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Original Article

Energy intake restriction significantly improves POCD after internal fixation of tibial fractures in mice

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Abstract: Objective: To investigate the effect of energy intake restriction on postoperative cognitive dysfunction (POCD) after internal fixation of tibial fractures in mice. Methods: Thirty mice were divided into model groups of internal fixation of tibial fractures with 0%, 20%, 30% and 40% energy intake restriction and sham operation group (n = 6). Novel object recognition task and elevated plus maze test were used to assess the ability of recognition memory and anxiety-related behavior before and one week after surgery. The blood samples were collected from mice on days 1, 3 and 7 after surgery, and the mice were euthanized on the 8th day after surgery. RT-PCR and Western blot were employed to detect the expression of AMPK-SIRT1 pathway-related genes and proteins in the hippocampus. ELISA was used to detect the levels of inflammatory factors in the peripheral blood of mice. Hematoxylin-eosin (H&E) staining and immunofluorescence (IF) staining were used to detect the proliferation, differentiation and injury of hippocampal cells. Results: The results showed that 20% and 30% energy intake restriction significantly improved the POCD after internal fixation of tibial fractures in mice. Significantly, 30% energy intake restriction reduced the expression of AP-1, NF-κB, CD45, IBA-1, and inflammatory factors IL-1β, IL-6, IL-8 and TNF-α, and increased the expression of AMPK and SIRT1 after the operation. H&E and IF staining showed that 30% energy intake restriction reduced postoperative hippocampal neuronal damage. Conclusion: Energy intake restriction can significantly improve POCD after internal fixation of tibial fractures in mice and may provide a new treatment paradigm for POCD patients.

Keywords: Energy intake restriction, POCD, tibial fractures, hippocampus

Introduction

Non-vertebral fractures are a serious and increasing source of morbidity and mortality in the elderly. Tibial fractures account for about 5%-8% of limb fractures and 3% of long bone fractures [1, 2]. With the population age, the incidence of limb fractures gradually increases, which results in significant morbidity and compromises the health [3-5]. Internal fixation surgery is currently the main treatment for the fractures, but this treatment can cause iatrogenic transient radial nerve injury. There is growing evidence that both anesthesia and surgery have long-term effects on cognition, e.g., postoperative cognitive impairment (POCD) with a longer duration after surgery. POCD mostly occurs in patients over 65 years old, manifested as confusion, anxiety, changes in personality, memory and concentration impairment, decreased intellectual function, etc. Permanent dementia and even death can occur with POCD [6-9]. Therefore, it is very important to establish suitable animal models of POCD. Fracture surgery is a widely used animal model of surgical trauma-induced POCD, which simulates clinical major orthopedic surgery in the elderly, and many POCD clinical studies also focus on such patients [10, 11]. The incidence of short-term POCD in elderly patients after major orthopedic surgery was as high as 17% [12]. In 2010, a research team used this operation for the first time to induce POCD and selected the left tibia as surgical site [13, 14]. In addition, some other research teams also used fracture surgery to establish POCD animal models [15, 16].

Epidemiological and early research has suggested that diet plays a central role in the

pathogenesis of many chronic diseases related to aging, and that the degree, timing and composition of energy restriction play important roles in health [17, 18]. This shatters the conventional wisdom that only energy intake can promote health. Increasing research reports have confirmed that restricting energy intake can have beneficial effects on the body through lowering cholesterol levels and reducing the incidence of several age-related diseases, including type II diabetes and cancers [19-23]. Rizza discovered that energy intake restriction increased mitochondrial biosynthesis regulator genes, such as: PGC-1α and NRF-1, promoted mitochondrial biogenesis, increased mitochondrial DNA content and increased cytochrome C oxidase activity, thereby alleviating aging-induced mitochondrial function decline [24]. The quantity and quality of mitochondria are closely related to human health. Csiszar found that energy restriction could activate endogenous antioxidant function, promote angiogenesis, anti-apoptosis and anti-inflammatory. In addition, the study also found that endothelial cells in the cerebral blood vessels of aged rats retained their youthful phenotype [25]. These changes in endothelial cell function and reactivity may contribute to the protective effect of energy restriction in aging cerebral vessels, and thus energy restriction may be useful in the prevention and treatment of vascular cognitive impairment in elderly patients [26, 27]. However, there is no systematic report at present on whether energy intake affects POCD.

In this work, we hypothesized that energy restriction has an effect on POCD induced by internal fixation of tibial fractures and subjected mice to energy restriction for 4 weeks. A mouse model of internal fixation of tibial fracture was then constructed to explore the cognitive ability of mice before and after surgery, inflammatory indicators in vivo, and the damage and proliferation of neurons in the hippocampus. Our goal was to determine whether energy restriction affects POCD resulting from tibial fracture fixation and whether this effect is beneficial.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University (2021-QuanYilun-165).

Materials

IL-1β detection kit (No. EMC001b), IL-6 detection kit (No. EMC004), IL-8 detection kit (No. EMC104) and TNF-α detection kit (No. EMC102a) were purchased from Neobioscience Biotechnology Co., Ltd. CD45 monoclonal antibody (No. 60287) and Iba-1 monoclonal antibody (No. 10904) were purchased from Proteintech Group (Wuhan). Total RNA extraction kit (No. AM1911), SYBRGreen PCR kit (No. F-415XL) and reverse transcription kit (No. K1622) were purchased from Thermo Fisher Scientific (China) Co., Ltd.

Animals

Male C57BL/6J mice (22 g ± 2 g) aged 7-8 weeks were used for all experiments. The mice were randomly divided into 5 groups (n = 6), and they were adaptively fed for 1 week under standard conditions (22-25°C, 12-h light/dark cycle).

Establishment of tibial fracture animal model

The mice were divided into control group (normal diet and sham surgery, 2.6 g/mouse/day), model group (normal diet and surgery, 2.6 g/mouse/day), low energy restriction group (20% energy restriction and surgery, 2.07 g/piece/day), medium energy restriction group (30% energy restriction and surgery, 1.82 g/piece/day) and high energy restriction group (40% energy restriction and surgery, 1.56 g/piece/day). Each group was kept feeding for 4 weeks as required, and then internal fixation of tibial fracture was performed. Briefly, pentobarbital sodium (40 mg/kg) was used to anesthetize mice. In the control group, only the epidermis was cut to separate the tibial and sutured. In other surgery groups, after the tibial was separated, the tibial was clipped off in the middle section, and Kirschner wires were inserted from the elbow joint of the tibial, and then moved up the medullary cavity to the distal tibial head. The excess Kirschner wires were cut off, and the epidermis was sutured layer by layer.

