



Original Article

Effects of Treadmill Exercise and Tryptophan Supplementation on Antioxidant Level, Synaptic mRNA Expression, and Memory Function in High-Fat-Diet Aging Rats

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ABSTRACT

Introduction: Exercise training with antioxidant and anti-inflammatory supplements plays an effective role in dementia progression, and tryptophan is an essential amino acid that can be supplied through the diet. The present study aimed to investigate the effects of treadmill exercise and tryptophan supplementation on brain antioxidant levels, *synapsin 1*, *brain-derived neurotrophic factor (BDNF)*, and *postsynaptic density-95 (PSD-95) mRNA* in rats fed a high-fat diet (HFD).

Materials and methods: A total of 75 male rats, 2-3 months old, with an average weight of 527 grams, were randomly assigned to five groups, with five rats per group and three replicates. The first group was administered the standard low-fat diet daily (LF), the second group was administered the HFD daily (HF), the third group was administered the HFD along with a tryptophan supplement at 250 mg/kg/orally (HFTS), the fourth group was given the HFD along with the tryptophan supplement and exercised daily (HFETS), and the fifth group was fed the HFD only and exercised (HFE). Exercise training was performed on a rodent treadmill, three days/week for eight weeks. At the end of the exercise protocol and cognitive analysis (light/dark maze and Barnes maze analyses), rats were euthanized, and brain tissue was collected for real-time polymerase chain reaction analysis of brain antioxidants, *synapsin 1*, *BDNF* and *PSD-95* mRNA.

Results: The HFD significantly raised serum total cholesterol and triglycerides compared to the low-fat diet, while the HFETS group reduced cholesterol to levels similar to the LF group. In the Barnes maze test, the HFETS group demonstrated the shortest escape time and fewest errors, indicating improved spatial learning, whereas no differences in anxiety-like behavior were observed in the light/dark maze analysis. Additionally, HFE and HFETS groups exhibited higher catalase levels and restored hippocampal *PSD-95* expression, while *SYN1* expression was increased in all HFD-fed groups than the LF group. *BDNF* expression did not differ significantly across all groups.

Conclusion: In aged rats, combining exercise with tryptophan supplementation partially mitigated HFD-induced metabolic disturbances and improved spatial learning and memory. Exercise alone was more effective than tryptophan supplementation in preventing the adverse effects of an HFD during aging.

1. Introduction

L-tryptophan is one of the eight essential amino acids that cannot be synthesized in the bodies of animals and

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humans and should be obtained from external sources¹. In humans, tryptophan has relatively low tissue storage, and the overall concentration of tryptophan within the body is the lowest among all amino acids, although only small quantities are required for general healthy nutrition¹. The positive effects of tryptophan on skeletal muscle and the central nervous system (CNS) have been found². It was observed that tryptophan metabolism was enhanced by exercise in the animal models³. Notarangelo et al.⁴ demonstrated that physical training may help the central nervous system by removing blood waste metabolites. It has been reported that the proportion of tryptophan used for serotonin production is very low, with estimates indicating that approximately 10% of the total tryptophan is converted into serotonin⁵.

Serotonin (5-Hydroxytryptamine) is a neurotransmitter in CNS⁶. Any reduction in serotonin signaling capacity leads to cognitive and memory impairment⁶. The serotonin deficiency due to aging causes neurodegeneration and necrosis of the hippocampus Cornu Ammonis 1 (CA1), CA3, and dentate areas⁶. The compromised serotonergic function may be an essential contributor to cognitive decline in Alzheimer's disease and aging⁷. It has been indicated that the bilateral injection of serotonin 3 receptor antagonist on the CA1 lateral hippocampus can reduce amnesia in morphine-administered mice⁸. Serotonin can affect nerve function, morphology, and even neuronal growth in the hippocampus⁹.

