Exercise Training Alleviates Cognitive Functions in Diabetic Rats through HMGB1/RAGE/NF-kB Pathway

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Research

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Abstract

Objective: To observe the effect of exercise training on cognitive functions of diabetic rat model. Methods: Male SD rats were given a high fat and high sugar diet, except for control group. After 4 weeks, 35 mg /kg STZ was intraperitoneally injected to establish type 2 diabetes model rats. After successful modeling, rats were randomly divided into the model group, model + exercise group. Animals performed 5 days of consecutive treadmill exercise (60 min/day) with 22 m/min speeds for 60 days. After 60 days, behavioral tests were conducted by Morris water maze method. then rats were weighed and blood samples were obtained to detect blood glucose. Some animals were sacrificed to prepare serum to detect glycosylated hemoglobin. Brain tissues were taken to detect the protein expressions of HMGB1-/RAGE-/NF-κB signal pathway by Western Blot. The brains of other animals were perfused and taken for RAGE and NF-κB immunohistochemical staining.

Results: Compared with control group, escape latency and probe distance in the model group were significantly prolonged, swimming time in the target quadrant was significantly shortened, and the number of crossing platform was significantly reduced. The average grayvalues of NF-κB and RAGE were significantly decreased. Expressions of HMGB1,RAGE,p-NF-κBp65 and p-IκBα were significantly up-regulated($P<0.05$ or $P<0.01$). Compared with the model group, escape latency and probe distance were significantly shortened, swimming time in the target quadrant was prolonged and increased the number of crossing platform, it also reduced the fasting blood glucose, increased body weights, reduced the level of glycated hemoglobin, and significantly increased the mean grayvalues of NF-κB and RAGE. The protein expressions of HMGB1, RAGE, p-NF-κBp65 and p-IκBα were decreased in model + exercise group.

Conclusion: Exercise training can ameliorate the cognitive dysfunction of diabetic rats, its mechanism may be related to reducing blood glucose, reducing the level of glycated hemoglobin and improving the HMGB1 /RAGE/NF-κB pathway in the brain tissue.

Introduction

Type 2 diabetes is one of the risk factors for dementia, which has a significant impairment on brain cognition since its early stage.[1,2]In recent years, the incidence of diabetes has increased year by year[3], and many studies have shown that diabetes is an independent high risk factor for cognitive impairment.[4] The probability of cognitive impairment in patients with diabetes is significantly higher than that in ordinary people.[5] With the prolongation of the course of the disease, the risk of cognitive decline increases, which seriously reduces the quality of life of patients.[6,7]Many researchers believe that the cognitive impairment of diabetes can be called type 3 diabetes[8], so the early treatment of type 2 diabetes is particularly important.

Some studies have shown that exercise intervention plays a certain role in preventing cognitive decline in the elderly, and the incidence of cognitive decline in the elderly who exercise regularly for a long time is relatively low.[9] Exercise can promote limb vasodilation, promote the improvement of hemodynamics,

correct neurophysiological abnormalities, improve the adaptive changes of the central nervous system, and then improve cognitive dysfunction. According to whether the muscle contraction energy comes from aerobic metabolism or anaerobic metabolism during exercise, exercise is subdivided into aerobic exercise and anaerobic exercise. At present, there are plenty intervention studies on the effects of aerobic training on patients with cognitive impairment. Aerobic exercise, also known as aerobic training or endurance training, refers to the physical training carried out by the human body under the condition of adequate supply of oxygen, in which the main large muscle groups of the whole body participate in continuous, rhythmic and lasting exercise for a long time. Aerobics is a low-cost, low-risk, simple and feasible physical activity. The common aerobic exercises include brisk walking, power car, swimming, aerobics and yoga. Previous studies have suggested that aerobic exercise can ameliorate the cognitive function of patients with mild cognitive impairment, including memory, executive ability and so on. [10,11,12]Anaerobic exercise, also known as resistance exercise or strength training, is a kind of anaerobic exercise that overcomes external resistance through continuous muscle contraction. It mainly includes free weight (dumbbells and barbells), push-ups, isometric exercises, elastic bands and strength training equipment.[13] Best et al.[14] carried out 2-year follow-up study on 155 aging women who participated in resistance training or balance adjustment. The results showed that resistance training could improve the executive function and memory of elderly women, and reduce cerebral cortex and white matter atrophy. The purpose of this study is to evaluate the effect of exercise training on diabetic cognitive impairment and its mechanism.

Materials And Methods

Animals

30 Male Sprague Dawley rats (250–280 g) were used in this study. The rats were maintained at an ambient temperature of 22–24°C and 50–60% humidity, under a 12 h light: 12 h dark cycle with food and water available *ad libitum*.The animals were randomly divided into three groups: (1) normal control group, (2) STZ injection group, and (3) STZ injection plus treadmill exercise group. The study was approved by the institutional ethics committee and complies with the Declaration of Helsinki.