Establishment of POCD model after tibial fracture

After the tibial fracture, the rats with successful modeling were screened by the Y-maze test. Referring to previous research [28], the upper

limit of 95% confidence interval of the average training length of rats in the Y maze test was used to determine whether the rats showed cognitive impairment after the operation. The experiment included a total of 158 fracture animals. Finally, 120 fracture animals with POCD were selected for follow-up experiments.

New object recognition (NOR) task

Mice in each group were subjected to NOR task one week before and one week after the surgery, and the effect of internal fixation of tibial fractures on the mice's ability to recognize new objects in the environment was evaluated. Briefly, two identical blue square objects were placed symmetrically at the two corners of the test box, and the mouse was put into the test box from a fixed position and allowed to move freely for 5 min. Then, one of the square objects was replaced, and the mouse was again put into the test box from the same position and allowed to move freely for 5 min. After the test for each mouse, the test box was entirely wiped with alcohol to avoid interference from odor clues. The recognition index was calculated as exploration of novel object - exploration of familiar object/total exploration time. The higher the recognition index, the stronger the learning and memory ability of mice.

Elevated plus maze (EPM) test

EPM is a common test method to evaluate the anxiety response of rodents [29]. Mice in each group were subjected to EPM test one week before and one week after the surgery. The test mouse was placed at the intersection of the open and closed arms of the elevated maze, with the mouse facing the open arm. The camera was controlled by the animal behavior analysis software to observe and analyze the activities of the mice in the platform within 5 min. The total number of times of entering the open arm and the closed arm shows the vitality of sports, and the anxiety state was evaluated by the proportion of the number of times of entering the open arm and the staying time at the open arm.

Enzyme-linked immunosorbent assay (ELISA) in peripheral blood of mice

After the internal fixation of tibial fractures, the peripheral blood of mice was collected on the 1, 3 and 7 days, and the levels of IL-1β, IL-6, IL-8 and TNF-α were detected by ELISA. After collection, 4% sodium citrate (10 μL) was added to 90 μL of blood and mixed for 20 min. The serum was collected by centrifugation (2000 g, 20 min), and the serum concentration of IL-1β, IL-6, IL-8 and TNF-α were detected according to the commercial ELISA kit instructions.

Western blotting (WB)

The mice were sacrificed 8 days after the internal fixation of tibial fractures. The hippocampus was taken out and quickly frozen with liquid nitrogen. The protein expression in the hippocampus were checked by WB. The hippocampal tissue of each group of mice were minced and suspended in Radio Immunoprecipitation Assay (RIPA) lysis buffer for 2 h. This mixture was centrifuged at 12000 g and 4°C for 10 min to collect the supernatant. The protein concentration of the supernatant was determined using the BCA protein quantitative detection kit. Samples containing equal amount of protein (30 μg/lane) were subjected to polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride (PVDF) membranes at 100 V for 1 h. The membranes were blocked in Tris-buffered saline with 5% non-fat milk and 0.1% Tween-20 for 1 h. PVDF membranes were incubated with the primary antibodies and second antibody, respectively. Enhanced chemiluminescence luminescent solution was used to visualize the target protein bands on the membrane. The same amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein was used as an internal reference control for protein standardization in WB.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The mice were sacrificed 8 days after the internal fixation of tibial fractures. The hippocampus was taken out and quickly frozen with liquid nitrogen. The mRNA levels of AMPK, SIRT1, IL-1β, NF-κB and AP-1 in the hippocampus of each group were detected by qRT-PCR. The hippocampus tissue was thoroughly ground and incubated with Trizol lysis buffer for 5 min at room temperature. Then the samples were added with pre-cooled chloroform (chloroform:Trizol = 1:5), shaked vigorously for 15 s, incubated at room temperature for 10 min, and centrifuged (15 min, 4°C, 12000 g) to collect the upper aqueous phase. An equal volume of isopropanol was added to the upper

aqueous phase (500 μL), incubated at -20°C for 30 min, and centrifuged (15 min, 4°C, 10000 g) to collect the RNA precipitate. The RNA pellet was washed with 650 μL of 75% ethanol in diethyl pyrocarbonate (DEPC)-treated water. The RNA was collected by centrifugation (5 min, 4°C, 8000 g), re-dissolved in 20 μl DEPC-treated water, and stored in a refrigerator at -20°C. The RNA was reverse transcribed into cDNA by using a reverse transcription kit. The qRT-PCR analysis was performed in a 20 μL reaction mixture, including 10 μL of SYBRGreen Mix, 0.4 μL of forward primer, 0.4 μL of reverse primer, 7.2 μL of double distilled water and 2 μL of cDNA template (**Table 1**). Data collection and analysis were performed by ABI Prism 7500 SDS software.

Hematoxylin-eosin (H&E) staining and immunofluorescence (IF) staining analysis

The mice were sacrificed 8 days after the internal fixation of the tibial fracture. The hippocampus was taken out and fixed with 4% paraformaldehyde for later use. After the hippocampal tissue was sectioned, it was stained with hematoxylin for 5 min and incubated with 1% hydrochloric acid ethanol for 2 s, and the excess reagent was washed away with distilled water. Then, the sections were stained with eosin (5 s), dehydrated with absolute ethanol and sealed with neutral glue. An inverted fluorescence microscope (Olympus IX71) was used to take pictures of tissue sections.

Hippocampal slices were immersed in antigen retrieval solution and incubated at 100°C for 15 min for antigen retrieval. The sections were incubated with 0.1% TritonX-100 for 10 min, 3% hydrogen peroxide for 15 min, 5% fetal calf serum for 30 min, and finally with primary antibodies (CD45 and IBA-1) at 37°C for 2 h. After washing 3 times with PBS, the sections were incubated with fluorescent secondary antibody for 1 h at 37°C and with Hochest for 15 min at room temperature. Finally, the slices were washed 3 times with PBS and imaged under an inverted fluorescent fluorescence microscope. The diagnostic criteria of pathological injury were referred to previous studies [30].