Incremental exercise and dietary tryptophan supplementation improve amino acid metabolism, serotonin status, and body composition in the animal model¹⁰. Aerobic exercise, such as swimming, induces *brain-derived neurotrophic factor (BDNF)* in older adult rats, thereby enhancing cognitive function¹¹. Furthermore, different aerobic exercises could improve memory in older adults with Alzheimer's disease¹¹.

Previous studies demonstrated that a routine high-fat diet (HFD) can impair cognitive and memory functions^{8,12,13}. Exercise training, alongside *BDNF*, enhances cerebral blood flow, elevates neurogenesis, and modulates neurotransmitter activity, all of which contribute to improved memory function in healthy individuals and patients¹⁴. In addition, different training loads have significant effects on the cognition and mental status of rats and can affect the mRNA and protein expression of *Postsynaptic density-95 (PSD-95)*¹⁵.

Postsynaptic density-95 is a major component of the postsynaptic density at excitatory synapses¹⁶. The genomic studies link *PSD-95* dysfunction to neuropsychiatric disorders such as schizophrenia, autism spectrum disorder, and intellectual disorder¹⁷. The hippocampus region is responsible for memory and cognition^{18,19}. The *BDNF*, *PSD-95*, and *Synapsin I (SYN1)* proteins help assess the severity of degenerative nerve disease in the animal model²⁰.

Exercise training in healthy individuals and patients can mitigate and manage the risk factors associated with aging in memory cognition¹¹. Advancing age and detrimental lifestyle habits, such as inadequate nutrition and high-fat consumption, contribute to erosive alterations in brain

tissue and compromise memory function. The present study aimed to investigate the effects of treadmill exercise and tryptophan supplementation on antioxidant levels, *SYN1*, *PSD-95* mRNA, and age-related cognitive decline in rats fed an HFD.

2. Materials and Methods

2.1. Ethical approval

The guidance from the National Institute of Health (NIH) for the care and use of laboratory animals (NIH publication No. 80-23, revised 1996), along with professional government guidelines, was followed in accordance with the Institutional Animal Care and Use Committee (IACUC) at Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

2.2. Study design

A total of 75 male Wistar rats aged 2-3 months, with an average weight of 527 g, were purchased from the laboratory animal care unit of Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran, and kept under laboratory conditions with free access to water and commercial food (BFM, Iran) with crude protein of 20% and 3100 Kcal/kg metabolizable energy. The adaptation period was conducted seven days before the start of the experiment. The rats have been kept in standard cages with a minimum humidity of 50%, a temperature of 24°C, and a 12-hour dark/light cycle, ensuring proper ventilation in each cage. After the pre-clinical health assessment, the rats were randomly divided into five experimental groups, with five rats per group and three replicates. The treatment groups included the first group, which received the standard low-fat diet daily (LF), the second group, which received the HFD daily (HF), the third group, which received the HFD with a tryptophan supplement daily (HFTS), the fourth group, which received the HFD along with the tryptophan supplement and exercised daily (HFETS), and the fifth group, which received the HFD only and exercised (HFE). Tryptophan supplements (Shaanxi TNJONE Pharmaceutical Co, China) were administered at a dosage of 250 mg/kg/orally²¹. The HFD was formulated based on the composition reported by Levin et al.²². The HFD contained 414.0 kcal/100 g metabolizable energy, with 43% of carbohydrate, 17% of protein, and 40% of fat (Table 1).

Table 1. Ingredient composition of the high-fat and basal diet fed to rats during 8 weeks of the study

Ingredient	High-fat diet (g/kg diet)	Basal diet (g/kg diet)
Corn starch	150	650
Casein	200	200
Tallow fat	400	0
Oil	0	50
Sucrose	150	0
Cellulose	50	50
Vitamin mix	10	10
Methionine	3	3
Salt	2	2

2.3. Exercise training

Following a two-week period of acclimating rats to the exercise training protocol, an 8-week exercise program was initiated for the exercise groups. Exercise training was performed on a rodent treadmill at speeds ranging from 12 to 56 meters per minute, three days per week for 8 weeks, and the speed increased daily (Table 2). Exercise training continued for up to 3 sessions/week, consisting of one minute of exercise and two minutes of rest (5 minutes/minute) for 10 minutes¹². The rats were euthanized at the end of the experiment (week 8), and the hippocampus was dissected as the sample tissue for molecular assays.

