Exercise rescues cognitive impairment through the fibrinogen pathway in diabetes

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Abstract

Background: Fibrinogen is proved to play a vital role in the activation of neuroinflammation. Exercise rescues the cognitive deficits due to diabetes. However, the molecular protective mechanism of exercise mediated by fibrinogen cascade following BBB leakage still remains elusive in diabetes.

Methods: This research focused on assessing the influence of fibrinogen on neuroinflammation and blood-brain barrier(BBB) and the role of exercise in improving cognitive function in diabetic rats by using stereotaxic FGG-AAV(fibrinogen gamma chain with adeno-associated viral vector)injection procedures.

Results: Exercise improved learning and memory function by inhibiting fibrinogen level in diabetic rats. Exercise intervention decreased the production and activation of microglia and astroglia of diabetic rats. Increased FGG expression in diabetic rats significantly inhibited the protective effects of exercise on hippocampal EB leakage, BBB-related protein ZO-1 and Claudin-5 expression, and MMP-9 protein expression level. The expression level of inflammatory factors IL-1β and TNFαwere inhibited in exercise group, while overexpressing FGG level would compromise the protective effects of exercise.

Conclusion: Exercise can improve cognitive impairment in diabetic rats by down-regulating the expression of fibrinogen, inhibiting the neuroinflammation process, and reducing blood-brain barrier damage.

Introduction

Hundreds of millions of people worldwide are living with diabetes and they have a higher risk to suffer from cognitive impairment and dementia[1]. Meanwhile, diabetes accounts for a high prevalence in patients with dementia. Follow-up studies have found diabetes could accelerate the process from mild cognitive impairment to dementia[2]. However, clinical drugs could barely postpone the deterioration process in the dementia stage, so it’s urgent to explore the mechanisms and seek for effective procedures in the earlier phase.

The mechanism of cognitive impairment of diabetes due to blood-brain barrier (BBB) beak-down is attracting more interests in recent years[3]. BBB disruption is considered to be the initial change in the process of cognitive deterioration[4]. BBB alters the brain micro-environment composition with blood proteins including fibrinogen leakage into the CNS when BBB breaks down. Recent researches have found that the blood coagulation protein fibrinogen plays a vital role in the activation of CNS inflammation. Fibrinogen was demonstrated in AD and MS models to have unique binding site with CD11b/CD18 integrin receptor on microglia, triggering subsequent neuroinflammatory cascade and promoting cognitive decline[5]. However, the molecular links between the fibrinogen cascade following BBB leakage, immune activation and cognitive function in diabetes still wait to be further exploited.

Exercise can improve attention, memory and increase pleasant mood[6]. Exercise could reduce glycemic level and insulin resistance, and produce cognitive protection by interfering with the pathophysiological mechanisms at different levels, including oxidative stress and low-grade inflammation[7]. Exercise also

plays a neuroprotective role by increasing prominent plasticity[8] and neovascularization in motor cortex[9]. However, the specific cognitive protective molecular pathway of exercise still needs to be elucidated in diabetes.

To date, no study has investigated the effects of exercise on cognition in diabetes models, relating to fibrinogen pathway with a focus on inflammatory cascade. A better understanding of fibrinogen may yield new insights into the pathogenesis of cognitive impairment due to diabetes, providing a new strategy for earlier and more effective prevention and treatment for dementia.

Materials And Methods

Animals and experimental design

Adult male Sprague-Dawley rats (weighing 180 ± 10 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd.(Beijing, China) and reared at a constant temperature (25 ± 1°C) and humidity (55 ± 5%) with 12 h day/night cycle.

All rats were randomly divided into five groups: a) Control; b) Diabetes; c) Diabetes + exercise; d)Diabetes + exercise + FGG-AAV(fibrinogen gamma chain with adeno-associated viral vector); e)Diabetes + exercise + Control-AAV. (Called group a b c d and e). Rats in Group a was fed with standard diets, and Group b,c,d and e were fed with high fat diets(HFD). After five weeks, rats in Group b,c,d,e were injected intraperitoneally with 2mL fresh STZ solution (35/40 mg/kg body weight) to construct diabetes rats, and rats in Group a were intraperitoneal injected with the same volume of saline. Two weeks before STZ injection, rats in Group c, d, e were subjected to stereotaxic injection. Those in the control-AAV group (Group e) was injected with AAV vector containing GFP alone, while those in FGG-AAV group were injected with AAV vector containing the genes for FGG(fibrinogen gamma chain)(Fig. 1).

Stereotaxic injection and AAV Procedures

Adeno-associated viral(AAV)vector was used for stereotaxic intra-hippocampal injection. AAV vectors contained the genes for (NM_001113197[NC_000069.7]) or GFP alone (Hanheng, Shanghai, China). The titer used were 1.0×10^{12} for AAV-FFG and 1.0×10^{12} for AAV-NC(containing GFP alone). Stereotaxic injection was proceeded 14 days before injection of STZ, rats in Group d and e). Diabetes + exercise + Control-AAV groups were stereotaxically injected with the Control-AAV, and Diabetes + exercise + FGG-AAV group (Group d) was stereotaxically injected with the overexpression of FGG-AAV Rats were anaesthetized with 1% Pell to barbitalum Natricum (40mg/kg, intraperitoneal(i.p.)), and then placed in a stereotaxic frame. A total volume of 8.0 μl AAV vectors (4.0 μl per side) with an injection rate of 0.5μl/min were delivered bilaterally into the CA1 region(-3.8 mm posterior to bregma, ± 2.3 mm from the midline and − 3.2 mm ventral to the surface of the skull[10, 11]. The needle was maintained in the injection site for 5 min each side, and then withdrawn very slowly.

Induction of diabetes model and exercise procedure

Type 2 diabetic rats were induced by high fat diet combined with Streptozocin (STZ). The rats in the Diabetes group(Group b, c, d, e) were fed with high-fat diet for 5 weeks and then were given STZ 35mg/kg intraperitoneally (dissolved in 0.1mmol/L Citrate Buffer pH 4.4), and the control group was intraperitoneally injected with the same volume of Buffer[12]. After 72h and a week, the blood glucose of tail vein was measured by a glucometer. Fasting blood glucose tests ≥ 7.8 mmol/L and postprandial blood glucose ≥ 11.1 mmol/L were considered as diabetic rats.

