Relationship between cognitive functions, levels of NR2A and NR2B subunits of hippocampal NMDA receptors, serum TGF-β1 level, and oxidative stress in rats fed a high-fat diet

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ABSTRACT. Although, excessive caloric intake is known to cause cognitive impairment, the possible mechanism behind this phenomenon is still unknown. Several studies have reported subunit composition changes in hippocampal N-methyl-D-aspartate (NMDA) receptors in rats fed a high-fat diet (HFD). The aim of this study was to test whether potential changes in hippocampal NMDA receptor subunits, which could occur as a result of a HFD, were associated with cognitive impairment, and to investigate their relationship with transforming growth factor-beta 1 (TGF-β1), a cytokine associated with inflammatory events and oxidative stress, which both have been shown to increase obesity. Two groups of rats were formed, one fed a HFD and the other standard chow. After feeding for 23 weeks, the rats' cognitive functions were evaluated using the Morris water maze test. The hippocampi of rats were homogenized and the density of NR2A and NR2B subunits of NMDA receptors was determined. Serum levels of TGF-β1 and malondialdehyde (MDA) were measured. While feeding a HFD caused cognitive impairment, decreased production of the hippocampal NR2B subunit protein, as well as increased serum TGF-β1 and MDA levels, it did not affect the production of the hippocampal NR2A subunit. In addition, a significant correlation was observed between impaired cognitive function and decreased NR2B concentration and increased MDA and TGF-β1 serum levels. Structural changes are likely to occur at the receptor level in the hippocampus as a result of events that increase oxidative stress and TGF-β1 levels in rats fed a HFD, thereby adversely affecting cognitive functions. TGF-β1 may be a signalling molecule that triggers cognitive impairment.

Introduction

In both developed and developing countries, the number of overweight and obese individuals is constantly increasing, and currently 39% of the world's adult population is overweight and more than 13% is obese (WHO, 2021). Although excessive caloric intake and inadequate physical activity are the main causes of weight gain, genetic predisposition, hormonal and metabolic problems, socio-cultural factors, psychological or neurological problems are also among the culprits (Romieu et al., 2017). Obesity is directly or indirectly related to many diseases, such as type 2 diabetes, atherosclerotic heart diseases, hypertension, liver and gallbladder diseases and cancer (Eyre et al., 2004).

It is known that excessive caloric intake and associated obesity and type II diabetes impair cognitive functions such as learning and memory (Duarte, 2014; Moheet et al., 2015; Agustí et al., 2018; Meo et al., 2019). However, the possible mechanisms of cognitive impairment have not been fully explained. Some studies have reported that endothelial dysfunction, blood-brain barrier disruption, neuroinflammation and the resultant neurodegeneration may underlie cognitive impairment (Buie et al., 2019; Liu et al., 2019; Rom et al., 2019; Tan and Norhaizan, 2019). The common rationale behind these studies is that the effect on cognitive functions may be associated with an increase in oxidative stress or inflammatory cascades.

High-fat diets (HFD) have been shown to cause oxidation damage in many tissues (Yuzefovych et al., 2013; Farhangi et al., 2017; Tan and Norhaizan, 2019; Prem and Kurian, 2021). An increase in the amount of fatty acids elevates redox reactions occurring in the cellular electron transport chain, resulting in a higher oxidative stress (Tan et al., 2018). In addition, release of tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) from leukocytes is stimulated, especially in response to saturated fat intake (Joffe et al., 2013). TNF-α and IL-6 enhance the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, increasing the formation of superoxide and H_2O_2 (Bagaitkar et al., 2015; Ding et al., 2019). The renin-angiotensin system is more active in obesity; angiotensin 2 also increases NADPH oxidase activity and superoxide and H_2O_2 formation (Seshiah et al., 2002). Elevated levels of oxidative products, as a result of a HFD, can accelerate the impairment of cognitive functions.

Another consequence of excessive caloric intake is an increased level of transforming growth factor-beta (TGF-β), a cytokine involved in inflammation (Silva Junior et al., 2017; Lee, 2018; Barretto et al., 2020). TGF-β is produced in many mammalian cells, mainly in immune cells. TGF-β is a versatile cytokine with both anti-inflammatory and pro-inflammatory properties (Prud'homme, 2007). Exposure to a HFD stimulates the production of cellular communication signals called adipokines in fat cells. Studies on adipokines have reported that adipose cell-derived TGF-β plays a role in the development of insulin resistance and metabolic syndrome (Hong et al., 2016; Lin et al., 2017).