Table 2. Progressive treadmill exercise protocol consisting of one-minute running and two-minute active rest cycles for rats over 8 weeks

Week	Session	Speed m/minute	Slope (degree)
1	1	12	0
	2	13.5	
	3	15	
2	1	17	0
	2	18.5	
	3	20	
3	1	22	± 5
	2	23.5	
	3	25	
4	1	27	± 5
	2	28.5	
	3	30	
5	1	32	± 10
	2	34	
	3	36	
6	1	38	± 10
	2	40	
	3	42	
7	1	42	± 15
	2	44	
	3	46	
8	1	48	± 15
	2	50	
	3	52	

2.4. Serum biochemical analysis

2.4.1. Total cholesterol and triglycerides

At the end of week 8, approximately 3 mL of blood was collected into EDTA and lithium heparin tubes via cardiac puncture. Intraperitoneal injection of Zoletil (tiletamine 15 mg/kg and zolazepam 15 mg/kg; Virbac, France) was administered to the rats to alleviate pain. To confirm hyperlipidemia induction, serum total cholesterol (TC) and triglyceride levels, which are indices of hyperlipidemia, were measured using an automated biochemical analyzer (Accent 200, China) in all groups.

2.4.2. Glutathione peroxidase and catalase

The blood samples were centrifuged for 15 minutes at 1,200 rpm to separate blood plasma. The antioxidant level and the activities of catalase (CAT) and glutathione peroxidase (GPX) enzymes in plasma samples were measured using a commercial colorimetric assay kit (Accent 200, China) according to the manufacturer's protocol.

2.5. Barnes maze test

The Barnes maze was applied in the last week of the

present study. Rats were released for 30 seconds in the middle of the maze. Upon locating the escape box, the latency to find the hole, the number of errors, and the distance traveled during exploration were recorded. Each trial duration was set to 90 seconds. Trials longer than 90 seconds were considered a failure. Errors were characterized by nose pokes and head deflections¹³. A video was recorded of the rats in the maze, and their movements were then analyzed.

2.6. Light/Dark maze test

The maze behavioral model, along with elevation, was used to measure anxiety. The tool was made of wood and had four arms, similar in shape to the plus sign (+). The dimensions of the open and closed arms were 10×50, and there were 40 cm high walls on both sides and at the end of the closed arm. The four arms were related to a central circumference with a 10 cm radius. The height of the maze was 50 cm. At the same time, the rats were placed in the central region facing an open arm. During the five minutes when the animal moved freely through different maze parts, four parameters were measured, including the number of entries into open or closed arms and the time spent in open or closed arms. Entering the open or closed arm means placing all four legs of the animal in the desired arm. The percentage of entries and the time spent in the open arm were calculated using formulas 1 and 2⁴.

$$\text{The percentage of entries to the Open arm} : \frac{\text{Number of entries to open arms}}{\text{Number of entries to open} + \text{Close arms}} \times 100$$

(Formula 1)

$$\text{The percentage of settlement to the open arm} : \frac{\text{Time of entries to open arms}}{\text{Time of entries to open} + \text{Close arms}} \times 100$$

(Formula 2)

2.7. Real-time PCR

The *BDNF*, *PSD-95*, and *SYN1* expression was evaluated by real-time polymerase chain reaction (PCR) analysis. Total RNA was extracted from the hippocampal tissue of rats using TRIzol reagent according to the manufacturer's protocol. The TRIzol reagent was used for RNA extraction, a common and established method that involves cell lysis and the separation of RNA based on its solubility in acidic phenol-chloroform. Subsequently, the PrimeScript RT kit (Takara Bio Inc., Japan) was employed for complementary DNA (cDNA) synthesis. This kit included reverse transcriptase enzymes that converted the extracted RNA into cDNA, a more stable form suitable for downstream applications such as PCR or sequencing. Then, primers were used with the cDNA templates for real-time PCR amplification, along with SYBR Premix2. The primer sequences used are shown in Table 3²⁵⁻²⁷. The ABI 7,500 Fast Real-Time PCR System (California, USA) was used for the present study.