Morris water maze(MWM) test

The MWM test were used to test cognitive impairment related to spatial learning and memory[13].The MWM equipment consisted of a circular pool(150cm in diameter and 60cm in height) filled with opaque water to a depth of 32cm at a temperature of 20 ± 1°C. The pool was divided into four equal-sized quadrants. A platform (9cm in diameter and 30cm in height) was placed in the middle of one(first) quadrant and was invisible in the water. The pool was located in a dimly lit room with several orientation cues placed around the pool. Rats experienced a learning phase with the platform hidden in the target quadrant and a probe test without the platform. In the learning phase, each rat was given four trials per day for five consecutive days. Rats were randomly placed into the pool from a different quadrant in each trial, facing the wall of the maze. The time for the rats from entering the water to finding the hidden platform was recorded if it was less than 60s. If the rats could not find the platform within 60s, the latency time was recorded as 60s. The latency time was recorded using an Animal Video Tracking Analysis System(AVTAS ver 4.0, Anilab Scientific Instruments Co., Ltd., Ningbo, China). All rats were placed on the platform to observe their surroundings for 20s after each trial. On the 6th day, a single 60s probe trial was conducted with the platform removed. The time the rats swam in the target quadrant (where the platform had been placed) was recorded.

Novel object recognition(NOR) Test

The NOR test was performed as described previously[14]. The test consisted of a test box (white square box, 65 cm × 45 cm × 40 cm) and two sets (two per set) of different objects(AA’,BB’, A,A’ were identical objects, B,B’ were identical objects). The test environment was quiet and the light in the test box was even without shadow. The whole test was separated to three stages. In the first stage (adaptation), a rat was placed in the test box with its back to the box and was allowed to move by itself for 10 min with no objects. The next day, two identical objects(AA’) were placed symmetrically in the box. The rat was placed with its back to the objects from the same distance point between the two objects and allowed to move by itself for 10 min. Then the rat was returned to its home cage. 1 hour later, two different objects (AB) were placed in the box in the same position, and the rat was left to explore the box for 5 min. The stopwatch software (Time Left 3) was used to record the exploration time of the old object (A) and the novel object (B) when different objects (AB) were placed. A discrimination ratio (DI) of exploring the novel object was calculated, expressed as DI = N/(N + F), where N was the time for exploring the novel object and F was the time for exploring the old object.

Assessment of BBB

Evans Blue (EB) can bind with albumin specifically which could penetrate into the brain when BBB injuried. Therefore, intravenously injecting EB and then measuring the content of EB in brain tissue have been used to evaluate the change of permeability after BBB injury[15]. The rats were injected with 2% EB (4 mL/kg, Sigma Aldrich, Saint Louis, MO, USA) through the tail vein. Two hours later, after anesthetized with 1% sodium pentobarbital, the rats was perfused by 50 mL heparinized saline from the left ventricle for 15 minutes to clear the remaining EB dye from the blood vessels. After that, hippocampal samples were taken out and soaked in formamide(3 mL/100 mg) at 60°C for 24 hours, centrifuged at 15000 g at 4°C for 30 minutes, and 0.2 mL of supernatant was obtained. Spectrophotometric determination of extravasated EB in the supernatant was assayed at 620nm. EB content was determined using a standard curve.

Western Blotting

A RIPA lysis buffer(AS1004;ASPEN) was used to extracted the hippocampus which contained protein inhibitors and then was centrifuged (12,000g, 5min, 4°C). The BCA protein assay was used to estimated protein concentrations. The samples(40μg protein each) were separated by sodium dodecyl sulfate-polyacrylamide gels and then transferred to a polyvinylidene difluoride membrane, and blocked for 1h in Blocking buffer at room temperature. Next, the membranes were sequentially incubated with primary antibodies against ZO-1(1:500, sc-33725, santa), claudin-5 (1:1000, abcam, ab131259), MMP-9 (1:1000, ab76003, abcam), GAPDH (1:10000, ab181602, abcam),at 4°C overnight. Then, after washing three times with TBST, the membranes were incubated with secondary antibodies horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:10000; AS1107;ASPEN) for 30 min. The freshly prepared ECL-mixed solution was added to the protein side of the membrane and exposed in a dark room. The exposure conditions were adjusted according to light intensities. The optical densities of specific bands were measured with Alpha Ease FC software

Enzyme-linked immunosorbent assay

The levels of IL-1β and TNFα in hippocampus were tested by Enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. IL-1β levels were determined using ELISA kit (ELK Biotechnology, ELK1272), with a detection range of 15.63–1000 pg/ml and sensitivity of 5.9 pg/mL. TNF-α levels were determined using ELISA kit (ELK Biotechnology, ELK1396), with a detection range of 15.63–1000 pg/ml and sensitivity of 6.1 pg/mL. The hippocampus tissue were washed by PBS at 4℃, then after homogenization and centrifugation, the supernatant was collected for further use. Seven gradient standards were configured using 1.0mL standard substance. The values were read at 450 nm in an ELISA reader, and IL-1β, and TNF-α concentration were calculated from specific calibration curves prepared with known standard solutions.

Immunofluorescence

The immunofluorescence was performed to evaluate hippocampus neuroinflammatory cells by labeling the GFAP+, Iba1 + and CD68 + cells. The coronal sections were used to evaluated neuroinflammatory cells

by labeling the microglia marker Iba1+/CD68+, astrocytes marker GFAP+. As a negative regulator of BBB permeability, MMP-9 was labeled to assess BBB permeability. The coronal sections were incubated with the primary antibodies for Iba-1 (rabbit anti-Iba-1, 1:300; 019-19741;WAKO), GFAP (rabbit anti-GFAP, 1:100; 16525-AP; Sanying), CD68(rabbit anti-CD68,1:200, BA3638, Boshide), and with DAPI (sc-3598; Santa Cruz) at 4 ∘ C overnight. Then sections were then labeled with fluorescent HRP goat anti-rabbit antibody (1:400),Cy3 labeled goat anti-mouse antibody (1:200) or 488 labeled goat anti-rabbit antibody (1:200), at room temperature for 50 min in the dark. Images were captured with confocal fluorescence microscope.