The hippocampus is a part of the limbic system and is a brain region with functions associated with response inhibition, episodic memory, and spatial cognition. The hippocampus is often involved in neurological diseases that affect memory functions. The most widely currently accepted model describing synaptic changes during memory formation is a phenomenon known as long-term potentiation (LTP). LTP is most prominently detected in the CA1 region of the hippocampus, where synaptic changes are dependent on N-methyl-D-aspartate (NMDA) receptors that use glutamate as a neurotransmitter. NMDA receptors are heterotetramer ion channels, and their subunits determine the function and pharmacological properties of individual receptors. A number of studies have previously been conducted on NMDA receptors with NR2A and NR2B subunits, and reported that these receptors play important roles in synaptic plasticity, learning and memory (Jung and Suh, 2010; Luciano-Jaramillo et al., 2019; Franchini et al., 2020). However, few studies analysed NMDA receptor subunits in high-weight obesity models. Yilmaz et al. (2011) reported that the expression of the hippocampal NR2A and NR2B subunits decreased in obese rats. A more recent study showed that HFD caused a decrease in the levels of hippocampal NR2B subunit, but NR2A was not included in this study (Davis et al., 2020).

In view of the information summarized above, changes in the synthesis of hippocampal NMDA receptor subunits may be related to cognitive impairment observed in high-calorie diet regimens. Moreover, TGF-β – a cytokine associated with inflammatory events – may be associated with possible changes occurring in the synthesis of hippocampal NMDA receptor subunits and may contribute to the onset of cognitive impairment.

The present study aimed to examine the possible effects of a HFD on cognitive functions, NR2A and NR2B subunits of hippocampal NMDA receptors, serum levels of TGF-β1, the prototypical member of the TGF-β family, and oxidative stress (if any), and to determine the relationship of these parameters with each other.

Material and methods

Experimental animals

The approval of the Adnan Menderes University Animal Experiments Local Ethics Committee was obtained prior to the experiments (Permission number: 64583101/2014). The study used 25 male Wistar rats, approximately 8–10 weeks old. Rats were obtained from the Adnan Menderes University,

Faculty of Medicine, Experimental Animals unit. During the experiment, all rats were housed in cages made of polycarbonate material with stainless steel tops, in a relative humidity of 40–60%, optimal temperature of 22 °C and a 12 h light/dark cycle.

Preparation of high-fat feeds

To prepare 1000 g of feed, 600 g of flour-formed standard feed (Bil-Yem, Nukleon, Ankara, Turkey) and 400 g of beef tallow (Alp Et Ürünleri, Aydın, Turkey) were mixed and shaped into pellets. Thus, feeds containing approximately 40% fat were prepared for the HFD group. The control feed was also prepared as pellets, but beef tallow was not added to the mix. The feeds were prepared every other day and stored at 4 °C in a refrigerator. The approximate energy and macronutrient contents of the feeds prepared for the control and HFD groups are given in Table 1.

Table 1. Approximate energy and macronutrient contents of the feeds prepared for the control and high-fat diet (HFD) groups*

Energy and macro-nutrient	Control group diet		HFD group diet	
	g %	calorie %	g %	calorie %
Carbohydrate	54.1	69.8	32.5	25.5
Protein	20.0	23.0	13.3	9.26
Lipid	2.7	7.2	40.3	65.2
Total energy, cal/g	3.49		5.74	

*energy and macronutrient contents of the diets were obtained from the manufacturers/nutrient labels on the packages

Groups and feeding

The rats used in the study were divided into two groups: HFD (n = 15) and control (n = 10). The HFD group was fed a specially prepared high-fat feed, and the control group was fed a standard feed for 23 weeks without food or water restrictions.

Weight measurements

The weight and height of the rats were recorded at the beginning of the experiment and at the end of week 23. The distance between the tip of the nose and the beginning of the tail of the rats was considered as height. A precision balance (Fakir-Scala, Cavory Industrial Co., Hong Kong) was used for weight measurements.

Body mass index (BMI) and Lee index were calculated based on rats' body weights and lengths on day 1 and at the end of week 23 using the following formulas:

$$BMI = body\ weight\ (g)\ /\ square\ of\ body\ length\ (cm^2)$$

$$Lee\ index = cube\ root\ of\ body\ weight\ (g^{1/3})\ /\ body\ length\ (cm)$$

Morris water maze test

The Morris water maze test, which lasted a total of 5 days, was performed at the end of week 23 of the experiment. A Morris water maze with a diameter of 160 cm and a height of 60 cm was used to test learning and memory. The Morris water maze is a large circular water-filled tank with a hidden escape platform (Figure 1A). The Morris water navigation task is a behavioural experiment designed to evaluate spatial memory in rats. After several repetitions of the tasks, reductions in the time spent to reach the escape platform are considered an indicator of the rat's learning and memory abilities.