Statistical methods

Data were expressed as means ± SEM. Statistical analyses were performed using Stata 16 software (StataCorp) and GraphPad Prism 8 software (GraphpadCorp). We performed two-tailed Student's t-test for two-sample comparisons and repeated measurement ANOVA for comparisons among multiple time points, with post hoc Newman-Keuls testing following ANOVA analysis. We tested the data for normal distribution with the d'Agostino and Pearsonomnibus test and equality of variances with the F-test. In each case, $P<0.05$ was considered significant.

Results

Effects of energy restriction on NOR in surgical mice

Energy restriction directly affects mouse body weight. As shown in **Figure 1A**, the weight loss rate of mice was positively correlated with the rate of energy restriction, and the weight of mice after energy restriction was significantly lower than that of the unrestricted group. To assess the long-term clinical consequences of peripheral injury on the central nervous system, NOR test was performed. The recognition index results showed (**Figure 1B**) that there was no significant difference in the index between each group before surgery, and energy restriction had no effect on the recognition index of normal mice. The postoperative recognition index was significantly reduced, and energy restriction could improve the recognition ability of the mice after surgery. Compared with the control group, the 20% energy restriction group had a lower index, but there was no significant difference. The recognition index decreased further as the rate of energy confinement increased.

Effects of energy restriction on anxiety state in surgical mice

The EPM was used to test the animal's exploration of the new environment and the fear of the elevated to examine the animal's anxiety state. The data of the EPM experiment (**Figure 2A**, **2B**) showed that there was no significant difference in the proportion of mice entering the open arm and the duration of staying at the open arm before surgical modeling, indicating that energy restriction had no effect on the anxiety state of normal mice. After surgical modeling, the proportion of mice entering the open arm and the duration of staying at the open arm were significantly reduced, and the values in the 40% energy restriction group were significantly lower than those in the control group. Compared with the control group, the

Table 1. The primers were used for qRT-PCR analysis

Gene	Forward	Reverse
GAPDH (mouse)	GGTGAAGGTCGGTGTGAACG	CTCGCTCCTGGAAGATGGTG
AMPK (mouse)	GTTCAGGCACCCTCACATC	TCGTCCAACCTTCCATTTT
sirt1 (mouse)	GTTTCTGTCTCCTGTGGG	GAATGGTCTTGGGTCTTT
IL-1β (mouse)	AGCACCTTCTTTTCCTTC	TTTTTGTTGTTCATCTCG
NF-κB (mouse)	CCAACCTGAAAATCGTGA	ACATCTGTGGGGGAAAAG
AP-1 (mouse)	GCCGCCCCTGTCCCCTAT	GCTGTGCCACCTGTTCCC

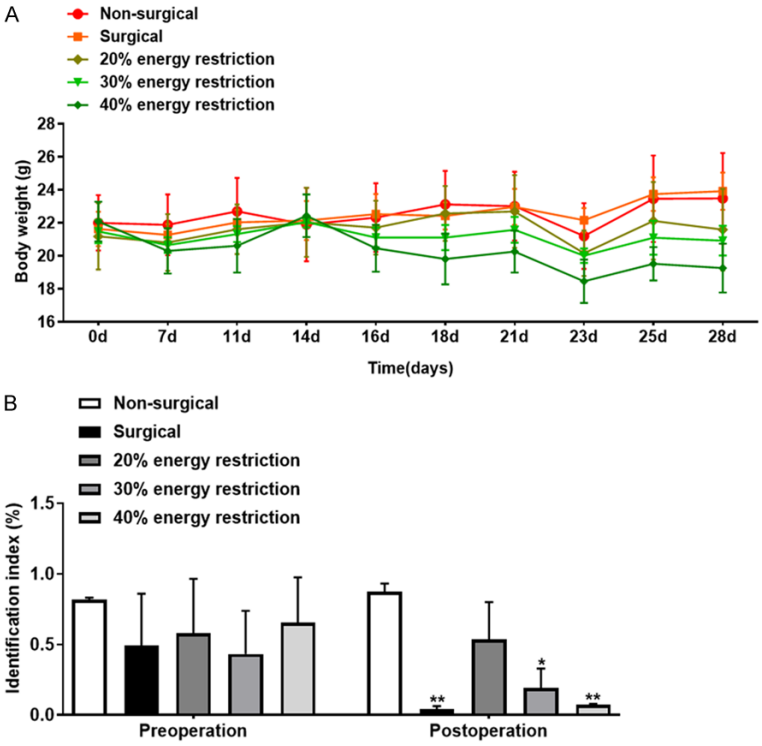

Figure 1. Changes in body weight of mice and evaluation of the ability of mice to recognize new objects in each group. A. Changes in body weight of mice in each group over 28 days of energy restriction. B. Evaluation of novel object recognition ability of mice in each group before and after fracture fixation surgery (n = 6, **P*<0.05, ***P*<0.01 Significant differences between non-surgical group and surgical group).

20% and 30% energy restriction groups did not significantly reduce the values, but compared with the model group, the values were significantly improved. It is indicated that appropriate energy restriction is beneficial to improve mouse anxiety.

Energy restriction reduces the secretion of postoperative inflammatory factors

Peripheral blood of mice was collected before and 1, 3, 7 days after fracture internal fixation operation for ELISA. As shown in **Figure 3**, the preoperative energy restriction did not affect the secretion of cytokines IL-1β (**Figure 3B**), IL-6 (**Figure 3D**), IL-8 (**Figure 3C**) and TNF-α (**Figure 3A**). The secretion of related inflammatory factors IL-1β, IL-6, IL-8 and TNF-α increased significantly after surgery. All indicators showed a downward trend after energy restriction, of which 30% energy restriction group showed the most significant declines.