Reagents and instruments

RAGE antibody (Cell SignalingTechnolo-gy); HMGB1,NF-κB-p65 antibody (American Santa Cruz Biotechnology Co., Ltd.) HMGB1, NF-κB-p65 antibody, IκBα, GAPDH antibody, immunohistochemical kit, glycosylated hemoglobin kit, PBS phosphate buffer, 4% paraformaldehyde (Nanjing Jiancheng Biology Co., Ltd.), streptozotocin (Sigma Co., Ltd.); Before streptozotocin was used, the solution was prepared with 0.1mol/L citric acid buffer (0℃ ~ 4℃, pH=4.2) at the ratio of 1 to 100. Morris water maze (Shanghai Jiliang Software Technology Co., Ltd.); Eon full-wavelength enzyme labeling instrument of Shanghai Jisun Software Technology Co., Ltd. (BioTek); Sanolian Blood glucose Meter (SanNuo Biosensor Co., Ltd.); BI-2000 medical image analysis system (Chengdu Tai Meng Software Co., Ltd.).

Induction of diabetes

To induce diabetes, Four weeks after high fat diet (Basic feed 59%, sucrose 20%, lard 18%, egg yolk 3%), a single intraperitioneal injection of STZ (35 mg/kg, dissolved in 0.01-M citrate buffer at pH 4.5; Sigma Chemical Co.) was given to each animal, as the previously described method.[15]One week later, rats fasted for 10 hours and then blood glucose levels were measured by a glucometer (Roche, Germany). The rats whose blood glucose exceeded 12 mmol/L had diabetes and were utilized for the following study. A total of 10 rats were enrolled in the group after exclusion of rats with substandard blood glucose. Morris water maze was utilized to detect whether the rat model had cognitive impairment[16] (compared with the blank control group, whether the platform could be found in the shortest time).

Exercise protocol

Before beginning the formal 60 day exercise protocol, animals were familiarized to treadmill running (5-20 min/day) for 5 consecutive days. After this period of habituation, the exercised animals performed 5 days of consecutive treadmill exercise (60 min/day) with 22 m/min speeds[17]. At the beginning of 60 min exercises, to warm up the rats, treadmill speed had been set at 5 m/min and progressively increased to 22 m/min. At the final of 60 minute exercises, the speed progressively decreased to 5 m/min to cool down. Mild electrical shock was accustomed the negligible amount to motivate animals to run. Control animals did not carry out treadmill exercise but were put on a nonmoving treadmill for 60 min/day for 5 days a week. Exercised animals were studied 24 h after their last exercise session.

Morris Water Maze Task

The Morris water maze task was used to evaluate memory function according to a previously described method.[16] Four marked points are identified in the middle of the four quadrants of the maze, and a black platform is set in the water. The morris water maze test was performed 1 hour after the last exercise. Each marked point was trained once in the morning and once in the afternoon, each training interval was more than 30 min for four consecutive days. On the 5th day, the platform was removed, and the rat was thrown into the water from the marked point of the quadrant I toward the pool wall. The target rat's swimming time in the target quadrant and the number of time crossed the virtual platform were recorded within 90 seconds.

Sampling and specimen handling

After the Morris water maze task, all rats were fasted for 12 hours, and their body weight and fasting blood glucose were recorded. The blood was obtained from the orbital venous plexus of 8 rats in each group, and the blood was centrifuged with 3500r/min for 10 minutes. The serum was taken to determine glycosylated hemoglobin according to the instructions of the kit. After blood samples were collected, 4 rats were randomly selected from each group for anesthesia (10% chloral hydrate 3 ml / kg), fully fixed with PBS intracardiac perfusion, and craniotomy was performed to take 3 mm to 4 mm tissue blocks in the coronary coronal position. Hippocampus DG area)[18,19,] placed in 4% paraformaldehyde, fixed at 4℃

for 7 days, routine paraffin embedding and coronal sectioning, RAGE and NF-κB immunohistochemical staining, the remaining brain tissues were frozen with liquid nitrogen for using.

HE staining of hippocampus DG region of brain tissue

The isolated hippocampus tissue was placed in 4% paraformaldehyde and fixed at 4 ℃ for 24 days. before dehydration, fixed tissue was washed overnight with tap water before dehydration. The hippocampus tissue was dehydrated in different gradients of ethanol, transparent with xylene, twice immersed in paraffin, and embedded in conventional paraffin. 5 μm of tissue sections were stained with HE, transparent with xylene, and sealed with gum for electron microscope observation.

Immunohistochemical detection

The isolated hippocampus tissue was placed in 4% paraformaldehyde and fixed at 4 ℃ for 7 days, and then the coronal sections were prepared after routine paraffin embedding. According to the instructions of the immunohistochemical kit, the sections were routinely dewaxed and eliminated the activity of endogenous peroxidase. After washing, antigen repair was carried out, and the sections were closed and incubated overnight with corresponding primary antibodies (target protein primary antibodies: RAGE, p-NF-κBp65) at 4℃. The blot was washed and incubated with the secondary antibody combined with the primary antibody at room temperature for 2 h. The sections were stained with hematoxylin, dehydrated with ethanol, transparent with xylene and coated with neutral glue.