In the experiment, the Morris water tank was divided into 4 equal quadrants by two imaginary perpendicular intersecting diameters. Visual cues were placed around the inner surface of the pool to help the rats learn to locate the platform. The escape platform was placed 20 cm inside the edge of the pool and was visible at a height of 1.5 cm from the water surface on the first day of practice, and 2 cm underwater and invisible in the practices carried out in the following days. The position of the platform was fixed in all individual practices.
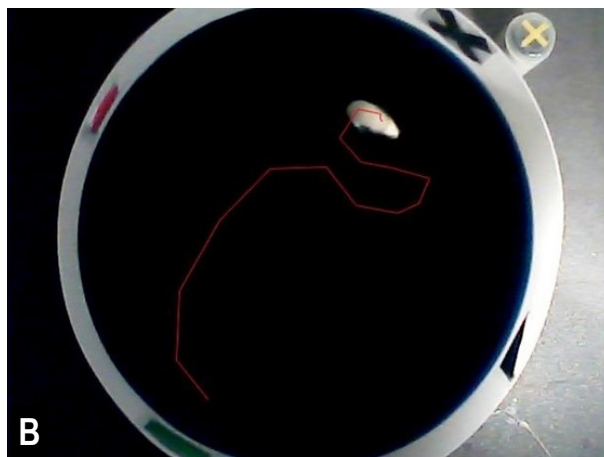

Figure 1. Morris water maze test A) Platform and visual clues, B) Evaluation of video recordings

The inside of the tank was filled with water and its colour was darkened with food dye, preventing the platform from being seen. Before each practice, the temperature of the water was adjusted to 22 °C using a kettle to heat the water and a thermometer to monitoring water temperature.

All water maze tasks performed by the rats were recorded on a computer using a video camera mounted on the ceiling covering the entire Morris water maze. Subsequently, the video recordings were evaluated using MATLAB software (version R2020a; MathWorks, Natick, MA, USA).

The Morris water maze experiments were planned as an adaptation practice on day 1 and learning period practices on days 2, 3, 4 and 5, for a total of 5 days. On the first day, the rats were placed on the platform and kept for 30 s to recognise the escape platform in the tank. The rats were then released into the water from the furthest point from the platform, facing the tank wall and floated for 90 s. During this time, the rats were expected to find the platform. The rats that were unable to find the platform within 90 s were taken out of the water and placed on the platform, and were expected to locate the platform using clues on the walls for 20 s. Subsequently, they were taken from the platform, dried, and placed in their cages. The rats were subjected to two trials on each consecutive experimental day, with a five-minute break between trials. The rats were released into the water from a different quarter of the tank in each practice. The video recordings were then analysed and the time each rat spent to find the platform (escape latency, EL) was calculated (Figure 1B).

Blood collection, rat decapitation and hippocampus removal

One day after the Morris water maze experiments were completed, the rats were anaesthetized by intraperitoneal injection of 10% ketamine HCl (90 mg/kg)-2% xylazine HCl (10 mg/kg). Blood was collected by cardiac puncture with a 20-gauge needle syringe. Blood was transferred to a serum separation tube and centrifuged at 1 200 g at room temperature for 5 min. Sera were aliquoted into Eppendorf tubes and stored at −80 °C until measurements.

After blood collection, the rats were decapitated and brain tissue was exposed by carefully removing the skull bones of each rat starting from the midline on an ice battery soaked with cold phosphate buffer. The two lobes were then separated from each other, and a straight edge was obtained by making small incisions in each of the frontal lobes. The brain tissue was lifted upright on the frontal part and the hippocampus was separated from the brain tissue.

Hippocampus homogenisation

Rat hippocampi were placed in individual Eppendorf tubes filled with cold phosphate buffer. After each hippocampus sample was weighed, it was homogenised by 20 strokes of a glass Teflon homogenisation apparatus in a homogenisation buffer containing antiprotease to ensure adequate protein concentration. Homogenates were centrifuged at 10 000 g at 4 °C for 15 min (Jouan B4i Centrifugeur, St. Herblain, France), portioned into 5 pieces and stored at −80 °C (Nüve DF-490 Deep Freeze, Ankara, Turkey).