Effects of energy restriction on postoperative protein expression levels of AMPK, SIRT1, IL-1β, NF-κB and AP-1

As shown in **Figure 4**, the effect of energy restriction on POCD after internal fixation of tibial fractures in mice was explored from a protein level. The protein content of AMPK (**Figure 4B**) and SIRT1 (**Figure 4C**) decreased significantly, and the protein content of IL-1β (**Figure 4D**), NF-κB (**Figure 4E**) and AP-1 (**Figure 4F**) increased significantly in the model group. With energy restriction, the expression of energy metabolism-related protein levels increased and inflammation-related protein levels decreased, but the overall trend was different from the control group.

Effects of energy restriction on postoperative mRNA expression levels of AMPK, SIRT1, IL-1β, NF-κB and AP-1

As shown in **Figure 5**, qRT-PCR was used to evaluate the mRNA expression of the target genes. The results of RT-qPCR showed that the mRNA levels of energy metabolism-related genes AMPK (**Figure 5D**) and SIRT1 (**Figure 5E**) decreased significantly, and the content of IL-1β (**Figure 5C**), NF-κB (**Figure 5B**) and AP-1 (**Figure 5A**) mRNA in the inflammation-related genes increased significantly in the model

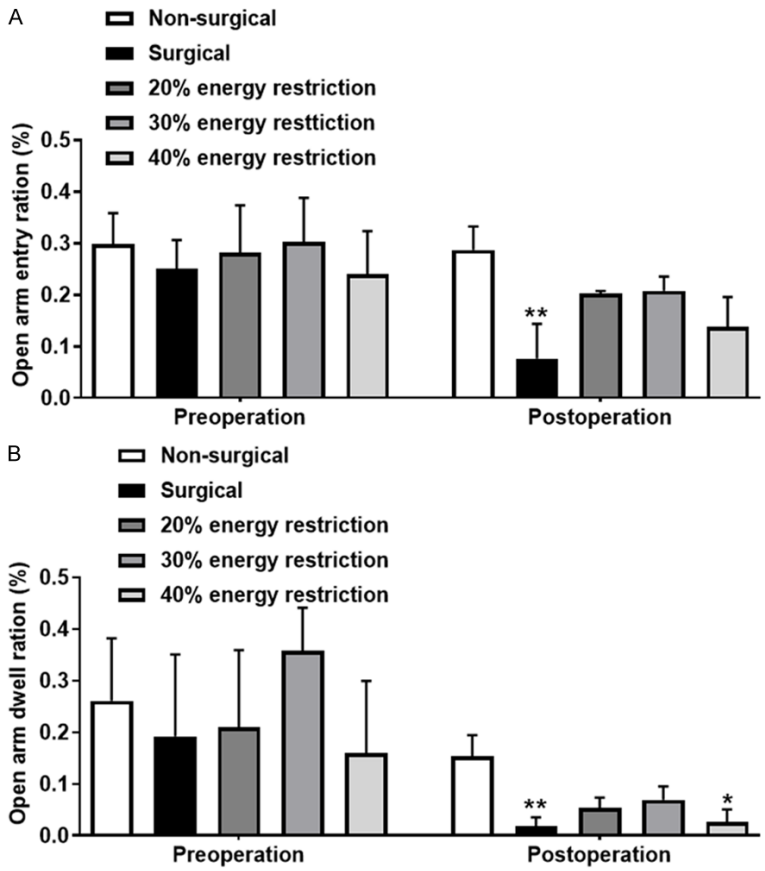

Figure 2. Elevated plus maze test was used for the proportion of mice in each group entering the open arm (A) and the time staying at the open arm (B) to reflect the anxiety state and activity ability of the mice in each group before and after surgery (n = 6, **P*<0.05, ***P*<0.01 Significant differences between non-surgical group and surgical group).

group. With energy restriction, the expression of AMPK and SIRT1 increased, and the expression of inflammation-related genes decreased, and the energy restriction of 30% showed the best effect.

Energy restriction reduces postoperative hippocampal injury

The HE staining results (**Figure 6A**) demonstrated that compared with the control group, the model group showed hyperplasia in some glial cells after tibial fracture, a small number of broken and necrotic cells, loose structure, no obvious red blood cell exudation, and wrinkled neurons in the hippocampus. With different degrees of energy restriction, the 3 energy restriction groups showed reduced hyperplasia in glial cells, tight structure, and reduced number of neuronal contractions in the hippocampus region, of which the 30% energy restriction group had the least degree of injury. The HE staying quantitative analysis results (**Figure 6B**) demonstrated that compared with the control group, serious injury was shown in the model group (n = 3, *P<0.05). With different degrees of energy restriction, the tissue damage was reduced (n = 3, #P<0.05). Diagnostic level: 0 point, normal; 1 point, slight damage; 2 points, mild injury; 3 points, moderate injury; 4 points, severe injury.

Energy restriction reduces postoperative CD45 and IBA-1 expression

As shown in **Figure 7**, the expression levels of CD45 (**Figure 7B**) and IBA-1 (**Figure 7C**) in the hippocampal slices were examined using IF analysis. The model group exhibited the highest expression levels of CD45 and IBA-1. The expression of CD45 and IBA-1 were significantly decreased with the influence of energy restriction, with 30% energy restriction group showing the lowest expression, but still higher than that of the control group. This indicates that 30% energy restriction was more beneficial to cognitive repair of POCD after tibial fracture.

Discussion

POCD refers to changes in cognitive abilities such as orientation thinking, memory and attention after surgery. POCD is a common postoperative complication, and it is more common in elderly patients [6, 31]. It is a post-operative complication relating to the peripheral central inflammatory response caused by preoperative trauma from surgery and anesthesia. This is because that abnormal protein regulation and amyloid accumulation may occur in elderly patients on the basis of the degeneration of the central nervous system [32, 33].

