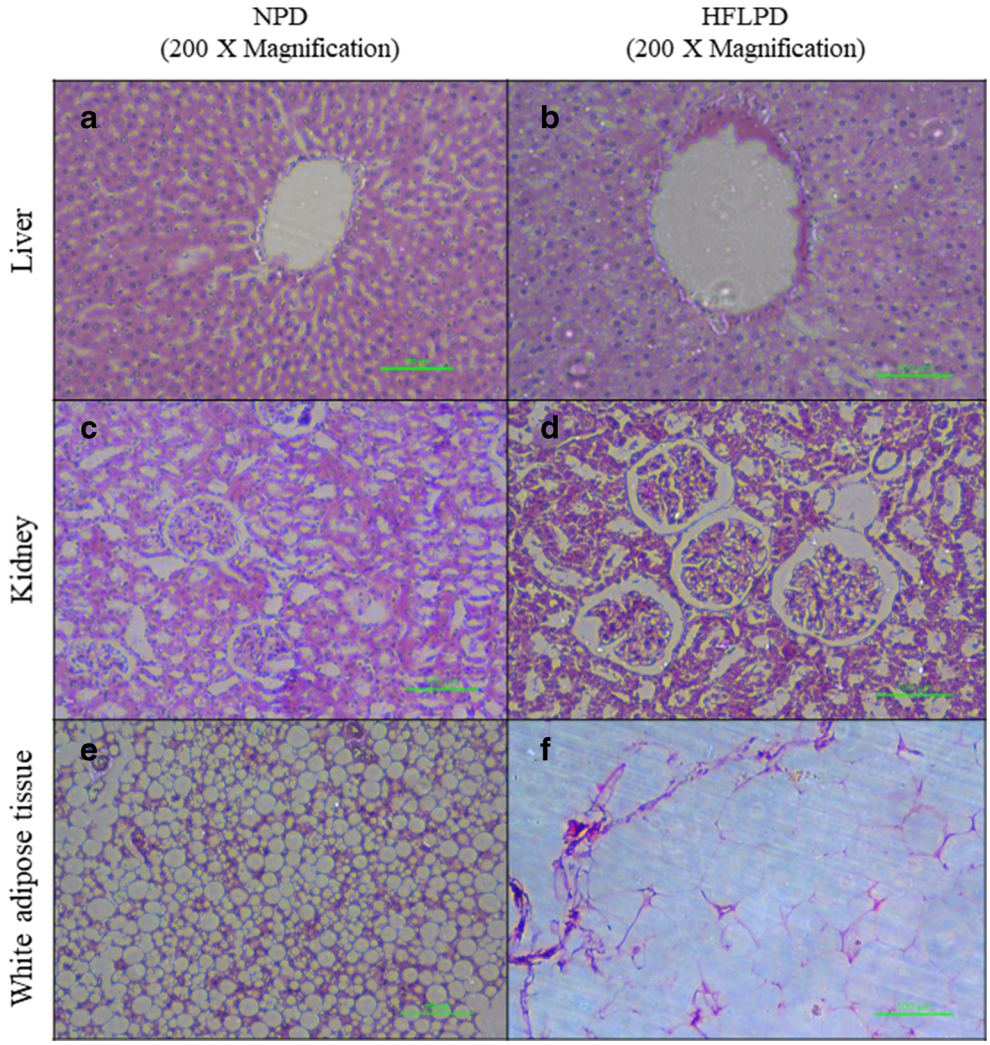

Fig. 9 Effects of HFLPD feeding for 24 weeks on anatomical structures of vital organs. The microphotographs of H&E stained NPD liver (**a**), Kidney (**c**), and WAT (**e**). HFLPD liver (**b**), Kidney (**d**) and WAT (**f**). Magnification-200× and scale bar: 100 μm

reduction in the BAT levels. However, the rise in rectal temperature may be due to the metabolic endotoxemia as a result of altered gut microbiota of HFLPD fed animals (Cani et al. 2007; Cani 2008; Kim et al. 2012).

In this 24-weeks study, HFLPD significantly altered the serum lipid profile from W4 to until the end of the study in a time-dependent manner. Reduced serum HDL, hypertriglyceridemia, increased total cholesterol, and LDL are the consequences of high amounts of saturated and long chain fatty acids present in coconut oil and butter (Wood et al. 1993; Eyres et al. 2016). A meta-analysis on effects of various oils and solid fats on blood lipid levels also concluded that coconut oil and butter are similarly as potent as lard and beef tallow to contribute to the raised blood LDL (Schwingshackl et al. 2018).