Western blot protocol: NR2A and NR2B NMDA receptor subunit analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was based on Laemmli's method (Laemmli, 1970). The appropriate homogenates were dissolved before electrophoresis and proteins were determination from their supernatants using a commercial kit compatible with Beckman Coulter AU5800 (Beckman Coulter Inc., Brea, CA, USA). Tissue homogenates were then mixed with sample buffer (Laemmli buffer) at a ratio of 1:1, and 9% lower and 4% upper gels were prepared daily.

Sample volumes to be seeded were calculated at a concentration of 25 µg of protein per well. The samples and marker samples (Prestained Molecular Weight Marker, Sigma-Aldrich, St. Louis, MO, USA) were activated in an oven at 95 °C for 5 min, and loaded on a 10-well gel with the marker on top. Electrophoresis was performed for 1.5 h by applying an electric current of 110 V and 17 mA per gel.

The samples in the SDS-PAGE procedure migrated on the gel according to their molecular weight. Gel-wide polyvinylidene difluoride (PVDF) membrane (Immobilon-P PVDF Membrane, Merk, Darmstadt, Germany) was activated by soaking in methanol and distilled water for 2 min for 30 s and then transferred to a transfer tank along with the gel. The transfer process was carried out for 1.5 h using an electric current of 100 V, 300 mA.

After the transfer procedure, the membranes were individually soaked overnight in solutions containing anti-β-actin + anti NR2A and anti-β-actin + anti NR2B. After overnight incubation, the membranes were incubated for 1 h with secondary antibody at a 1/10000 dilution (Sigma-Aldrich, St Louis, MO, USA) and kept in freshly prepared 5-bromo-4-chloro-3-indolyl-phosphate (BCIP)/4-nitro blue tetrazolium chloride (NBT) solution until sufficient staining was achieved. The resulting bands were scanned using a Kodak Image Station

2000MM (Eastman Kodak Company, New Haven, CT, USA) and blot densities were analysed using KODAK 1D Image Analysis software (version 3.5, Eastman Kodak, Rochester, NY, USA). The obtained band intensities were evaluated for each receptor subunit after normalisation with β-actin and compared with the control group.

Determination of serum malondialdehyde (MDA) and TGF-β1 levels

The BioVision Lipid Peroxidation (MDA) Fluorometric Assay Kit (Cat. No: #K739; BioVision Inc., Milpitas, CA, USA) was used to determine MDA content; the Invitrogen Rat TGF beta 1 ELISA Kit was used for TGF-β1 determination (Cat. No: BMS623-3; Thermo Fisher Scientific, Waltham, MA, USA). The manufacturers' instructions were followed for both assays. Measurements were performed using a Spectramax-i3 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Statistical analysis

All data obtained were evaluated using Graph-Pad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Sample size calculations were performed to avoid type I and type II errors. The D'Agostino-Pearson and the Shapiro-Wilk normality tests were used to determine whether the data followed the normal distribution. The Mann-Whitney U test was used for comparisons between groups and the Spearman correlation test was used to evaluate the relationships between parameters. Bonferroni-corrected two-tailed P-values < 0.05 were considered as significant, < 0.01 very significant, and < 0.001 extremely significant. Scatter plots were drawn using Python 3.10 and its matplotlib 3.5.1 module (downloadable from https://www.python.org/ and https://matplotlib.org/).

Results

Observations of qualitative changes during the experiment

Two rats, one from the HFD group and one from the control group, died during the 23-week experimental period. Other rats maintained a healthy appearance throughout the experiment. Both groups of rats exhibited similar behaviour in terms of their mobility, water consumption and eating habits. It was observed that the rats from the HFD group gradually gained weight when compared to the control group at the end of the experiment.

Height and weight measurements

The body weights and heights of all rats were measured on the first day and at the end of week 23. The BMI and Lee index values calculated from these two values are given in Table 2.

Table 2. Body weight and height of the rats from the high-fat diet (HFD) and control groups on the first day and at the end of week 23

		Control group	HFD group	P-value
Day 1	Weight, g	277 ± 46.8	278 ± 20.1	0.98
	Height, cm	21.0 ± 1.25	20.6 ± 0.663	0.38
	BMI	62.4 ± 5.30	65.3 ± 4.93	0.20
	LEE I	0.310 ± 0.007	0.316 ± 0.010	0.11
Week 23	Weight, g	448 ± 61.2	495 ± 29.2	0.023*
	Height, cm	26.7 ± 1.12	25.6 ± 0.594	<0.01**
	BMI	62.8 ± 5.26	75.4 ± 3.05	<0.001***
	LEE I	0.286 ± 0.007	0.309 ± 0.005	<0.001***

body mass index (BMI) and Lee index (LEE I) calculated based on body weight and height values; data are presented as mean value ± SEM (standard error of the mean); *,**,*** – means within a row with different superscripts are significantly different (* $P < 0.05$ significant, ** $P < 0.01$ very significant, *** $P < 0.001$ extremely significant)