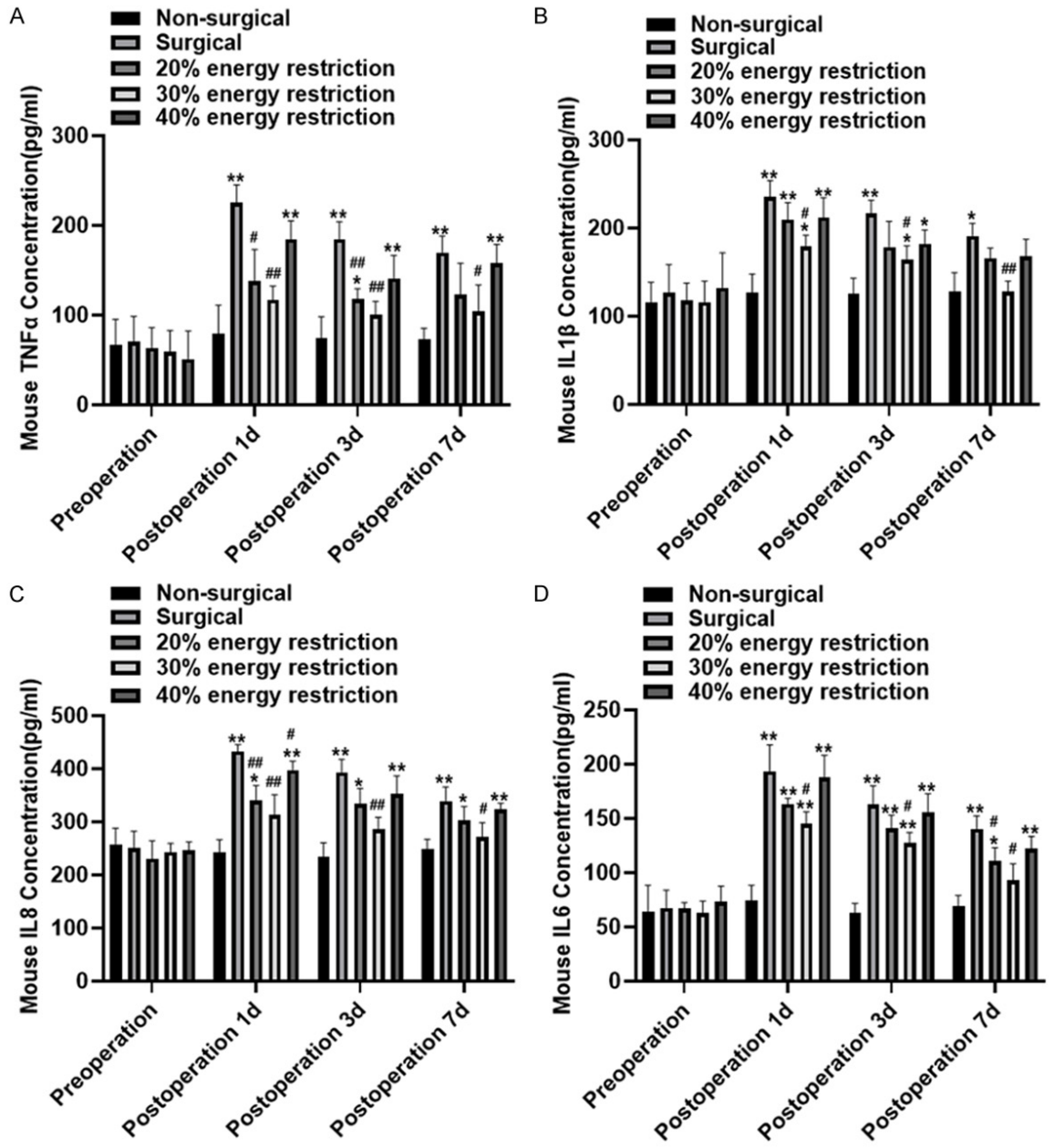

Figure 3. The expression of inflammatory factors TNF-α (A), IL-1β (B), IL-8 (C) and IL-6 (D) in the peripheral blood of mice in each group were detected by ELISA before surgery and 1 day, 3 days and 7 days after surgery. (n = 6, **P*<0.05, ***P*<0.01 Significant differences between non-surgical group and surgical group; #*P*<0.05, ##*P*<0.01 Significant differences between surgical group and energy restriction group).

Surgical trauma can activate the endogenous immune system leading to a peripheral inflammatory response and the expression of inflammatory factors, such as IL-1β, IL-6 and TNF-α. Inflammatory factors can activate the massive expression of NF-κB, thereby promoting the cytokines and inflammatory enzymes, and the released inflammatory mediators and harmful substances can damage the blood-brain barrier and neurons [34-36]. Then it causes brain tissue edema, nerve cell damage and central nervous system dysfunction.

In this study, the internal fixation of tibial fracture affected the recognization ability of mice. Then, restriction of energy intake effectively improved POCD. However, energy intake should not fall below a certain range. Our results