The presence of high amounts of carbohydrates in HFLPD and 15% oral fructose feeding might be responsible for the hyperglycemic condition in animals. Uncontrolled and chronic feeding on carbohydrates (Doddigarla et al. 2016) and refined sugars such as fructose (Sachdeva et al. 2018) are few of the significant factors behind hyperglycemia. Chronic feeding of high amounts of sugars and fats in the diet leads to hypertriglyceridemia (Li et al. 2014; Sachdeva et al. 2018). Prolonged hyperglycemia, along with hypertriglyceridemia, results in an insulin resistant condition.

Chronic feeding of HFLPD resulted in elevation of glucose and TG levels in the blood. The excessive glucose and fats converted into adipose tissue, which over the time started to expand through adipocyte hypertrophy, thereby leading to an increase in peritoneal WAT density (Hariri and Thibault 2010).

Elevation of SBP in HFLPD fed rats might be due to the high amounts of saturated fats and carbohydrates in the diet (Hamsi et al. 2015). Chronic feeding of high calorie consumption causes obesity and hypertension (Hall et al. 2014) via various mechanisms such as vasoconstriction due to elevated fat deposition in peripheral blood (Matova and Vihert 1976), activation of the renin-angiotensin system and elevation of oxido-nitrosative stress, thereby leading to cardiovascular remodeling which might be the other reasons for raised SBP (Panchal et al. 2011; Spence and Voskuhl 2012; Hamsi et al. 2015).

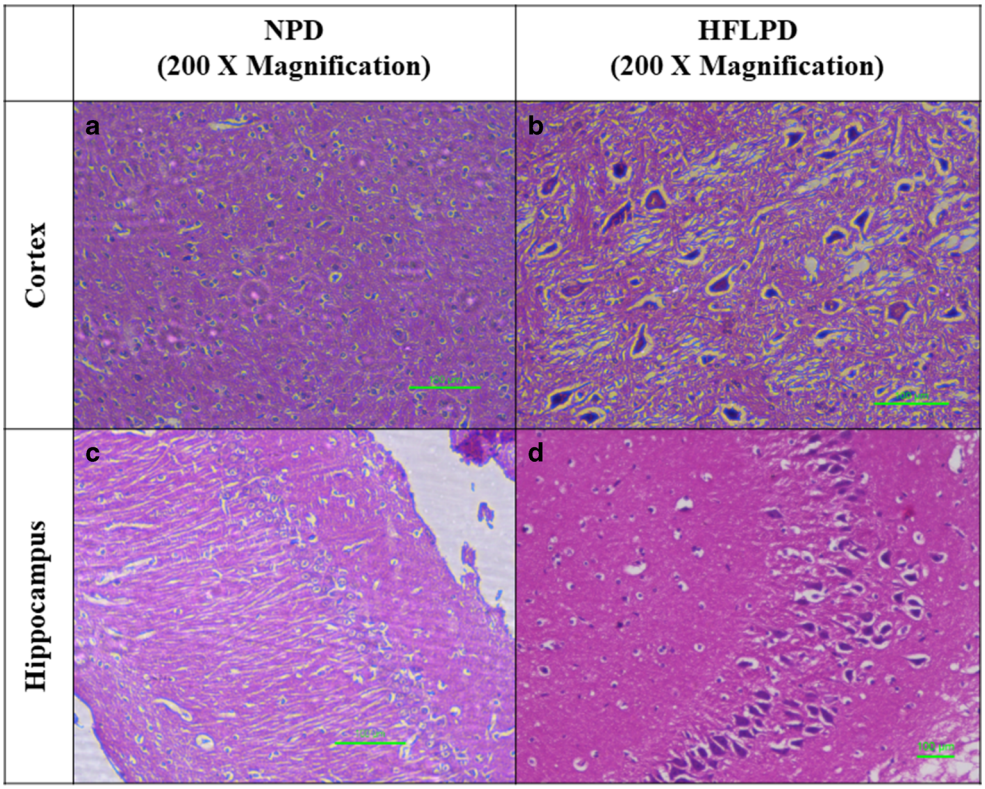

Fig. 10 Effectsof chronic HFLPD feeding on neuroanatomical structures. The H&E stained microphotographs show massive loss of neurons in the cortex (**b**) and hippocampal region (**d**). Magnification-200× and scale bar: 100 μm

The adverse effects of the western diet and cafeteria diet on cognitive functions are well recorded. However, our observations reveal that chronic HFLPD feeding has exerted differential effects on cognitive tasks over time. Rats fed with HFLPD have significantly reduced locomotion, resulted in increased anxiety-like behavior. But, in our context, HFLPD feeding for 12 weeks didn't alter the task learning capacity of the animals despite the HFLPD fed animals showed a reduced escape latency and shorter pathlength to reach the hidden platform in the Morris water maze task. In probe trials, the time spent in target quadrant by the HFLPD fed rats was significantly high at W16 and W20, later, which the TSTQ of HFLPD group seemed to decrease at the W24 (W16 > W20 > W24) and similar type of findings were observed in the case of frequency of entries into TSTQ by HFLPD fed animals (W16 > W20 > W24). These findings show that the HFLPD may facilitate a better task learning and spatial memory retention in the initial periods, but will impact memory retention negatively after W24. This short-lived beneficial effects of HFLPD may be due to the presence of coconut oil in the diet (Spence and Voskuhl 2012). The female reproductive hormones (Estrogen & Progesterone), they have been reported to be neuroprotective in the previous literature (Wang et al. 2010). However, these hormones have not been measured in our study.