Both groups of rats did not differ statistically in terms of weight, height, BMI and Lee index on the first day of the experiment. At the end of week 23, the weight gain of the HFD rats was significantly higher ($P = 0.023$). On the other hand, their height was significantly shorter ($P = 0.007$). We expected that the height and weight of the rats from the HFD group would increase. There could be several reasons for this result. On the first day of the experiment, the weights were measured after the rats randomization into two groups as HFD and control. Although there was no statistical difference, the control group was 4 mm taller at baseline. At the end of the experiment, this difference reached 11 mm and was statistically significant. One of the hormonal height determinants is insulin, which increases protein synthesis and accelerates bone development. While the amount of fat in the diet administered to the HFD group was high, the carbohydrate content, which increased insulin synthesis, was relatively low. Perhaps the relatively low carbohydrate content, not the high-fat level itself, may have caused this result. At the end of week 23, both BMI and Lee indices were quite high in the HFD group ($P < 0.001$). Our results in terms of body weight, BMI, and Lee indices were expected, and thus we observed that high-calorie feeding caused overweight in rats from the HFD group.

Morris water navigation tasks

At the end of week 23 of the experiment, Morris water navigation tasks, which lasted for a total of

5 days, were performed. The escape latency (EL) values of both groups of rats in the Morris water maze test are given in Table 3, and the daily changes in EL are shown in Figure 2. There was no statistical difference between the two groups in terms of EL on day 1, which was the exercise day, and the following days 2 and 3 ($P = 0.88$, $P = 0.93$, $P = 0.53$, respectively). On day 4 of the experiment, HFD rats had longer ELs compared to the control group, but this difference did not fall within the accepted significance limits ($P = 0.081$). On day 5 (final), i.e. the last day of the Morris water test, ELs of the HFD group were statistically longer compared to the control group ($P = 0.022$).

Table 3. Escape latency values of the rats from the high-fat diet (HFD) and control groups in the Morris water maze test

	Day				
	1	2	3	4	5 (final)
HFD	47.0 ± 32.8	37.1 ± 26.5	27.0 ± 15.0	21.9 ± 9.12	13.5 ± 11.0
Control	44.6 ± 38.6	35.9 ± 30.7	31.7 ± 18.9	14.9 ± 8.62	5.56 ± 3.09
P-value	0.88	0.93	0.53	0.081	0.022*

data are presented as mean value ± SEM (standard error of the mean);
* – means within a row with different superscripts are significantly different (* *P* < 0.05 significant)
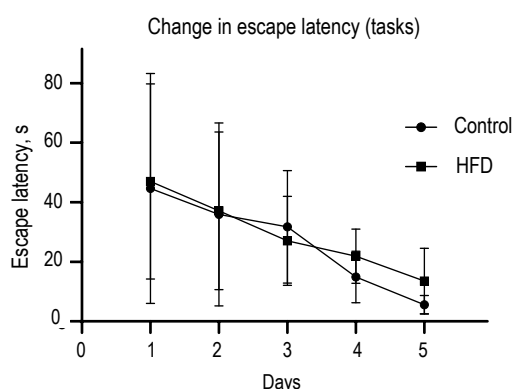

Figure 2. Daily variation in escape latency in the Morris water maze test of the rats from the high-fat diet (HFD) and control group

Analysis of the hippocampal NR2A and NR2B receptor subunits

After SDS-PAGE, the samples of hippocampal tissues were labelled with anti-β-actin + anti-NR2A and anti-β-actin + anti-NR2B antibodies. The bands stained with the secondary antibodies are shown in Figure 3. After all band intensities were analysed using an image analysis software, the intensities of NR2A and NR2B receptor bands were normalized to β-actin band intensities determined in the same wells. For each receptor subunit, the average band intensity values in the control group were adjusted to 100, and band intensities of the NR2A and NR2B receptors of the rats from the HFD group were
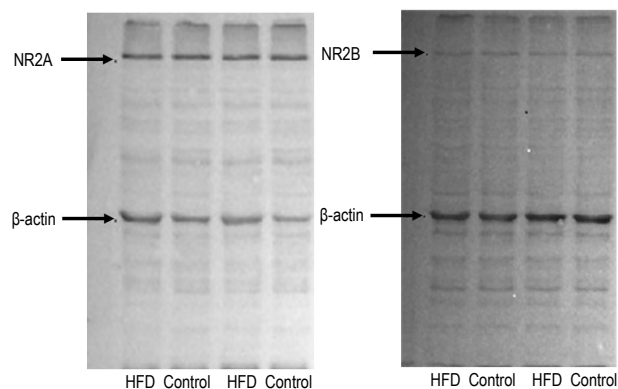