Western blot analysis

The frozen brain tissue was washed with PBS and incubated in lysis buffer, and then phosphatase inhibitor was added, the tissue was ground with a grinding rod until completely crushed, centrifuged twice at 4°C, and the supernatant was taken, the brain protein concentration was quantified using the BCA protein assay kit, then mixed with the loading buffer and heated with 2-mercaptoethanol at 100 ℃ for 5 min. The extracted protein was separated by SDS-PAGE and transferred to PVDF membrane. In TBST, the nonspecific binding sites were blocked by 5% skimmed milk powder, and then incubated overnight at 4℃ with corresponding primary antibodies (target protein primary antibodies: HMGB1, RAGE, p-NF-κBp65, p-IκBα, internal reference protein first antibody: NF-κBp65, IκBα, GAPDH). The blot was washed and incubated with the secondary antibody combined with the primary antibody at room temperature for 2 h. The ultra-sensitive ECL chemiluminescence kit and Clinx chemiluminescence imaging system display specific bands. The grayscale analysis of WB bands was carried out by ImageJv1.8.0, and the results were introduced into Excel. GAPDH (if the target protein is phosphorylated, total protein is used) as a reference, the gray ratio of bands is used as the expression result, and SPSS is used to analyze the results of each group.

Statistical analysis

Data are presented as the mean ± standard error of the mean. SPSS software version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. One way ANOVA was used when the normal

distribution was uniform and the variance was homogeneous. Nonparametric test was used when the normal distribution was not uniform or the variance was not homogeneous. One way ANOVA was employed to the test results.

Results

Effect of sports training on the hidden platform of Morris water maze

On the first day of hidden platform test, there was no significant difference in escape latency and probe distance among the three groups, but from the second day, compared with the control group, the escape latency and probe distance of the model group were significantly prolonged, while on the third day, the escape latency and probe distance of the exercise group were significantly shorter than those of the model group. On the fourth day, escape latency and probe distance of the exercise group were significantly shortened ($P < 0.01$). See Table 1 and table 2.

Compared with the control group, the swimming time in the target quadrant of the model group was significantly shorter ($P < 0.01$), and the number of crossing platform was significantly reduced ($P < 0.01$); Compared with the model group, the swimming time in the target quadrant of the exercise group was significantly longer ($P < 0.05$), and the number of crossing platform in the exercise group was significantly increased ($P < 0.05$). See Table 3 and figure 1.

Effects of exercise training on blood glucose, body weight and glycosylated hemoglobin

Compared with the control group, the levels of blood glucose and glycosylated hemoglobin in the model group increased significantly ($P < 0.01$), and the body weight decreased significantly ($P < 0.01$). Compared with the model group, the blood glucose in the exercise training group decreased significantly ($P < 0.01$), the body weight in the exercise training group increased significantly ($P < 0.01$), and the level of glycosylated hemoglobin decreased significantly ($P < 0.05$). See Table 4.

Effect of exercise training on brain tissue

Compared with the control group, the HE staining of the hippocampus histopathological sections showed that the cells of granular layer in the DG area of the model group were sparse and arranged irregularly. Compared with the model group, the cells in the exercise training group were dense and arranged neatly. See Figure 2.

Effect of exercise training on the expression of NF- kappa B and RAGE protein in hippocampus

The results of immunohistochemical staining showed that compared with the control group, the number of NF- κB immunoreactive positive cells in granular cell layer and cortical neurons in the hippocampus DG region of the model group was more and deeply stained, and the gray value of NF- κB decreased significantly ($P < 0.01$). Compared with the model group, exercise training significantly reduced the NF-κB immunoreactive positive cells in the granular cell layer and cortical neurons of the hippocampus DG area,

and the average gray value of NF-κB was significantly increased (P <0.05), see Figure 3 , Table 5; Immunohistochemical staining showed that compared with the control group, the number of RAGE immunopositive positive cells in the DG area of the hippocampus of the model group was significantly increased, and the coloration was significantly deepened. Compared with the model group, the number of RAGE immunopositive positive cells in the hippocampus DG area of the sports training group was significantly reduced, and the coloring was lighter; compared with the model group, the average gray value of RAGE in the exercise training group was significantly increased (P< 0.05) Figure 4, Table 5. (Note: The arrows in the figure below refer to immunoreactive positive cells).

Effect of exercise training on the expression of HMGB1/RAGE/NF- κ B pathway protein in brain tissue

Compared with the control group, the expression of HMGB1, RAGE, p-NF-κBp65, p-IκBα protein in the model group was significantly up-regulated (P < 0.01). Compared with the model group, the exercise training group could significantly down-regulate the expression of HMGB1, RAGE, p-NF-κB p65, p-IκBα protein. See figure 5, Table 6.

Discussion

Long-term hyperglycemia can increase the damage of oxygen free radicals, cause metabolic disorders, increase the level of inflammatory factors, and produce advanced glycation end products, which affect the nervous system. Cukierman-Yaffe et al.[20] showed that for every 1% increase in glycosylated hemoglobin, the score of digital symbol learning test and mini mental state examination scale in patients with type 2 diabetes decreased by an average of 1.75 points and 0.2 points respectively. Another study confirmed that the higher the glycosylated hemoglobin, the lower the cognitive test score of type 2 diabetic patients. After 3 years of follow-up, the cognitive impairment of type 2 diabetic patients with high glycosylated hemoglobin was further aggravated.[21] The current research results show that exercise training can reduce the fasting blood glucose of diabetic rats, improve cognitive impairment caused by diabetes, and improve the learning and memory function in rats.