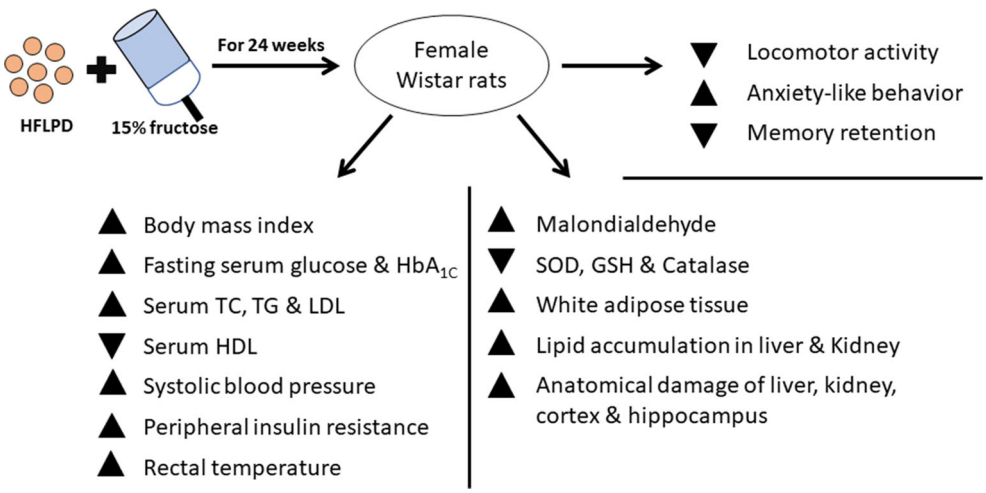

Fig. 11 Feeding of HFLPD and 15% fructose solution led to the alteration of various physiological, metabolic, histological, cognitive, and oxidative stress parameters

We found that the MDA levels in cortex, hippocampus, liver, and kidney of HFLPD rats were increased by many folds as compared to the NPD group. Additionally, HFLPD pointedly reduced the endogenous antioxidant enzyme levels in these organs. Previous studies on HFD feeding reported that the increased oxidative stress is one of the major contributing factors in the pathogenesis of various metabolic (Wei-Chuan et al. 2004; Roberts and Sindhu 2009; Hopps et al. 2010; Noeman et al. 2011; Matsuda and Shimomura 2013) as well as cognitive diseases (Pistell et al. 2010; Cordner and Tamashiro 2015; Kothari et al. 2017). Moreover, the hyperglycemia, hypertriglyceridemia and increased peripheral insulin resistance were linked to cognitive decline in animals (Gregg et al. 2000; Convit 2005; Gunathilake et al. 2016).

Feeding HFLPD for over 24 weeks, leads to lipid accumulation in the liver (Asai and Miyazawa 2001; Meng et al. 2004). A prominent fat accumulation in the kidneys is evident by the glomeruli hypertrophy (Jiang et al. 2005). Fat accumulation in vital organs is due to altered lipid metabolism and hyperphagic conditions. Histopathological microphotographs of brain sections have shown severe anatomical changes in the cortex and hippocampus. HFLPD feeding to the rats led to significant neuronal loss in the cortex and hippocampal structures. These histopathological observations were highly evident as the basis behind the negative impacts of HFLPD on cognitive functions (Fig. 11). Further, more studies are required to confirm the negative effects and underlying mechanisms of low protein diet induced metabolic and cognitive alterations.

Conclusions

The HFLPD is quite different from the western diet and cafeteria diet especially in terms of composition. Moreover, it is fairly relevant to the clinical diets to induce animal model to study MetS and associated abnormalities. Despite having a relatively high amounts of protein, HFLPD has the potential to induce the metabolic and cognitive disorders. Additional 15% fructose in drinking water also exaggerated the development of metabolic and cognitive alterations. we conclude that the time has arrived for us to analyze about our dietary habits and make a transition towards a diet with high amounts of protein and fewer amounts of carbohydrates and fats.

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Author's contributions RND, KC, MB, and KKK had conceived the idea and designed the study. RND & SA did the experimental work, data collection, data analysis, and manuscript preparation. KC, MB, and KKK reviewed and revised the manuscript.

Compliance with ethical standards

Conflict of interest Authors declare no conflicts of interest.

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