Figure 3. Western-Blot analysis of hippocampal NR2A and NR2B receptor subunits isolated from the rats fed the high-fat (HFD) and control diet

calculated numerically in proportion to the control group (Table 4). According to the data obtained, it was observed that the intensity of the NR2A receptor bands did not differ significantly between the groups ($P = 0.33$); however, the intensity of the NR2B receptor bands was significantly reduced in the HFD group ($P < 0.001$).

Table 4. Numerical values of band intensities of NR2A and NR2B receptors isolated from the rats from the high-fat diet (HFD) and control groups

	NR2A	NR2B
HFD	98.3 ± 3.21	80.2 ± 5.73
Control	100 ± 4.33	100 ± 5.78
P-value	0.33	<0.001***

band intensities were normalized to actin control; data are presented as mean value ± SEM (standard error of the mean); *** – means within a row with different superscripts are significantly different (*** *P* < 0.001 extremely significant)

MDA and TGF-β1 analysis

The study used MDA levels as an indicator of oxidative stress. The subject of the current work was TGF-β1, a prototypical member of the TGF-β superfamily. Serum MDA and TGF-β1 levels in the HFD and control group are listed in Table 5. Both MDA and TGF-β1 concentrations were significantly higher in the HFD group compared to the control group ($P < 0.01$, $P < 0.001$, respectively).

Table 5. Serum malondialdehyde (MDA) and transforming growth factor-beta 1 (TGF-β1) levels isolated from the rats fed the high-fat (HFD) and control diet

	MDA, nmol/ml	TGF-β1, pg/ml
HFD (n = 14)	52.9 ± 3.82	13.9 ± 3.55
Control (n = 9)	44.3 ± 6.28	6.13 ± 0.516
P-value	<0.01**	<0.001***

data are presented as mean value ± SEM; **,*** – means within a row with different superscripts are significantly different (** *P* < 0.01 very significant, *** *P* < 0.001 extremely significant)

Correlation matrix

A correlation matrix was created for both groups using body weights, heights, BMIs, Lee indices, final ELs of the water maze tasks, hippocampal NR2A and NR2B receptor densities, and serum MDA and TGF-β1 levels obtained in the study. The correlation matrices of the HFD and control group are shown in Figure 4 and 5, respectively.

In the HFD group, there was a high positive correlation between BMI and MDA, a moderate correlation between BMI and TGF-β1, and a negative correlation between BMI and NRB2 receptor density. In addition, BMI showed a weak relationship with final EL. These parameters, which were associated with BMI, also showed a similar correlation with weight and Lee's index, but the correlation coefficient values were lower compared to BMI. NR2A receptor density was not correlated with any parameter. However, NR2B receptor density demonstrated a high negative correlation with TGF-β1 and a moderately negative correlation with BMI, weight, final EL and MDA. In addition, a moderately positive correlation was observed between MDA and TGF-β1. Scatter plots of the TGF-β, MDA and NR2B values in the HFD group are shown in Figure 6.
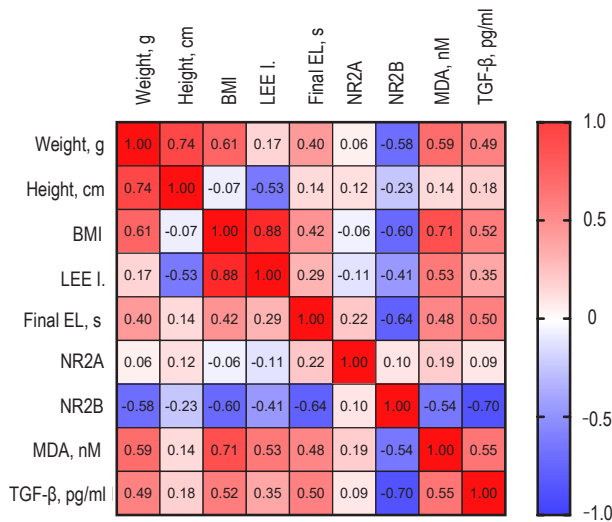