Studies have shown that aerobic exercise is related to cognitive function in brain structure.[22] The hippocampus in the brain is the field responsible for learning and memory, and once the structural and functional integrity of the hippocampus is destroyed, it can lead to a decline in cognitive function. Studies have shown that aerobic exercise can increase the volume of hippocampus, gray matter and white matter.[23] Studies have shown that after 6 months of aerobic exercise intervention, the left, right and all hippocampus volumes of the elderly with mild cognitive impairment increased significantly.[24] Exercise can change the adaptability of brain structure and function, and then maintain or ameliorate the cognitive function of the elderly.[25] Erickson et al.[23] showed that after one year of aerobic exercise intervention, the left and right hippocampus capacity of the elderly increased by 2.12% and 1.97%, respectively.

High mobility group protein B1 (HMGB1) is an important late inflammatory factor, which can act as an endogenous pyrogen in the central nervous system.[26] Increased levels of HMGB1 in the brain can induce

memory abnormalities, which may be mediated by advanced glycation end products receptor (RAGE).[27]

The results of this study show that compared with the model group, exercise training can significantly down-regulate the protein level of HMGB1,RAGE in the brain and effectively improve the memory abnormality of model rats. Other studies have shown that (RAGE), nuclear transcription factor (NF- κB), a receptor for advanced glycation end products, plays an important role in the pathogenesis of diabetic cognitive impairment. The combination of HMGB1 and RAGE can lead to oxidative stress in neurons and activate the expression of NF- κB. Hofmann et al confirmed the activation of NF- κB in patients with diabetes for the first time. This study showed that compared with the model group, the protein expression of p-NF-κB p65 and p-IκBα in the exercise training group was significantly down-regulated. Due to the down-regulation of the protein level of HMGB1,RAGE in the brain, the activation of downstream NF- κB pathway was also inhibited. Therefore, the protein levels of p-NF-κB p65 and p-IκBα in the exercise training group were also significantly down-regulated. The results of pathological sections of hippocampus tissue showed that compared with the model group, exercise training could increase the average gray values of NF- κB and RAGE of diabetic cognitive impairment rats, and effectively improve the development of hippocampus pathological changes. In addition, the results of this study also show that exercise training can improve the cognitive ability of diabetic cognitive impairment rats, and significantly reduce the levels of blood glucose and glycosylated hemoglobin, indicating that exercise training may improve the cognitive impairment of diabetic rats by reducing blood glucose, reducing the level of glycosylated hemoglobin and improving HMGB1/RAGE/NF- κB pathway in the brain tissue of diabetic rats. It also shows that exercise training may have the effect of improving cognitive impairment mediated by RAGE.

Limitations

In this study, the form of single exercise may not achieve the better effect of exercise intervention. There was no healthy exercise group, but the benefit of exercise was considered in the experiment.

Conclusions

This study suggests that exercise training can effectively improve the cognitive impairment of diabetic rats and slow down the development of hippocampus tissue damage caused by diabetes. The results of this study can serve as a basis for the follow-up study of exercise intervention on cognitive impairment of diabetes. It is suggested that exercise training can reduce blood sugar, reduce glycosylated hemoglobin level and improve HMGB1 /RAGE/ NF-κ B pathway in diabetic rats. and other pathways can improve cognitive impairment in diabetic rats. It also shows that exercise training may improve the cognitive impairment mediated by RAGE.

Declarations

Author Contributions: Wang Chengji participated in the study design analysis report development and interpretation of study findings.**Xu jichao** participatedin writing the report.

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CONFLICTS OF INTEREST

The author declared that there are no conflicts of interest.

References

1. Sun DM, Ma Y, Sun ZB, Xie L, Huang JZ, Chen WS, Duan SX, Lin ZR, Guo RW, Le HB, Xu WC, Ma SH. Decision-making in primary onset middle-age type 2 diabetes mellitus: a BOLD-fMRI study. Sci Rep. 2017 Aug 31;7(1):10246.
2. Hayashi K, Kurioka S, Yamaguchi T, Morita M, Kanazawa I, Takase H, Wada A, Kitagaki H, Nagai A, Bokura H, Yamaguchi S, Sugimoto T. Association of cognitive dysfunction with hippocampal atrophy in elderly Japanese people with type 2 diabetes. Diabetes Res Clin Pract. 2011 Nov;94(2):180–5.
3. Fernández RDV, Díaz A, Bongiovanni B, Gallucci G, Bértola D, Gardeñez W, Lioi S, Bertolin Y, Galliano R, Bay ML, Bottasso O. D'Attilio L. Evidence for a More Disrupted Immune-Endocrine Relation and Cortisol Immunologic Influences in the Context of Tuberculosis and Type 2 Diabetes Comorbidity. Front Endocrinol (Lausanne). 2020 Mar 20;11:126.
4. Garcia CC, Potian JG, Hognason K, Thyagarajan B, Sultatos LG, Souayah N, Routh VH, McArdle JJ. Acetylcholinesterase deficiency contributes to neuromuscular junction dysfunction in type 1 diabetic neuropathy. Am J Physiol Endocrinol Metab. 2012 Aug 15;303(4):E551-61.