Cell quantification and statistical analysis

Experimental data were expressed as the mean ± SEM. Statistical analyses were conducted using Graph-Pad Prism Software. Differences in escape latency were analyzed via a two-way repeated-measures ANOVA followed by a Tukey's multiple-comparisons post hoc test was used to compare means of different groups in this study. Tamhane's T2 post hoc test was used to perform a multiple comparison analysis of the results of the probe trial. Analysis of the other data was performed via a one-way ANOVA followed by a post-hoc Tukey test for multiple comparisons. Statistical significance was defined as $P < 0.05$.

Results

Exercise downregulated the expression level of FGG in diabetic group

The recombinant AAV9-CMV-R-FGG-3XFlag-GFP virus contained a green fluorescent protein (GFP). 14 days after the virus injection, we evaluated the location of viral injection in brain tissue by fluorescence tags of the GFP in frozen sections. The results showed that a large amount of green fluorescence (GFP expression) was observed in the hippocampal CA1 region of the FGG-AAV group, while no green fluorescence was observed in the hippocampal CA1 region of the control group, indicating that FGG overexpressed virus was successfully transfected into the hippocampal CA1 region and the virus was expressed (Fig. 2A).

The expression levels of fibrinogen in the hippocampus and blood were detected by ELISA. Compared with the Control group, the expression level of FGG in diabetes was elevated both in hippocampus and blood, while exercise downregulated the FGG level. The FGG expression was upregulated obviously in hippocampus by overexpression of FGG, but not in blood. The AAV-NC didn't influence the level of FGG.

Exercise improved learning and memory ability by inhibiting fibrinogen level in diabetic rats.

Rats were trained in Morris Water Maze(MWM) for 5 consecutive days, and the latency from entering the water to find the platform in 4 quadrants was recorded every day. Mean escape latency was defined as the average time of latency in 4 quadrants. As shown in Fig. 3B, compared with the control group,

diabetic rats spent longer time searching for the hidden platform on 3rd, 4th, 5th ($p < 0.05$, $p < 0.05$, $p < 0.05$) day. Exercise significantly reduced the escape latency of diabetic rats. There was no significance in the escape latency between the control group and exercise intervented diabetic group($p < 0.05$). In addition, the escape latency of the rats in the FGG-AAV group was prolonged. There was no significant difference between diabetic group and FGG-AAV group, while groups between control and exercise intervention had no significance.

On day 6, through observation of the probe trials with the platform removed, we found that the time spent in the platform quadrant of the diabetic group was significantly lower than that of the control group and the diabetic rats with exercise intervention, and the difference was statistically significant ($p < 0.05$). There was no significant difference in target quadrant exploration time between control-AAV and exercise intervention.

The novel object recognition experiment (Fig. 3D) showed that the time ratio of the diabetic group to explore new objects was significantly lower than that of the control group and the diabetic rats with exercise intervention, and the difference was statistically significant ($p < 0.05$). There was no significant difference between control-AAV and exercise intervention.

Effects of exercise on inflammatory cells in diabetic rats.

The production and activation of microglia and astroglia in diabetic rats were significantly higher than those in control and diabetic rats with exercise intervention (Fig. 4A), and the difference was statistically significant ($p < 0.05$) (Fig. 4B, C and D). The activation of exercise-inhibited glial cells was significantly up-regulated in the FGG overexpression group, while there was no significant difference between the virus control group and the exercise training group (Fig. 4E). These results indicated that exercise can inhibit the activation of microglia and astrocytes, and the up-regulation of FGG expression antagonizes the effect of exercise on the inflammatory activation of diabetic rats.

Protective effects of exercise on BBB in diabetic rats.

The content of Evans-blue (EB) in hippocampus was quantitatively analyzed by chemical colorimetry to investigate the leakage of EB in hippocampus of diabetic rats. Compared with the control group, hippocampal EB leakage significantly increased in diabetic rats ($p < 0.01$), while exercise significantly reduced it ($p < 0.05$) (Fig. 5B). Increased FGG expression in diabetic rats significantly inhibited the protective effects of exercise on EB leakage ($p < 0.01$), while there was no significant difference between the control-AAV group and exercise intervention group.

We evaluated BBB-related protein expression levels including ZO-1 and Claudin-5 in hippocampus of rats using Western blot. Compared with the control group, the levels of ZO-1 ($p < 0.01$) (Fig. 5D) and Cluadin-5 ($p < 0.01$) (Fig. 5E) were decreased in diabetic rats, while exercise significantly improved the damage of BBB-related protein expression. In FGG overexpression group, both ZO-1 ($p < 0.05$) and Claudin-5 ($p < 0.01$) were significantly lower than those of exercise intervention, which indicated that high level of FGG

would disrupt the protective effect of exercise on diabetes. There was no significant difference between the control-AAV group and exercise intervention group ($p > 0.05$).

Effects of exercise on MMP-9 expression in diabetic rats.

The level of MMP-9 in diabetic rats was evaluated by immunofluorescence (Fig. 6A) and Western blot (Fig. 6B). Compared with the control group, the number of MMP-9 positive cells and the expression level of MMP-9 protein were significantly increased in diabetic rats ($p < 0.01$), and exercise could significantly reduce the expression level of MMP-9 in diabetic rats ($p < 0.01$ for MMP-9 positive cells, $p < 0.05$ for protein content)(Fig. 6C,D). FGG-AAV group disrupted the inhibitory effect of exercise on MMP-9 level, and there was no significant difference between control-AAV group and exercise intervention group.

After evaluating the level of IL-1β and TNFα by ELISA, we observed that the inflammatory factors were obviously upregulated ($p < 0.05$ for IL-1β, $p < 0.001$ for TNFα) in diabetes(Fig. 6E,F). In exercise group, the expression level of IL-1β ($p < 0.05$)and TNFα ($p < 0.001$) were inhibited, while overexpressing FGG level would compromise the protective effects of exercise of diabetic rats ($p < 0.01$ for IL-1β, $p < 0.05$ for TNFα). There was no significant difference between control-AAV group and exercise intervention group ($P > 0.05$).