Figure 4. Correlation matrix of the data for the high-fat diet group (> 0.7 strong relationship, 0.5–0.7 moderate relationship, 0.3–0.5 weak relationship, < 0.3 negligible). BMI – body mass index, LEE I – Lee index, Final EL – final escape latency, MDA – malondialdehyde, TGF-β1 – transforming growth factor-beta 1
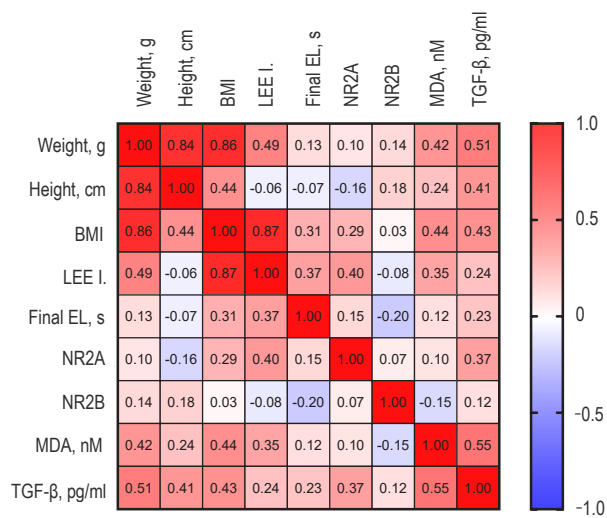

Figure 5. Correlation matrix of the data for the control group (> 0.7 strong relationship, 0.5–0.7 moderate relationship, 0.3–0.5 weak relationship, < 0.3 negligible). BMI – body mass index, LEE I – Lee index, Final EL – final escape latency, MDA – malondialdehyde, TGF-β1 – transforming growth factor-beta 1
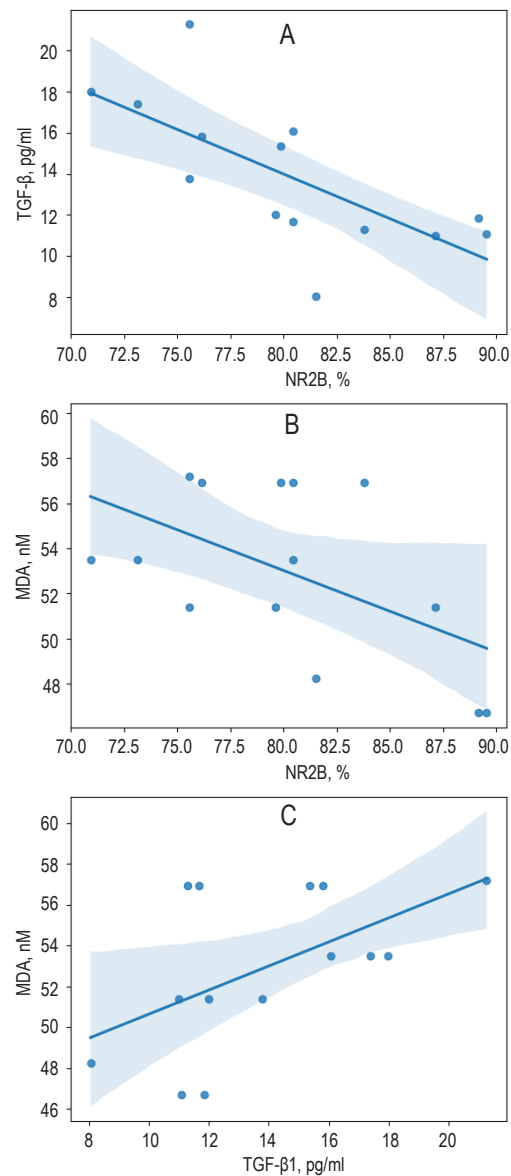

Figure 6. Scatter plots of transforming growth factor-beta 1 (TGF-β1), malondialdehyde (MDA) and NR2B levels of the rats from the high-fat diet (HFD) group. A) Relationship between TGF-β1 and NR2B, B) Relationship between MDA and NR2B, C) Relationship between TGF-β and MDA

In the control group, there was a moderate or mild correlation between BMI and weight values and TGF-β1 and MDA. However, the correlation of BMI and body weight with the final EL and NR2B receptor density observed in the HFD group was not detected in the control group.

Discussion

The present study aimed to examine the possible effects of HFD on cognitive functions, NR2A and NR2B subunits of hippocampal NMDA receptors, oxidative stress, and TGF-β1 levels in an animal model, and to determine the potential interrelationships of these parameters. To summarize the data obtained in the study, feeding a HFD prolonged EL and decreased the number of hippocampal NR2B receptors. The results showed that there was a relationship between decreased NR2B density, oxidative stress and immune processes elevating MDA and TGF-β1 levels. Moreover, cognitive functions were negatively affected by structural changes in the level of receptors in the hippocampus.