5. Casolla B, Caparros F, Cordonnier C, Bombois S, Hénon H, Bordet R, Orzi F, Leys D. Biological and imaging predictors of cognitive impairment after stroke: a systematic review. J Neurol. 2019 Nov;266(11):2593–604.
6. Damanik J, Mayza A, Rachman A, Sauriasari R, Kristanti M, Agustina PS, Angianto AR, Prawiroharjo P, Yunir E. Association between serum homocysteine level and cognitive function in middle-aged type 2 diabetes mellitus patients. PLoS One. 2019 Nov;6(11):e0224611. 14(.
7. Strachan MW, Reynolds RM, Marioni RE, et al. Cognitive function, dementia and type 2 diabetes mellitus in the elderly. Nature reviews Endocri- nology, 2011, (7): 108–114.
8. de la Monte SM. Insulin Resistance and Neurodegeneration: Progress Towards the Development of New Therapeutics for Alzheimer's Disease. Drugs. 2017 Jan;77(1):47–65.
9. Laurin D, Verreault R, Lindsay J, et al. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol. 2001;58(3):498–504.
10. Lautenschlager NT, Cox KL, Flicker L, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease:a randomized trial. JAMA. 2008;300(9):1. 027 – 1 037.
11. Cui MY, Lin Y, Sheng JY, et al. Exercise intervention associated with cognitive improvement in Alzheimer' s disease. Neural Plast, 2018, 2018:9234105.
12. Song D, Yu DSF, Li PWC, et al. The effectiveness of physical exercise on cognitive and psychological outcomes in individuals with mild cognitive impairment:a systematic review and meta-analysis. Int J Nurs Stud. 2018;79:155–64.
13. Roberto C, Marta I, Ileana T, et al. May the force be with you: why resistance training is essential for subjects with type 2 diabetes mellitus without complications. Endocrine. 2018;62(1):14–25.
14. Best JR, Chiu BK, Liang Hsu CL, et al. Long-term effects of resistance exercise training on cognition and brain volume in older women:results from a randomized controlled trial. J Int Neuropsychol Soc. 2015;21(10):745–56.
15. Kim DY, Jung SY, Kim TW, Lee KS, Kim K. Treadmill exercise decreases incidence of Alzheimer’s disease by suppressing glycogen synthase kinase-3β expression in streptozotocin-induced diabetic rats. J Exerc Rehabil. 2015;11:87–94.
16. Li H, Luo Y, Xu Y, et al. Meloxicam Improves Cognitive Impairment of Diabetic Rats through COX2-PGE2-EPs-cAMP /pPKA Pathway. Molecular pharmaceutics, 2018, (15):4121–4131.
17. Taylor RP, Ciccolo JT, Starnes JW. Effect of exercise training on the ability of the rat heart to tolerate hydrogen peroxide. Cardiovasc Res. 2003;58(3):575–81.
18. Campos EJ, Martins J, Brudzewsky D, et al. Neuropeptide Y system mRNA expression changes in the hippocampus of a type I diabetes rat model. Annals of anatomy = Anatomischer Anzeiger: official organ of the Anato- mische Gesellschaft. 2020;227:151419.
19. Paxinos G, Chai SY, Christopoulos G, et al. In vitro autoradiographic localization of calcitonin and amylin binding sites in monkey brain. J Chem Neuroanat. 2004;27:217–36.

20. Cukierman-Yaffe T, Gerstein HC, Williamson JD, et al. Relationship between baseline glycemic control and cogni- tive function in individuals with type 2 diabetes and other cardiovascular risk factors:the action to control cardiovascular risk in diabetes-memory in diabetes(ACCORD-MIND) trial. Diabetes Care. 2009;32(2):221–6.
21. Umegaki H, Kawamura T, Kawano N, et al. Factors associated with cognitive decline in elderly diabetics. Dement Geriatr Cogn Dis Extra. 2011;1(1):1–9.
22. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci USA. 2011;108(7):3 017–3022.
23. Tarumi T, Zhang R. Cerebral hemodynamics of the aging brain: risk of Alzheimer disease and benefit of aerobic exercise. Front Physiol. 2014;5:6.
24. Ten Brinke LF, Bolandzadeh N, Nagamatsu LS, et al. Aerobic exercise increases hippocampal volume in older women with probable mild cognitive impairment:a 6-month randomised controlled trial. Br J Sports Med. 2015;49(4):248–54.
25. Chang YK, Chung-Ju H, Chen KF, et al. Physical activity and working memory in healthy older adults:an ERP study. Psychophysiology. 2013;50(11):1174–82.
26. Lawrimore CJ, Coleman LG, Zou J, Crews FT. Ethanol Induction of Innate Immune Signals Across BV2 Microglia and SH-SY5Y Neuroblastoma Involves Induction of IL-4 and IL-13. Brain Sci. 2019 Sep;10(9):228. 9(.
27. Julia L, Sobesky, Heather M, D'Angelo MD, Weber, et al. Glucocorticoids Mediate Short-Term High-Fat Diet Induction of Neuroinflammatory Priming, the NLRP3 Inflammasome, and the Danger Signal HMGB1. eNeuro. 2016 Jul-Aug; 3(4): ENEURO.0113-16.2016.