Discussion

In this study, we found that fibrinogen gamma chain contributed to cognitive deterioration in diabetic rats through destroying BBB and regulating inflammatory response, while swimming exercise could rescue this process. Furthermore, after we upregulated FGG through adeno-associated virus injection, the protective effect of exercise on cognition was significantly inhibited, accompanied by aggravated BBB injury and inflammatory response. Therefore, we speculate that exercise could improve cognitive deficit of diabetic rats by targeting FGG pathway to inhibit inflammatory factors and regulate the BBB function.

Individuals with diabetes exhibit greater cognitive decline, especially on executive function[16]. Diabetes-related microvascular dysfunction is associated with a higher risk of cognitive dysfunction[17]. Accumulating evidence indicates that oxidative stress and chronic inflammation contribute to the development of diabetic encephalopathy[18]. Clinical studies indicated that physical exercise has potential to elicit cognitive benefits[19, 20]. Exercise is an important contributor to diabetes remission and could reduce brain inflammatory response[21]. Our study confirmed again from animal experimental perspective that exercise can improve cognitive impairment in diabetes.

Cross-talks between peripheral and central inflammation are common in diabetes. Increased BBB permeability is a critical neurovascular complication of diabetes[3]. Damage to the BBB can lead to immune cells infiltrating into the brain, exacerbating the central inflammation, while neuroinflammation also contributes to BBB damage in the other way, leading to cognitive dysfunction[22]. Physical exercise could diminish BBB permeability by reinforcing antioxidative and anti-inflammatory capacity[23]. On the other hand, exercise was reported to rescue cognitive dysfunction in diabetic rats, decreasing IL-1β, IL-6 and TNFα expression level, and alleviating proinflammatory microglia M1 activation[18, 24, 25].Our

results are consistent with those previous evidence that cognitive deficits in diabetes were accompanied with synergistic activation of neuroinflammation and BBB damage and further demonstrated that swimming exercise could slow down the cognitive deterioration through constraining these two process.

Fibrinogen, a blood-derived coagulation protein, was demonstrated to have the unique function to bind with the CD11b/CD18 integrin receptor and induce subsequent pathogenic microglia activation, triggering neuroinflammatory response and leading to fibrin deposition in the brain[26, 27]. Clinical studies have shown that higher baseline blood fibrinogen level was associated with greater subsequent cognitive decline in older diabetic patients[28]. FGG expression level was reported to increase in diabetes rats[29]. BBB also compromised in DM in the vivo model and associates with memory deficits and neuroinflammation[30]. In our study, we found that the expression level of gamma-chain fibrinogen was upregulated both in the blood and hippocampus of diabetic rats, and the activation of inflammation cascades further increased the permeability of the BBB, while exercise decreased the FGG expression and protected the BBB function. After upregulating hippocampal FGG level through stereotaxic AAV vector injection, we observed that the neuroprotective effects of exercise were attenuated, meanwhile, the inflammatory factors were activated and BBB permeability increased subsequently. Hence, our findings indicate that exercise could probably play a protective role through the FGG pathway, suppressing inflammatory activation, maintaining the integrity of BBB, and ultimately improving cognitive function in diabetic rats.

Fibrinogen serves as an inflammatory factor with the function of upregulating the expression of IL-1β and MMP-9 secretion[26, 31]. MMP-9 can break down the various components of the BBB and is regarded as a key marker for BBB breakdown. In this study, we confirmed that the increased FGG induced by diabetes is accompanied with multiplied expression of IL-1 β and MMP-9, leading to an increase in BBB permeability, while exercise could rescue the pathological process. The protective effect of exercise on BBB was attenuated by overexpressing FGG level, and IL-1 β and MMP-9 also accordingly increased. Therefore, exercise may regulate IL-1β/MMP-9 pathway to improve BBB permeability by inhibiting FGG expression level, and thus play a cognitive protective role in diabetic rats.

One limitation of this study is that we didn’t use the transgenic animal model of fibrinogen and that will be our future research plan. What’ more, although STZ induction method is a recognized model of diabetes, it can’t fully represent the complex pathophysiological process of clinical cases[32].

Summary

This study mainly explored the role of fibrinogen in the cognitive protection mechanism of exercise in diabetic rats models.We proved that FGG over-expressed could further activate the inflammatory response, broke down the blood-brain barrier, and lead to cognitive dysfunction in diabetes. Exercise had cognitive protective function by downregulating the expression of FGG, inhibiting the neuroinflammation process, thus to protect blood-brain barrier. Further research is needed as the impact of exercise is yet to be fully elucidated.

Abbreviations

BBB
blood-brain barrier
EB
Evans Blue
MWM
Morris water maze
STZ
Streptozocin
AAV
Adeno-associated viral
GFP
fluorescent protein
ELISA
Enzyme-linked Immunosorbent Assay
NOR
Novel object recognition
FGG
fibrinogen gamma chain

Declarations

Author contributions

DW.L, D.S and JJ.Z designed the experiments. CJ.Q and MF performed the experiments. CJ.Q and DW.L analyzed the data and wrote the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

All animal protocols were approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Ethics Committee of Wuhan University(permit number 2019110).The rats involved in this experiment were handled in strict accordance with the regulation for the use and care of laboratory animals. All efforts were made to minimize animal suffering.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