The study that inspired us to carry out this research was the work of Yılmaz et al. (2011), who showed that the expression of hippocampal NR2A and NR2B subunits was reduced in obese rats. We continued this line of research and aimed to find an association of these receptors with cognitive functions (tested by Morris water maze), and with markers such as MDA and TGF-β1 that provide information about oxidative stress and inflammatory events. While our study was ongoing, another study was published by Davis et al. (2020) confirming that HFD reduces the level of the hippocampal NR2B subunit. Our study showed that feeding a HFD reduced the formation of N2RB protein, a hippocampal NMDA receptor subunit, and this decrease was correlated with both oxidative stress and TGF-β1 levels. There was no decrease in NR2A receptor subunit levels in our study, which contradicted findings of Yılmaz et al. (2011) regarding NR2A.

The hypothalamus, i.e. the centre of homeostatic mechanisms, maybe the first region in the brain to be affected by oxidative stress and inflammation in the deterioration of cognitive functions caused by high-calorie nutrition. Since some regions of the hypothalamus lack an effective blood-brain barrier, fenestrations in the endothelial layer in these regions are large enough to allow free passage of large molecules (Haddad-Tóvolli et al., 2017). Free fatty acids such as palmitate, free oxygen radicals, inflammatory cytokines and activated immune cells, whose levels increase as a result of a HFD, reach the hypothalamus through the blood, where they initiate local inflammation, including microglial proliferation (Gyengesi et al., 2012; Miller and Spencer, 2014; Kashima and Hata, 2018; Liu et al., 2019; Melo et al., 2020). This local inflammation can cause synaptic remodelling and neurodegeneration in the hypothalamus, altering the output of the hypothalamus to the hippocampus, amygdala, and reward processing centres, thereby affecting memory, learning, motivational and emotional functions (Miller and Spencer, 2014). In addition to the above, it is likely that oxidative stress and inflammation directly affect neurons associated with cognitive functions through endothelial dysfunction and disruption of the blood-brain barrier (Buie et al., 2019; Liu et al., 2019; Rom et al., 2019; Tan and Norhaizan, 2019a).

We have not encountered any information in the literature on the possible causes of the downregulation of hippocampal NR2B receptor subunit at the molecular level. It is conjectured that synaptic abnormalities between the hypothalamus and hippocampus may have caused downregulation of NR2B receptor expression. Perhaps oxidative stress and inflammatory processes may directly affect the hippocampus, resulting in inhibition of the expression of NR2B genes. We hope that possible mechanisms will be clarified in future research.

TGF-β1 induces differentiation of T helper 17 cells, which causes both inflammation and increased autoimmune events (Korn et al., 2009). However, there is a common opinion in the literature that TGF-β1 expression increases as part of an anti-inflammatory reaction that develops as a response to inflammation (Sanjabi et al., 2009). Scientists reported that TGF-β1 acted as a neuroprotective and neurotrophic factor and even claimed that it played an important role in hippocampal synaptic plasticity and memory functions (Caraci et al., 2015).

In addition to being one of the end products of lipid peroxidation, MDA can also be generated by the breakdown of arachidonic acid and larger polyunsaturated fatty acids as by-products in enzymatic processes during the biosynthesis of thromboxane A2 and 12-l-hydroxy-5,8,10-heptadecatrienoic acid (Ayala et al., 2014). In addition, MDA production may be mediated by non-enzymatic oxidation mechanisms that have not yet been fully elucidated. Therefore, MDA is considered a general indicator of oxidative stress rather than

a specific marker of lipid peroxidation (Trevisan et al., 2001). Our findings suggest that oxidative stress may be responsible for these changes in the hippocampus.

Present study found that TGF-β1 expression increased as a result of feeding a HFD and was negatively associated with cognitive functions. Perhaps locally secreted TGF-β1 in tissues exerted an anti-inflammatory effect, while TGF-β1 originating from distant organs could have induced a pro-inflammatory reaction in target tissues. Other alternative could involve a gradual increase in TGF-β1 secretion to counteract or counterbalance the effects of oxidative stress or inflammatory events induced by a HFD. We have not found any studies in the literature that would answer these questions. We hope that the possible mechanisms of TGF-β1-induced deterioration in cognitive functions of rats fed a HFD will be revealed in future studies.

Conclusions

A high-fat diet reduced the concentration of hippocampal NR2B receptors. The reason for this decrease may be oxidative stress and immune processes that cause an increase in MDA and TGF-β1 levels.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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