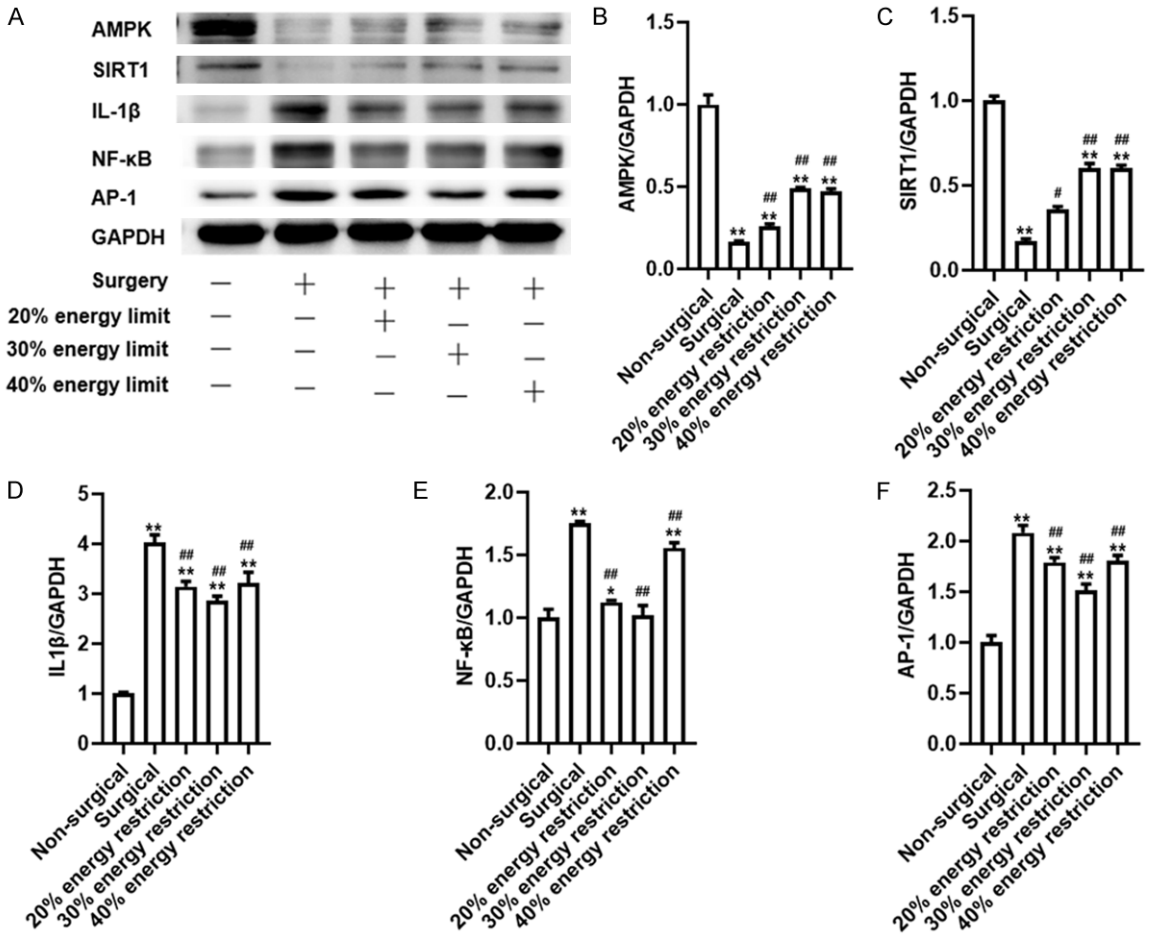

Figure 4. Effects of energy restriction on postoperative protein expression levels of AMPK, SIRT1, IL-1β, NF-κB and AP-1. (A) The protein expression levels of AMPK, SIRT1, IL-1β, NF-κB and AP-1 in the hippocampus of mice in each group were detected by Western Blot on the eighth day after surgery, and the expression of GAPDH was used as a loading control. Quantitative analysis of protein expression levels of AMPK (B), SIRT1 (C), IL-1β (D), NF-κB (E) and AP-1 (F) in the hippocampus of mice. (n = 3, **P*<0.05, ***P*<0.01 Significant differences between non-surgical group and surgical group; #*P*<0.05, ##*P*<0.01 Significant differences between surgical group and energy restriction group).

showed that the best effect in the 30% energy restriction group. The NOR test showed (**Figure 1**) that energy restriction could improve the recognition ability of the mice after surgery. The data of the EPM experiment (**Figure 2**) showed that the proportion of mice entering the open arm and the duration of staying at the open arm were significantly reduced after surgical modeling, and this behavior was effectively improved after energy restriction. This indicates that energy restriction can improve POCD in mice. The expression levels of related genes in peripheral blood and hippocampus of mice after modeling were detected by ELISA (**Figure 3**), WB (**Figure 4**) and RT-qPCR (**Figure 5**) experiments. ELISA results showed that the expression of inflammatory factor IL-1β, IL-6, IL-8 and TNF-α increased after operation. This suggests that surgical trauma can activate the endogenous immune system and lead to peripheral inflammatory response, resulting in the expression of inflammatory factors. WB and RT-qPCR results showed that the expression of AMPK and SIRT1 decreased, and IL-1β, NF-κB and AP-1 were significantly increased after surgery. This suggests that tibial fracture fixation surgery can cause inflammation of the central nervous system. AMPK, as a cellular energy regulator, plays a crucial role in energy metabolism. Decreased activity of AMPK and AMPK-activated silent information regulator SIRT1 leads to disturbance of cellular energy metabo-