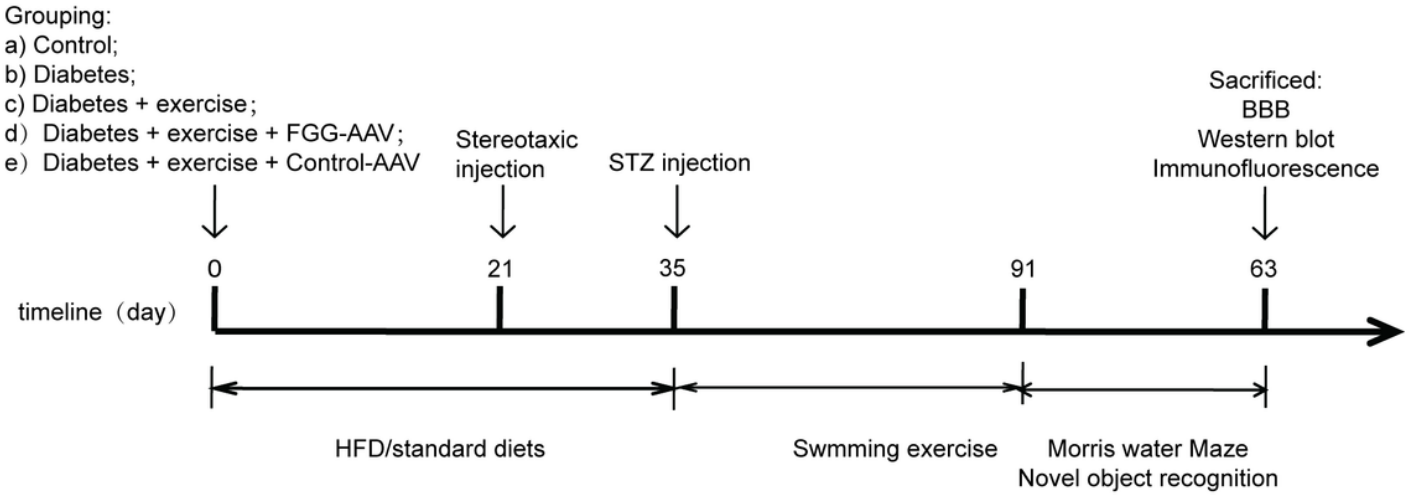

Figure 1

Timeline for experiments

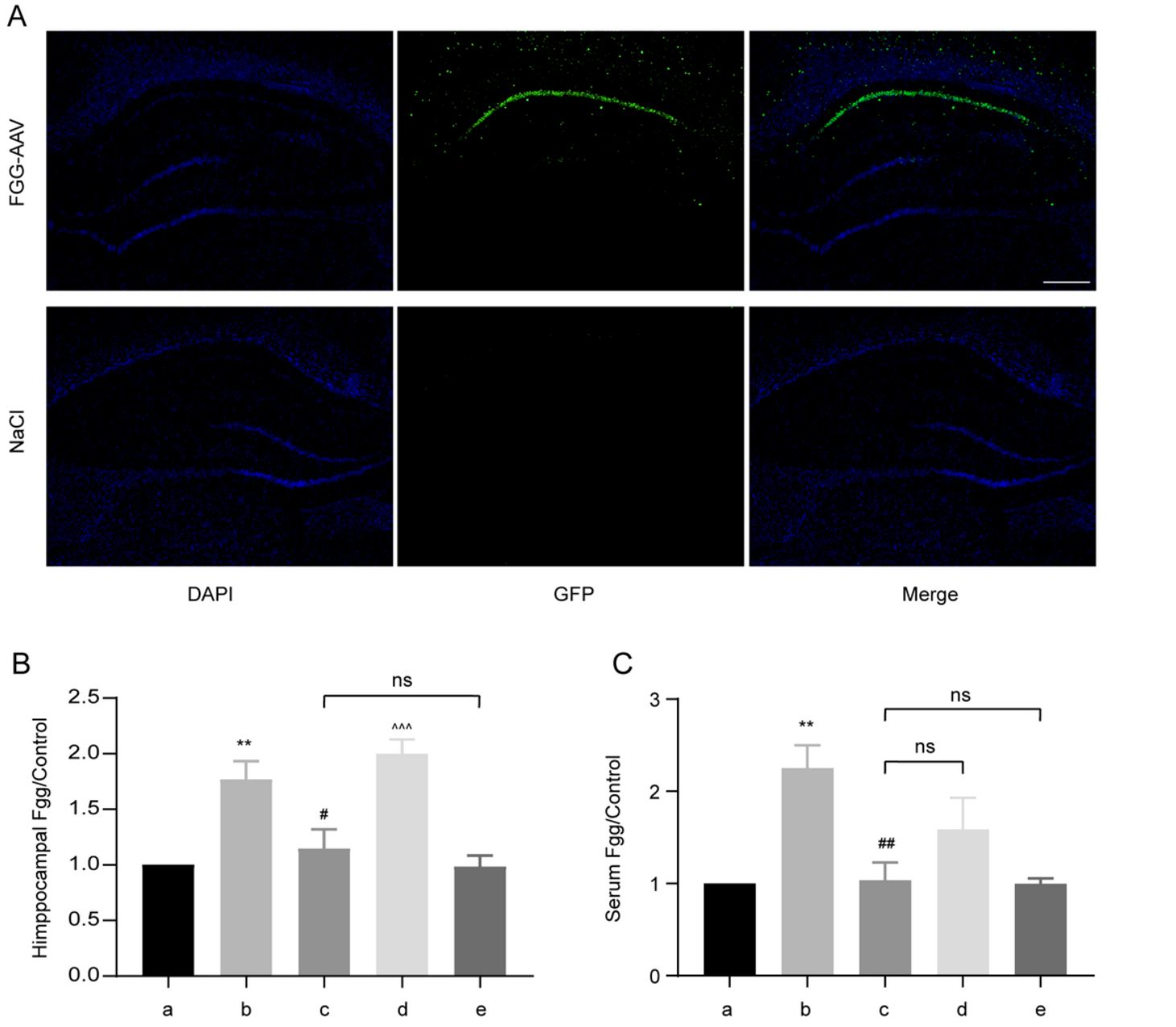

Figure 2

Exercise downregulated the expression level of FGG both in diabetic group. **A** Representative immunofluorescence image of hippocampal CA1 region of rats 14 days after normal saline or FGG-AAV was injected into hippocampal regions by stereotactic injection. **B,C** Expression level of FGG in hippocampal(**B**) or blood(**C**) in five groups. a, b, c, d, e represent the Control, Diabetes, Diabetes + exercise, Diabetes + exercise + FGG-AAV, Diabetes + exercise + Control-AAV respectively. Data are expressed as the mean±SEM.*p < 0.05, **p<0.01,***p<0.001 compared to the Control group(group a). p < 0.05, p < 0.01, p < 0.001 compared to the Diabetes group (group b). ^p< 0.05, ^^p<0.01,^^^p<0.001 compared to the Diabetes + exercise group(group c).