Table 3. Primer sequences used in the present study for real-time PCR

Gene	Product length (Base pairs)	Primer sequences	Reference
<i>SYN 1</i>	202	F: GCAGTTTGGTCATTGGGCTG R: ACAGGGTATGTTGTGCTGCT	(25)
<i>BDNF</i>	165	F: ACCCTGAGTTCCACCAGG R: CCAGAGTCCCATGGGTCC	(26)
<i>PSD-95</i>	132	F: AGATGAAGACAGCCCCCTC R: CCTCTGTTCCATTCACCTGC	(27)

BDNF: Brain-Derived Neurotrophic Factor, *PSD-95*: Postsynaptic density-95, *SYN1*: Synapsin I, F: Forward, R: Reverse

2.8. Statistical analysis

The data are presented as mean $\pm$ standard deviation (SD). To verify whether the data followed a normal distribution, the Shapiro-Wilk test was used (IBM SPSS Statistics 20). The comparison of the brain variable contents between groups was performed using Two-Way ANOVA. The significance level was set at a p-value less than 5 % ($p < 0.05$).

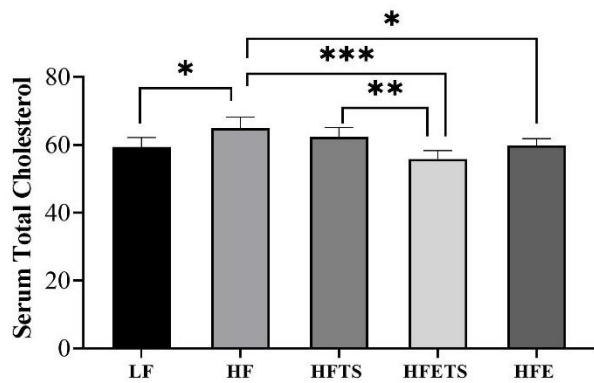


Figure 1. Serum fat levels of the rats in experimental groups at the end of week 8. LF: Low-fat diet, HF: High-fat diet, HFTS: High-fat diet with tryptophan supplement, HFETS: High-fat diet with tryptophan supplement and exercise, HFE: High-fat diet with exercise. The cholesterol rate is significantly higher in the HF group than in the HFETS and HFE groups. Values are presented as mean $\pm$ SD.

Table 4. Fat molecules in the serum of the rats in the experimental groups at the end of week 8

Group	TC (mmol/L)	TG (mmol/L)
LF	1.45 $\pm$ 0.14	0.68 $\pm$ 0.14
HF	4.36 $\pm$ 1.70 ^a	0.91 $\pm$ 0.10 ^a
HFTS	2.15 $\pm$ 0.23 ^b	0.70 $\pm$ 0.22 ^b
HFETS	1.47 $\pm$ 0.19 ^c	0.72 $\pm$ 0.13 ^b
HFE	2.35 $\pm$ 0.40 ^b	0.75 $\pm$ 0.26 ^b

LF: Low-fat diet, HF: High-fat diet, HFTS: High-fat diet with tryptophan supplement, HFETS: High-fat diet with tryptophan supplement and exercise, HFE: High-fat diet with exercise. TC: Total cholesterol, TG: Triglyceride. ^{abc} Mean different superscript letters in the same column indicate significant differences at $p < 0.05$.