Tables

Table 1 The effect of exercise training on the latency time of hidden platform test

Group	escape latency(s)			
	First day	Second day	Third day	Forth day
A	87 ± 5	66 ± 9**	52 ± 7**	31 ± 10**
B	86 ± 7	88 ± 3	76 ± 12	54 ± 7
C	86 ± 7	78 ± 10	59 ± 5*	41 ± 6**

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Table 2 effect of exercise training on Hidden Platform test

Group	Probe distance / (m)			
	First day	Second day	Third day	Forth day
A	22.5 ± 5.0	13.8 ± 3.0**	9.2 ± 2.7**	5.7 ± 1.6**
B	21.5 ± 4.1	21.5 ± 2.5	19.8 ± 6.2	13.7 ± 3.3
C	23.1 ± 3.0	17.81 ± 9.1	10.6 ± 7.5*	7.1 ± 2.9*

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Table 3 Effect of sports training on swimming time in the target quadrant (the first quadrant) and crossing platform times

Group	Swimming time in quadrant □/□%□	Times of crossing platform
A	38.1 ± 5.8**	4.3 ± 2.5**
B	20.9 ± 4.3	1.3 ± 1.7
C	30.6 ± 9.4*	3.5 ± 1.7*

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Table 4 effects of exercise training on blood glucose, body weight and glycosylated hemoglobin

Group	blood sugar (mmol /L)	Weight (g)	glycosylated hemoglobin (g /ml)
A	4.4 ± 0.4**	488.5 ± 47.0**	3.9 ± 1.0**
B	17.6 ± 2.8	247.5 ± 50.6	8.7 ± 2.2
C	13.5 ± 1.2**	370.8 ± 57.8**	6.3 ± 1.9*

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Table 5 effect of exercise training on the average gray value of RAGE,NF- κ B in hippocampus

Group	NF-κB average gray value	RAGE average gray value
A	127 ± 4**	130 ± 9**
B	93 ± 6	98 ± 7
C	110 ± 7*	119 ± 9**

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Table 6 effect of exercise training on the expression of HMGB1/RAGE/NF- κ B signal pathway protein in brain tissue

Group	HMGB1 /GAPDH	RAGE /GAPDH	P-NF-κBP65 /NF-κBP65	P-IkBa /IkBa
A	0.0941 ± 0.0019**	0.0633 ± 0.0021**	0.1651 ± 0.0210**	0.348 ± 0.021**
B	0.2139 ± 0.0075	0.1728 ± 0.0057	0.4797 ± 0.0407	1.033 ± 0.069
C	0.1395 ± 0.0078**	0.0788 ± 0.0061**	0.5003 ± 0.0039**	0.598 ± 0.028*