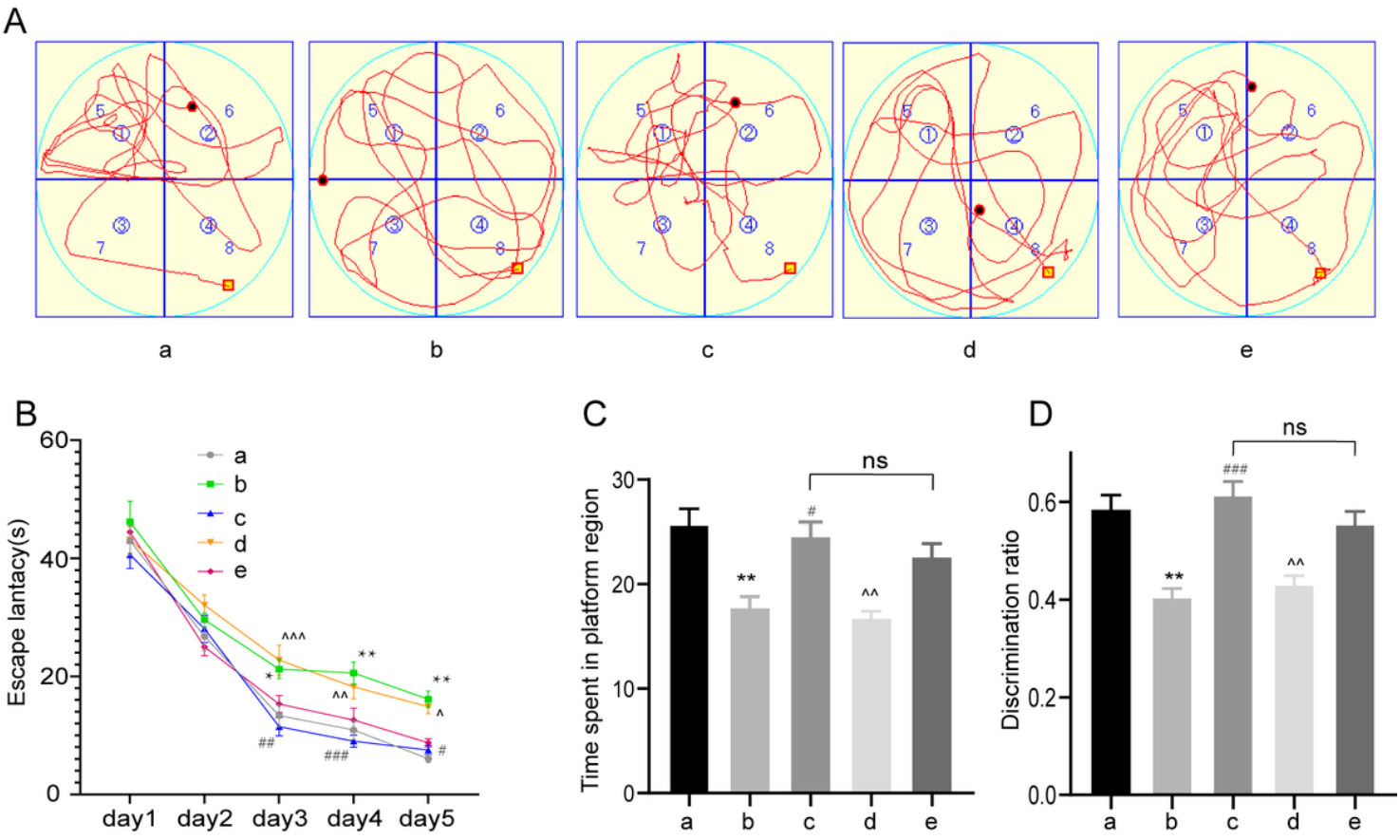

Figure 3

Exercise improved learning and memory ability by inhibiting fibrinogen level in diabetic rats. **A** Representative swimming paths of each group during the probe trial. **B** Changes in the average escape latency on days 1–5 in the MWM with a platform located in the upper left-hand quadrant. **C** Time spent in the target quadrant during the spatial probe test with the platform removed. **D** Discrimination ratio of Novel object recognition. a, b, c, d, e represent the Control, Diabetes, Diabetes + exercise, Diabetes + exercise + FGG-AAV, Diabetes + exercise + Control-AAV respectively. Data are expressed as the mean±SEM. p<0.05 was set as the threshold for significance. *p < 0.05, **p<0.01,***p<0.001 compared to the Control group(group a). p < 0.05, p < 0.01, p < 0.001 compared to the Diabetes group (group b). ^p< 0.05, ^^p<0.01,^^^p<0.001 compared to the Diabetes + exercise group(group c).