3.2. Serum antioxidants

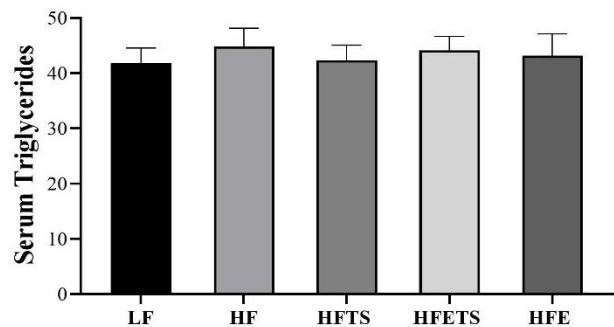
In comparison to GPX, which exhibited no indications of storage, the CAT level demonstrated a significant difference between the groups ($p < 0.05$). The HFE and HFETS groups had significantly higher CAT values than the other treatment groups ($p < 0.05$). Additionally, the LF and HF groups had significantly lower CAT values than the other treatment groups ($p < 0.05$). The HFTS group did not demonstrate any

3. Results

3.1. Serum biochemical results

3.1.1. Serum fat level

At the end of week 8, the HF group had significantly higher total TC (4.36 ± 1.70 mmol/L) and triglycerides (0.91 ± 0.10 mmol/L) than the LF group (TC: 1.45 ± 0.14 mmol/L; TG: 0.68 ± 0.14 mmol/L; $p < 0.05$). The HFTS and HFE groups had significantly lower TC values (2.15 ± 0.23 mmol/L, 2.35 ± 0.40 mmol/L, respectively) than the HF group ($p < 0.05$). The most significant effect was observed in the HFETS group, where TC (1.47 ± 0.19 mmol/L) was comparable to that of the LF group. Regarding triglycerides, HFTS (0.70 ± 0.22 mmol/L), HFETS (0.72 ± 0.13 mmol/L), and HFE (0.75 ± 0.26 mmol/L) groups had significantly lower triglyceride levels than the HF group (0.91 ± 0.10 mmol/L; $p < 0.05$), with no significant differences among the exercised and supplemented groups ($p > 0.05$). Rats in the exercised groups (HFETS and HFE) had significantly lower cholesterol levels than those in the HF and HFTS groups ($p < 0.05$; Figure 1; Table 4).



statistically significant differences compared with other treatment groups ($p > 0.05$).

3.3. Barnes maze test

The shortest time to reach the escape hole was recorded in the HFETS group. Both the HFETS and HFE groups had a significantly lower time to reach the hole compared to the HF group ($p < 0.05$). In contrast, the LF and HF groups demonstrated significantly longer times to reach the hole than the HFETS and HFE groups ($p < 0.05$; Figure 2). Regarding errors, rats in the HF group made the most errors in finding the hole, while the LF group and all other treatment groups (HFTS, HFETS, and HFE), especially the HFETS group, made a significantly lower number of errors compared to the HF group ($p < 0.05$; Figure 2).

3.4. Light/Dark maze test

The evaluation of the percentage of time spent on the open arm and the number of open arm entries revealed no significant differences in any group ($p > 0.05$; Figure 3).

3.5. Real-time PCR

The expression of *PSD-95* and *BDNF* in the hippocampus did not exhibit any significant difference ($p > 0.05$). In contrast, *PSD-95* expression in the HF group was

significantly lower than that in the HFETS and HFE groups ($p < 0.05$). The *SYN1* expression did not differ significantly among the groups, except the LF group, which exhibited lower levels than all other treatment groups (Figure 4).