A:control group; B:model group; C:model + exercise training group

* P<0.05, **P<0.01 vs model group

Figures

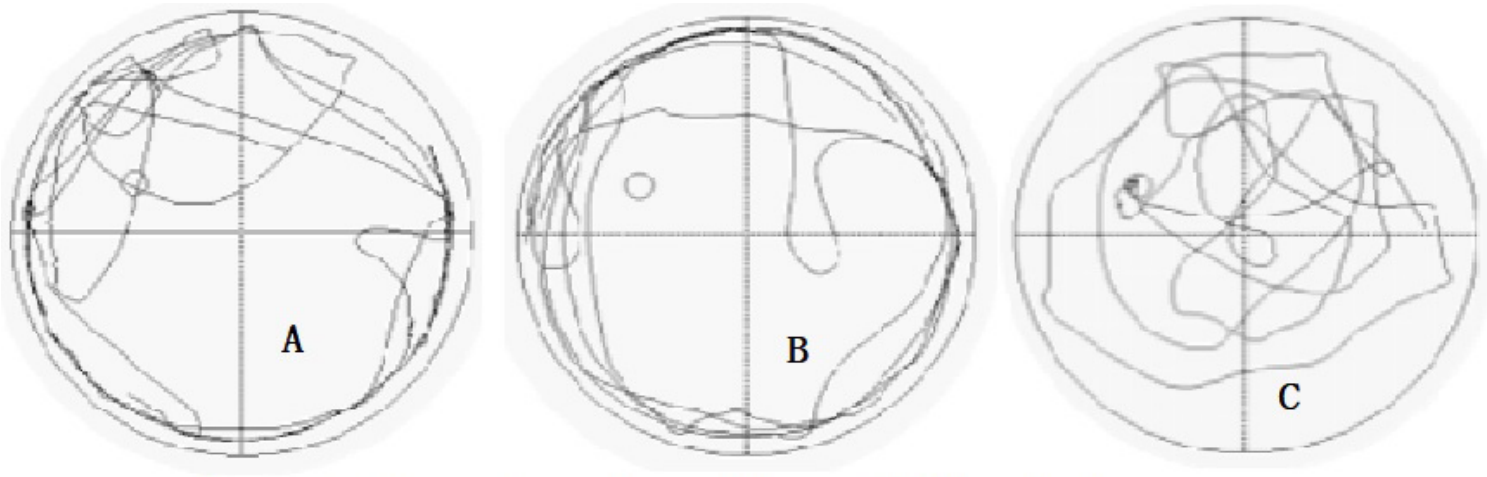

Figure 1

Effect of exercise training on hidden platform test

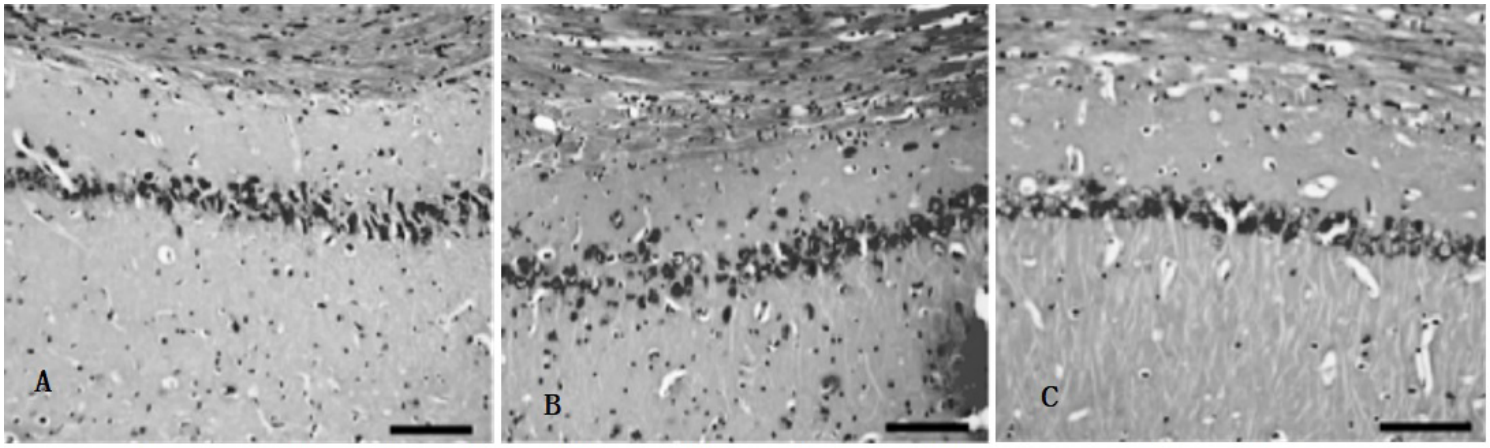

Figure 2

effect of exercise training on histopathology of hippocampus (HE, × 200)

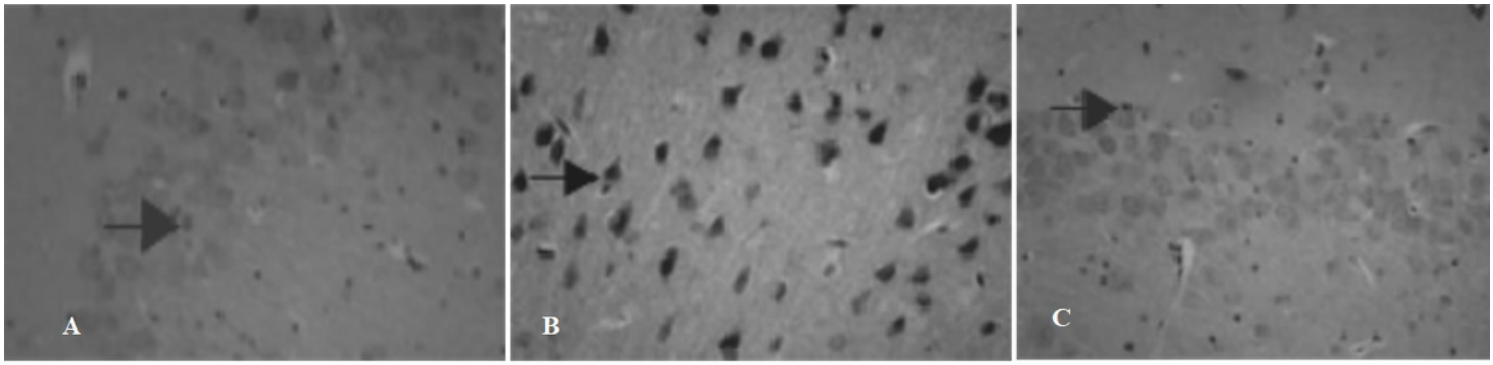

Figure 3

Effect of exercise training on the expression of NF- κB protein in hippocampus (immunohistochemistry, × 400)