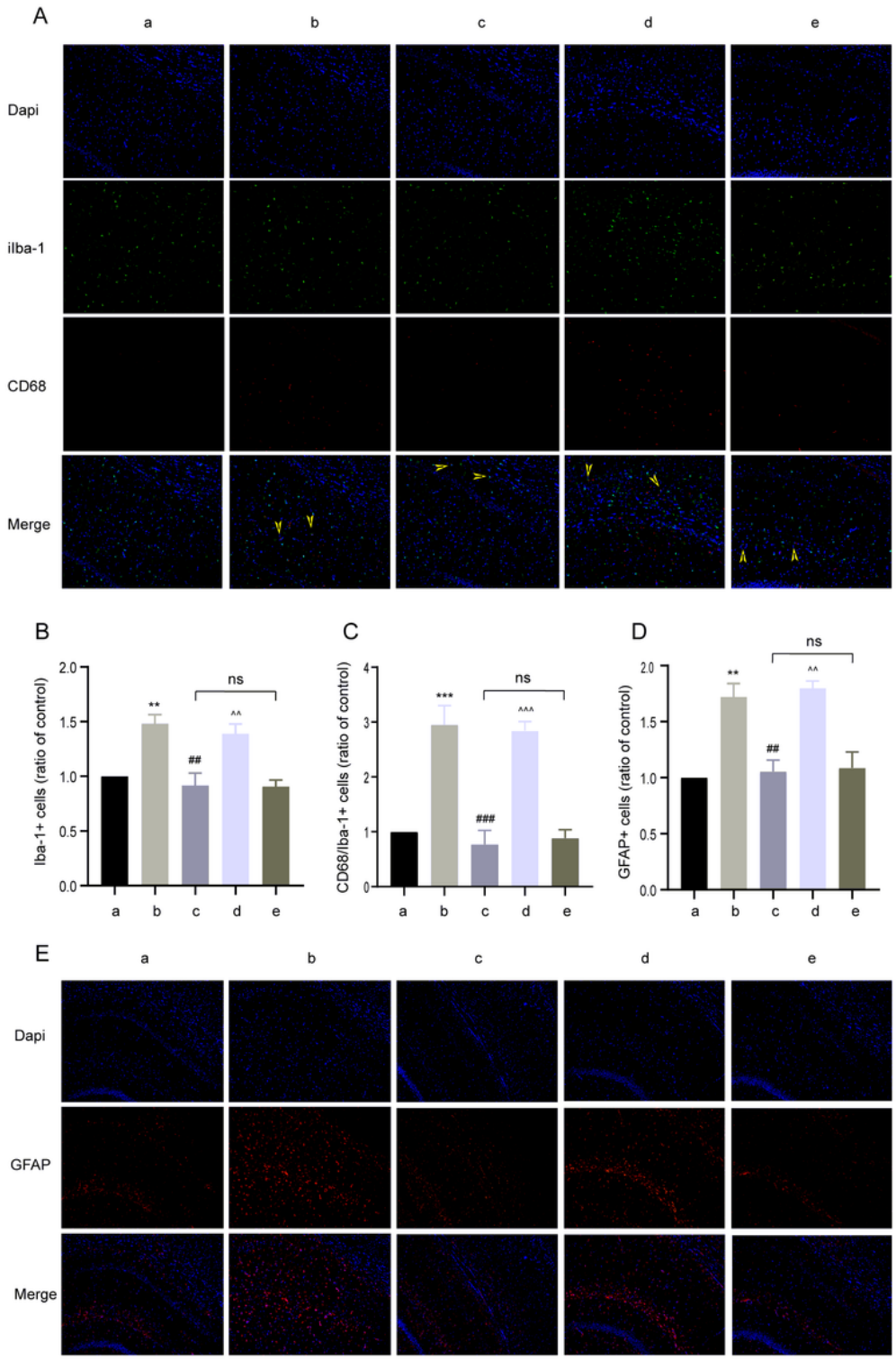

Figure 4

Effects of exercise on inflammatory cells in diabetic rats. **A,E** Representative images of Iba-1/CD68 double labelling and GFAP immunofluorescence in the CA1 are shown.Representative positive cells are indicated by arrows. **B** Quantification of Iba-1 positive cells. **C** Quantification of CD68/Iba-1 immunofluorescence. **D** Quantification of GFAP immunofluorescence. a, b, c, d, e represent the Control, Diabetes, Diabetes + exercise, Diabetes + exercise + FGG-AAV, Diabetes + exercise + Control-AAV

respectively. Data are expressed as the mean±SEM.*p < 0.05, **p<0.01,***p<0.001 compared to the Control group(group a). p < 0.05, p < 0.01, p < 0.001 compared to the Diabetes group (group b). ^p< 0.05, ^^p<0.01,^^^p<0.001 compared to the Diabetes + exercise group(group c).