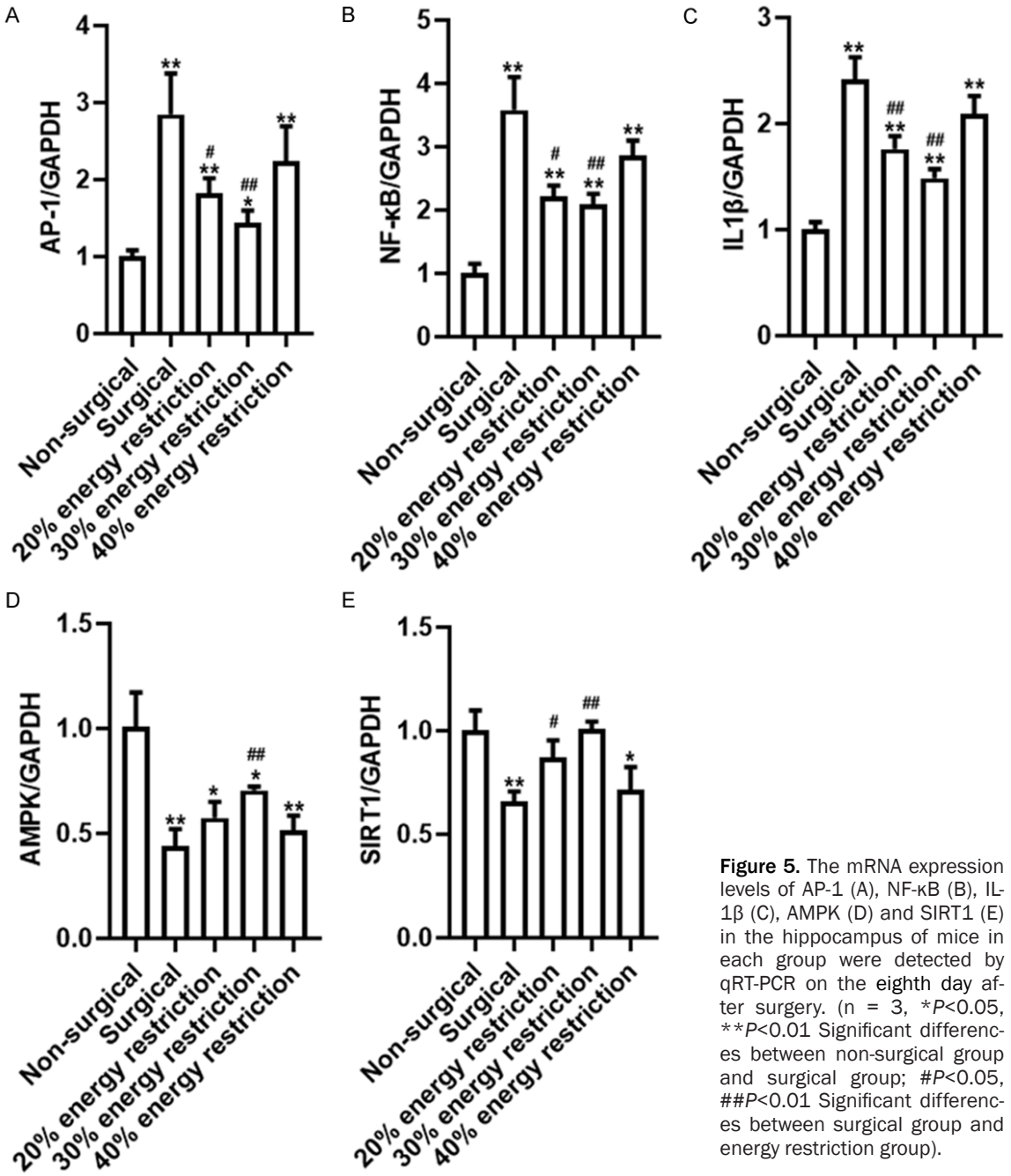

Figure 5. The mRNA expression levels of AP-1 (A), NF-κB (B), IL-1β (C), AMPK (D) and SIRT1 (E) in the hippocampus of mice in each group were detected by qRT-PCR on the eighth day after surgery. (n = 3, **P*<0.05, ***P*<0.01 Significant differences between non-surgical group and surgical group; #*P*<0.05, ##*P*<0.01 Significant differences between surgical group and energy restriction group).

lism after surgery. The expression of AMPK and SIRT1 increases after energy restriction, indicating that energy restriction helps to regulate the inflammatory response and cell metabolism disorder cause by surgery. HE (**Figure 6**) and IF (**Figure 7**) results confirm that peripheral immune cells were able to pass through the damaged blood-brain barrier and participate in immune inflammation in the hippocampus after surgery. With energy restriction, the groups showed reduced glial proliferation, reduced cell necrosis and reduced number of shrunken neurons in the hippocampus. CD45 [37] and IBA-1 [38] are both markers of microglia in the central nervous system and can be used to detect the activation of microglia. Microglia plays an important role in maintaining the stability of the central nervous system [39]. Proliferative and activated microglia can release a variety of inflammatory cytokines (such as TNF, IL-6, IL-8

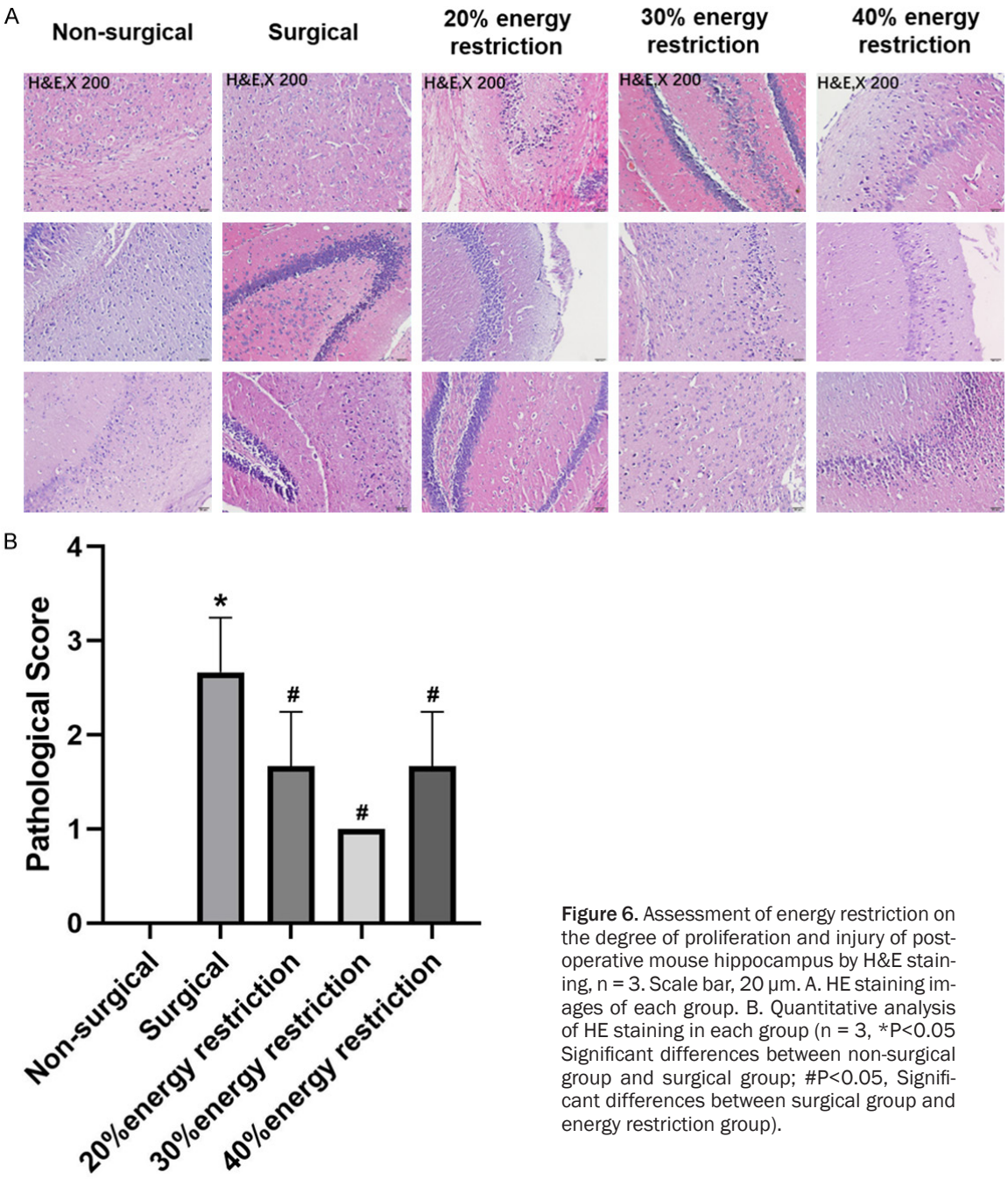

Figure 6. Assessment of energy restriction on the degree of proliferation and injury of postoperative mouse hippocampus by H&E staining, n = 3. Scale bar, 20 μm. A. HE staining images of each group. B. Quantitative analysis of HE staining in each group (n = 3, *P<0.05 Significant differences between non-surgical group and surgical group; #P<0.05, Significant differences between surgical group and energy restriction group).