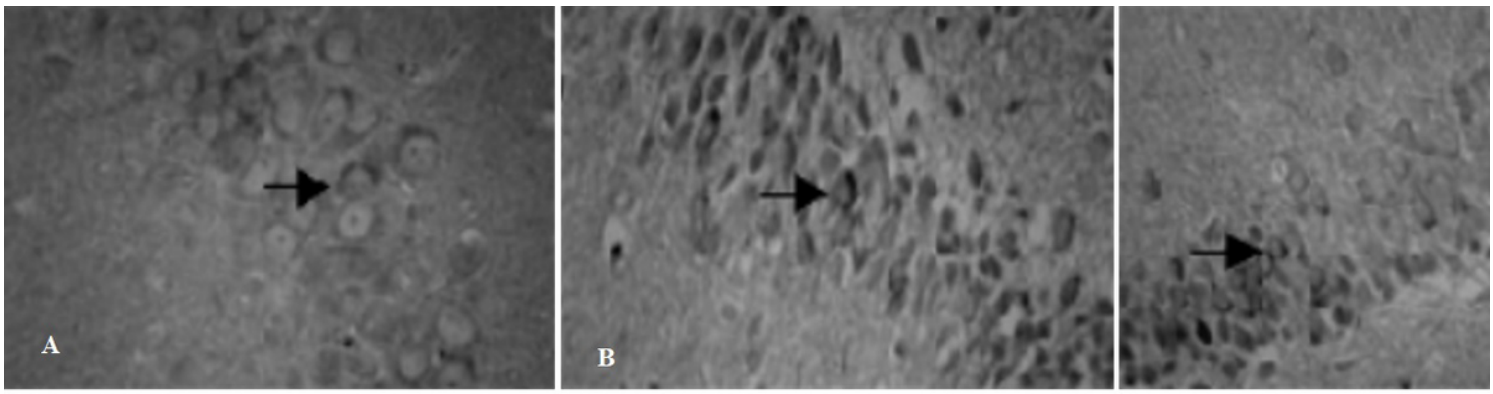

Figure 4

effect of exercise training on the expression of RAGE protein in hippocampus (immunohistochemistry, × 400)

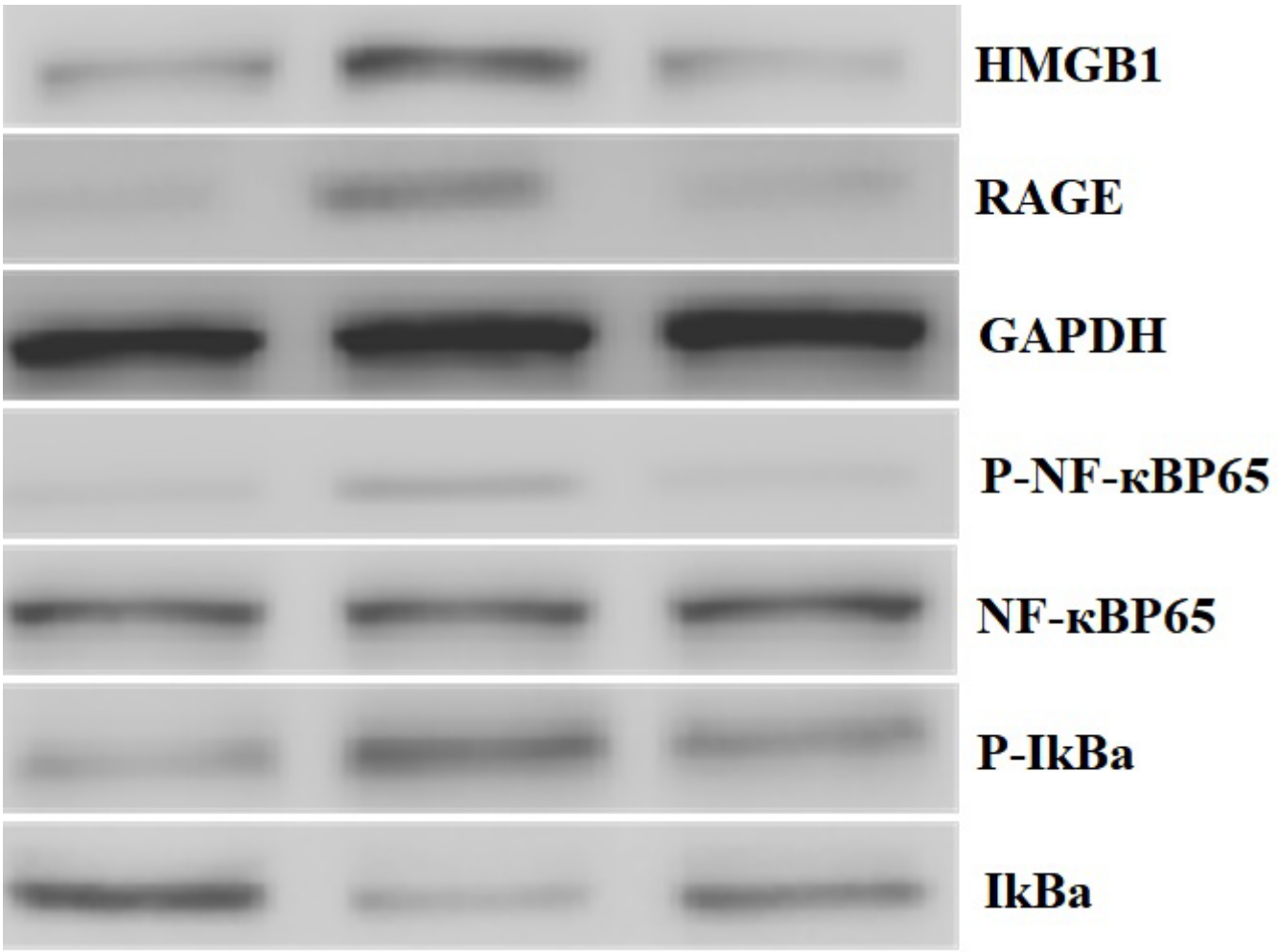

Figure 5

Effect of exercise on HMGB1 / rage / NF - κB signal pathway protein expression in brain tissue