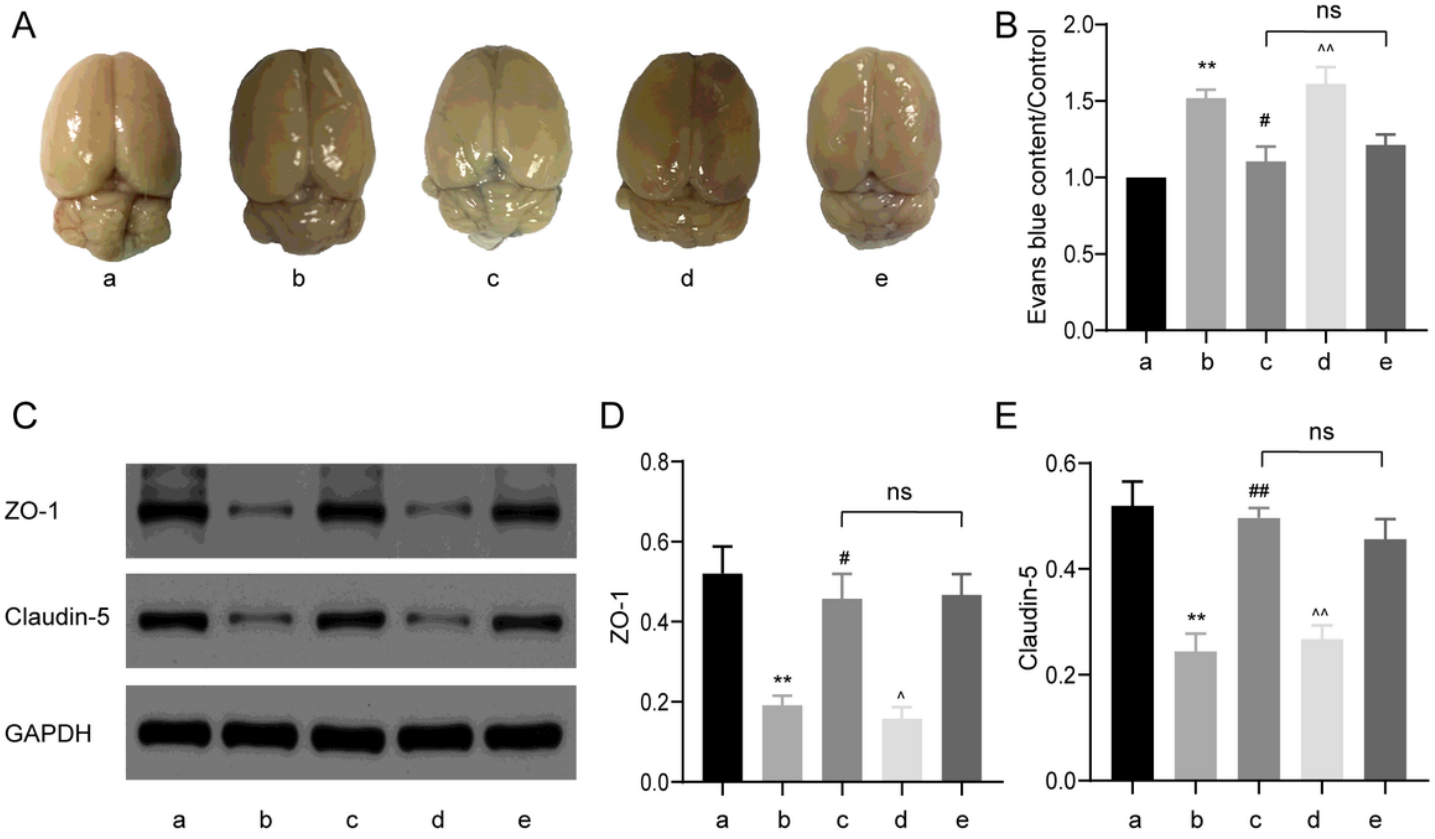

Figure 5

Effects of exerciseon BBB in diabetic rats. **A,B** Representative images of brain(A) and quantification of EB content(B) after injection of EB are shown. **C** Representative western blot images of the ZO-1/Claudin-5 proteins and GAPDH; **D,E** Quantification of the relative optical densities of hippocampal ZO-1 and claudin-5. a, b, c, d, e represent the Control, Diabetes, Diabetes + exercise, Diabetes + exercise + FGG-AAV, Diabetes + exercise + Control-AAV groups respectively. Data are expressed as the mean±SEM. *p < 0.05, **p<0.01,***p<0.001 compared to the Control group(group a). p < 0.05, p < 0.01, p < 0.001 compared to the Diabetes group (group b). ^p< 0.05, ^^p<0.01,^^^p<0.001 compared to the Diabetes + exercise group(group c).

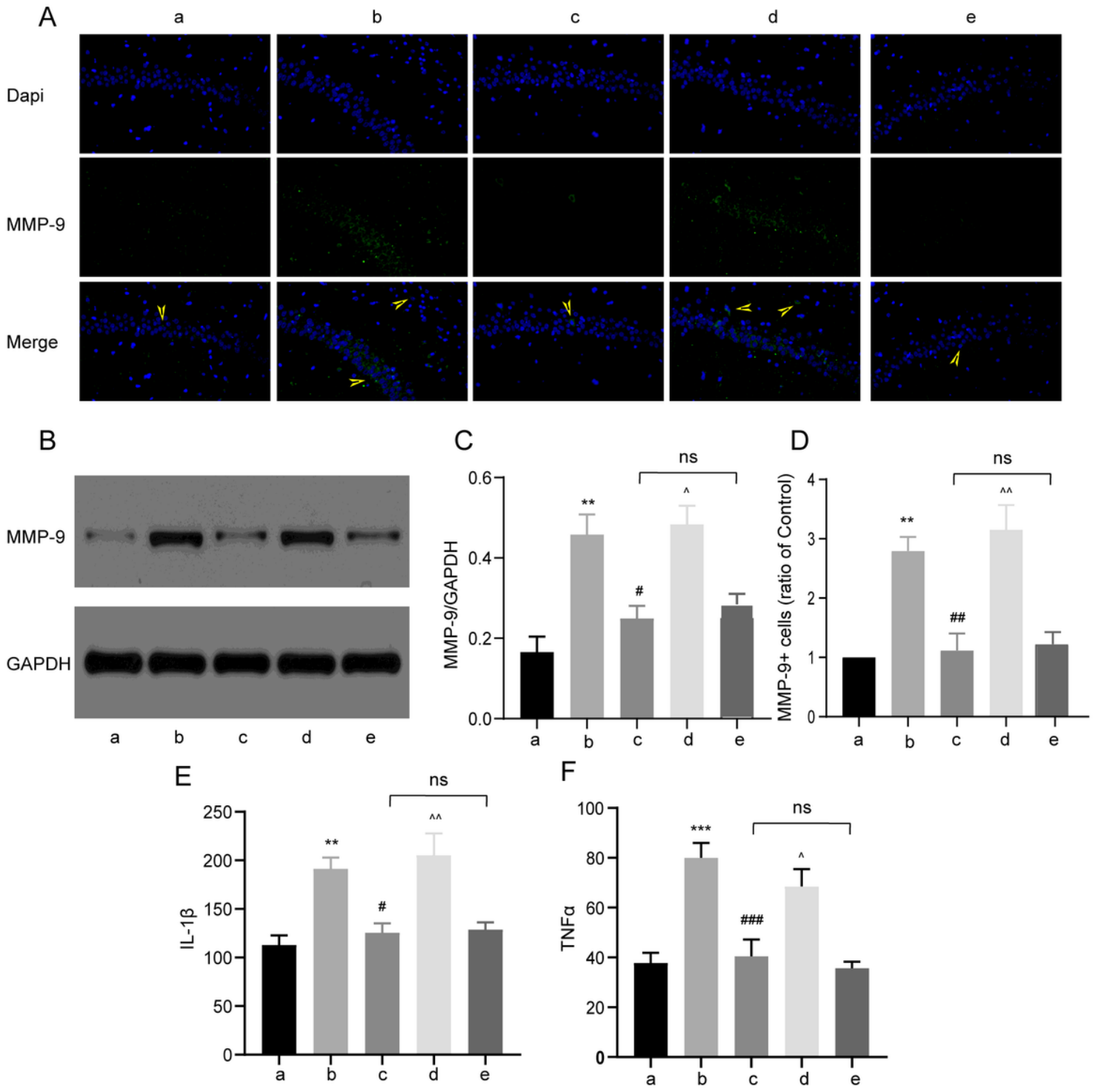

Figure 6

Effects of exerciseon MMP-9 expression in diabetic rats. **A** Representative images of MMP-9 immunofluorescence in the CA1 are shown. Representative positive cells are indicated by arrows. B Representative western blot images of the MMP-9 proteins and GAPDH. **C** Quantification of the relative optical densities of hippocampal MMP-9. **D** Quantification of MMP-9 positive cells. Group a, b, c, d, e separately represent the Control, Diabetes, Diabetes + exercise, Diabetes + exercise + FGG-AAV, Diabetes +

exercise + Control-AAV group. Data are expressed as the mean±SEM. *p < 0.05, **p<0.01,***p<0.001 compared to the Control group(group a). p < 0.05, p < 0.01, p < 0.001 compared to the Diabetes group (group b). ^p< 0.05, ^^p<0.01,^^^p<0.001 compared to the Diabetes + exercise group(group c).

Supplementary Files

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