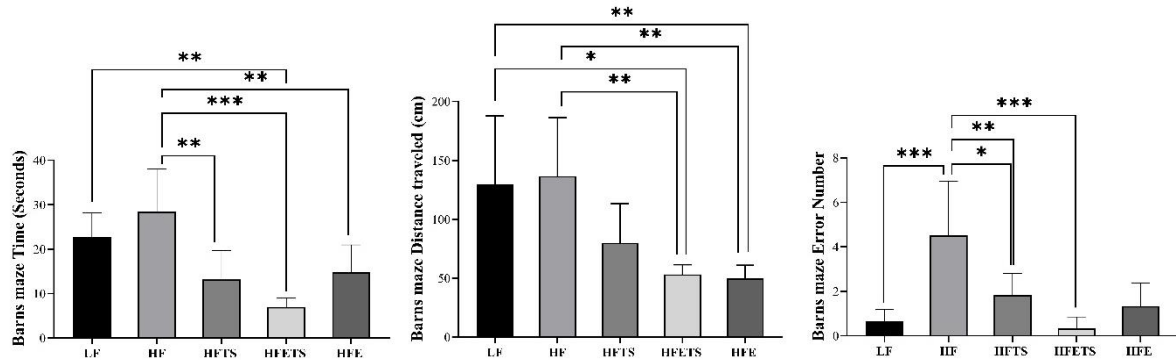


Figure 2. Barnes maze analysis in rats at the end of week 8. LF: Low-fat diet, HF: High-fat diet, HFETS: High-fat diet with tryptophan supplement, HFETS: High-fat diet with tryptophan supplement and exercise, HFE: High-fat diet with exercise. The time, distance, and number of errors were significantly higher in the HF group than in the HFETS, HFE, and HFETS groups. Values are presented as mean $\pm$ SD.

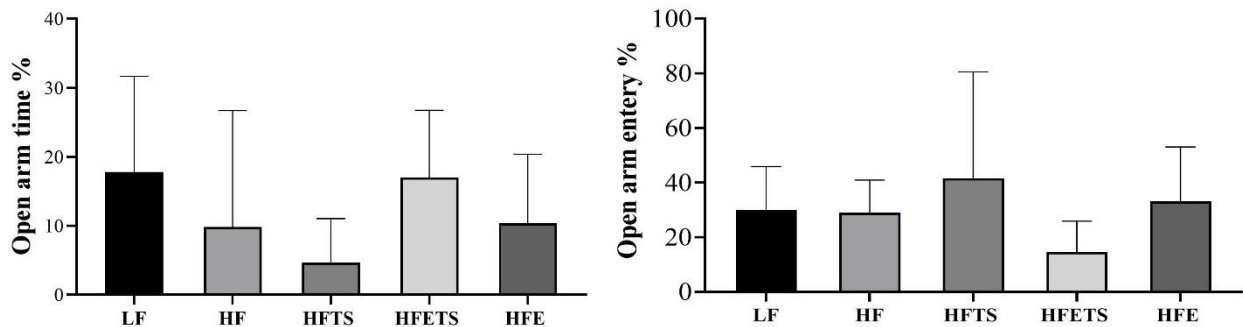


Figure 3. Mean values of the Dark/Light maze analysis in rats during 8 weeks. LF: Low-fat diet, HF: High-fat diet, HFETS: High-fat diet with tryptophan supplement, HFETS: High-fat diet with tryptophan supplement and exercise, HFE: High-fat diet with exercise. There were no significant differences between the treatment groups. Values are presented as mean $\pm$ SD.