and IL-1β) to mediate a variety of lesions of the central nervous system and induce POCD [40, 41]. The results showed that energy restriction reduced inflammatory cytokines that crossed the blood-brain barrier and damaged neurons.

Internal fixation of tibial fractures can activate peripheral inflammatory responses, and inflammatory factors can pass through the blood-brain barrier and cause inflammatory responses in the central system, leading to neuronal damage and decreased learning ability and memory, resulting in POCD [42-44]. However, energy restriction can reduce inflammatory cytokine production after internal fixation of tibial fractures, thereby reducing central neuron damage caused by peripheral inflammatory factors. Thus, energy restriction could serve as a new avenue for the treatment of POCD.

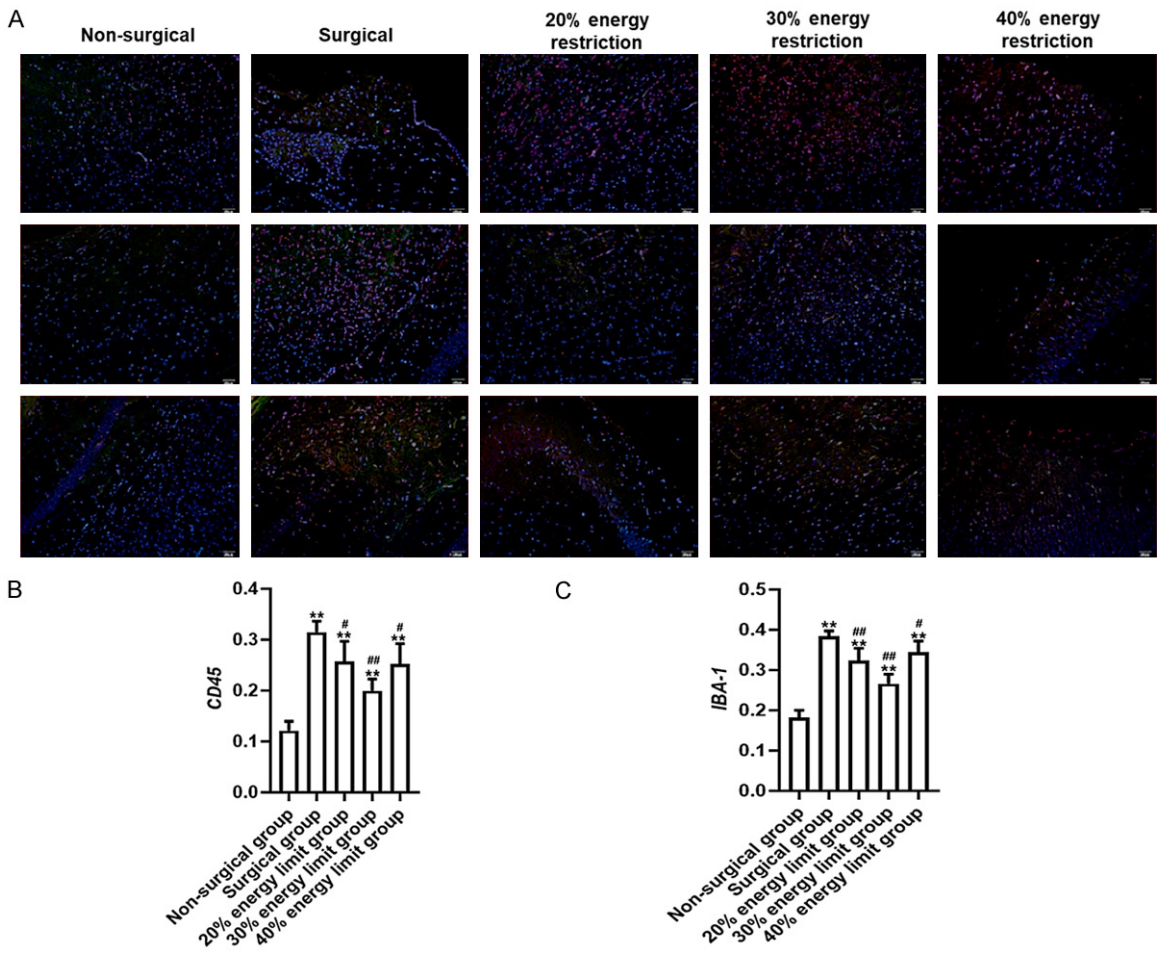

Figure 7. The expression levels of CD45 and IBA-1 in the hippocampal slices were examined using immunofluorescence analysis. (A) Immunofluorescence staining assessed the effect of energy restriction on the expression levels of CD45 (green fluorescence) and IBA-1 (red fluorescence) in the hippocampus of mice after surgery. Relative fluorescence intensity analysis of CD45 (B) and IBA-1 (C) (n = 3, **P<0.01 Significant differences between non-surgical group and surgical group; #P<0.05, ##P<0.01 Significant differences between surgical group and energy restriction group). Scale bar, 20 μm.

Conclusion

This study found that surgical injury from internal fixation of tibial fractures in mice can cause POCD. In addition, we also found that although energy restriction had a significant effect on improving POCD, it was not proportional, and 30% energy restriction was the best for improving POCD. The beneficial effects of early energy restriction may provide a new preventive or therapeutic paradigm for POCD patients.

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Disclosure of conflict of interest

None.

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