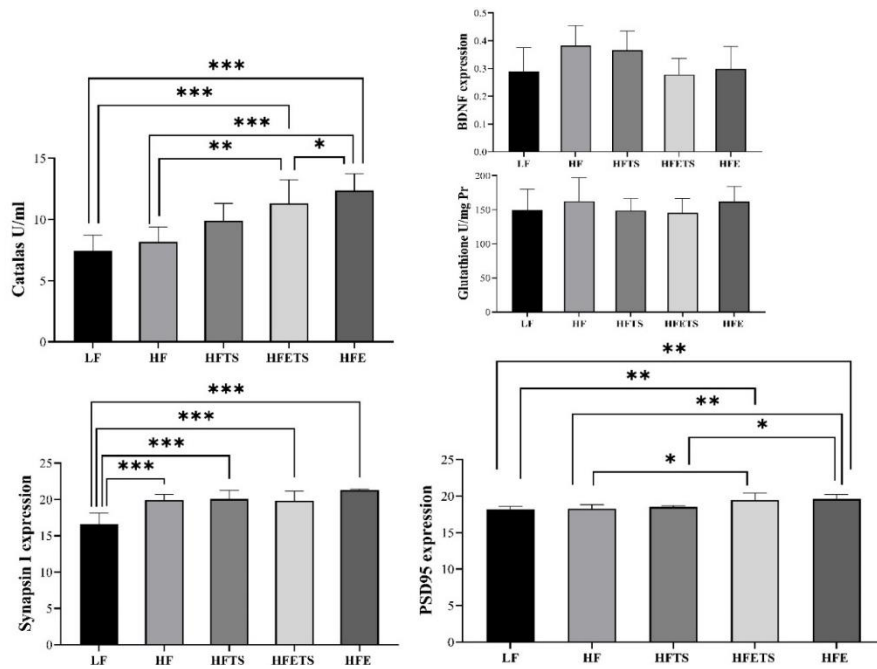


Figure 4. Serum antioxidants and gene assays of the rats in experimental groups at the end of week 8. LF: Low-fat diet, HF: High-fat diet, HFETS: High-fat diet with tryptophan supplement, HFETS: High-fat diet with tryptophan supplement and exercise, HFE: High-fat diet with exercise. There are no significant differences between the treatment groups in glutathione levels or *BDNF* levels. By contrast, a significant difference among the groups in *Synapsin 1* and *PSD-95* expression was observed. Values are presented as mean $\pm$ SD.

4. Discussion

Aging is a well-known and powerful promoter of cognitive decline. Furthermore, chronic stress, oxidative stress, and elevated pro-inflammatory cytokine expression in the hippocampus have similar effects, independent of the aging process²⁸. Additionally, HFD, Western diets, and obesity are linked to cognitive decline in humans, yet the precise mechanisms driving this relationship remain poorly elucidated, especially regarding the interplay between aging and exercise training²⁹. The current results of the behavioral and genetic analyses demonstrated that HFD decreased *SYN1* and *PSD-95* mRNA levels and memory and cognitive functions. Exercise with tryptophan supplementation has been shown to reduce memory and cognitive impairment. Prolonged feeding of rodents on an HFD may lead to changes in the behavior and immunology of neurons associated with obesity. Consistent with the present findings, previous studies have indicated that an HFD induced peripheral inflammation and nerve injuries in animal models³⁰. Similarly, the HFD enhanced reactive oxygen species (ROS) formation in the brain, resulting in cognitive and memory impairment³¹, which aligns with the observed memory deficits in the HF group in the present study. According to de Souza et al.³², high levels of exercise over 8 weeks were associated with greater antioxidant activity, suggesting that more ROS were produced³². Although melatonin and tryptophan have been reported to reduce free radicals and increase antioxidant levels³³, the present study found that tryptophan supplementation alone (HFETS group) did not notably elevate antioxidant levels (CAT or GPX) compared with other groups. However, when combined with exercise (HFETS group), a significant increase in CAT was observed. Therefore, combining tryptophan with exercise training can enhance the antioxidant effects of exercise and reduce the brain side effects caused by an HFD. Abbasnejad et al.³⁴ reported that six weeks of feeding an HFD altered spatial memory, with altered expression of P38, Akt, JNK, and ERK in the hippocampus. Furthermore, Ajayi et al.³⁵ indicated that a deficiency in tryptophan may result in increased anxiety and depression due to reduced serotonin synthesis.

According to the present results, exercise training with upregulating tryptophan can improve cognitive function. In other words, the combination of exercise and tryptophan administration over an 8-week period may decrease the duration, distance traveled, and error count in the Barnes Maze test. These findings were consistent with previous studies indicating that exercise and adequate dietary supplementation reduced anxiety and improved the Barnes Maze test index³⁵. Maintaining physical fitness supports proper blood flow to brain tissue, potentially lowering the risk of damage or deterioration³⁶. Animal and human studies demonstrated that aerobic exercise stimulated the release of growth hormones, which might improve brain function³⁷. Another mechanism through which exercise enhances brain function is by upregulating *BDNF*. However, in the current study, the *BDNF* gene did not exhibit notable differences among treatment groups. In contrast to the present results,

İSMAİL et al.³⁸ reported that the heterozygous *BDNF* gene in rats fed an HFD was downregulated in the cerebral cortex compared to rats fed a normal diet. The observed differences in outcomes were likely attributed to variations in the training and subject modality.

As previously stated, *PSD-95* was upregulated by exercise and by tryptophan supplementation at 250 mg/kg in the brains of aging rats. Mutated *PSD-95* leads to social, abnormal, redundant behaviors and anxiety-related confusion¹⁷. Madhu et al.³⁹ reported that blocking N-methyl-D-aspartate (NMDA) receptors reduced insulin resistance and hepatic steatosis in HFD-fed rats. This is consistent with the present findings, in which HFD decreased hippocampal *PSD-95* expression, whereas exercise (HFE) and tryptophan supplementation plus exercise (HFETS) significantly restored *PSD-95* levels. Activation of NMDA receptors may impair fatty acid oxidation⁴⁰. This aligns with the current findings, which demonstrated that the HF group experienced a notable decrease in hippocampal *PSD-95* expression. The lowered *PSD-95* levels may partly explain the observed decline in fast correct responses and overall behavioral performance in the HF group. The higher *PSD-95* expression in the exercised groups was probably due to the positive effects of exercise rather than the adverse effects of an HFD, as evidenced by the remarkable differences between the LF group and the exercised groups. The mice with the *SYN1* gene knockout were unable to inhibit synaptic transmission, whereas the mice with the *SYNII* gene knockout demonstrated the ability to enhance inhibitory transmission⁴¹. Consistent with present study, expression of *SYN1* in the present study was increased with exercise and tryptophan supplementation in the brain. Exercise has the potential to safeguard the brain against neurological impairments by activating an anti-inflammatory HSP70/NF-κB/IL-6/*SYN1* pathway in injured brains⁴². However, the anti-inflammatory HSP70/NF-κB/IL-6/*SYN1* pathway was considered in the brain of aging rats, and exercise appeared to mitigate the adverse effects of aging by upregulating the *SYN1* axis.

5. Conclusion

The impact of exercise training combined with tryptophan supplementation in mitigating the adverse effects of an HFD was confirmed in aging rats. However, a tryptophan-containing diet, when combined with exercise, did not improve memory deficits induced by an HFD. The current findings indicated that exercise was a more effective intervention than tryptophan supplementation alone in mitigating the detrimental effects of an HFD during aging. Nevertheless, the potential synergistic effects of combining exercise with tryptophan supplementation require further exploration, particularly in studies involving human participants.

Declarations

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Authors' contributions

Mohammadreza Dana designed the study and performed the sampling and practical procedures. Diana Abbasi revised the manuscript and corrected the language errors. Ali Heydari and Shayan Amini revised the manuscript draft and reported the molecular findings. Ali Mobaraki performed the statistical analysis. Marzieh Saki and Narges Safari wrote the draft of the manuscript. All authors checked the final proof, the statistical results, and the final edition of the manuscript before publication in this journal.

Availability of data and materials

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The data are available upon reasonable request from the corresponding author.

Ethical considerations

All authors have thoroughly reviewed the ethical considerations, including issues related to plagiarism, consent for publication, misconduct, data fabrication and/or falsification, duplicate publication and/or submission, and redundancy. No artificial intelligence tools were employed in conducting and preparing the present study.

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