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Impact of Early High-protein Diet on Neurofunctional Recovery in Rats with Ischemic Stroke

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Background: Ischemic stroke, featuring high incidence, morbidity, and mortality, is one of the three major diseases troubling human beings. The purpose of the study was to examine the impact of early high-protein diet on neurofunctional recovery in rats with ischemic stroke as well as their cerebral infarct areas and molecular expressions of oxidative stress.

Material/Methods: The middle cerebral artery occlusion model (MCAO) was established, and 48 adult, male Sprague Dawley (SD) rats of clean grade aged seven to eight months (250–280 g body weight) were randomized into four groups: the MCAO group with high-protein diet (MH), the MCAO group with standard-protein diet (MS), the sham group with high-protein diet (SH), and the sham group with standard-protein diet (SS). High-protein diet intervention started on the first day of the surgery, and the rats' body weights and their neurological deficit scores were measured on each postoperative day while the scores of motors coordination and balance ability were recorded every other day. In addition, their cerebral infant areas and the molecular expressions of oxidative stress injuries were detected as well.

Results: Compared to the MS group, the rats in the MH group gained faster weight growth ($p<0.05$), presented significantly lower neurological impairment scores ($p<0.05$), remarkably improved motor coordination and balance ability ($p<0.05$) as well as showed smaller cerebral infarct areas ($p<0.05$), increased expression of SOD (superoxide dismutase), and reduced expressions of MDA (malondialdehyde) and iNOS (inducible nitric oxide synthase). However, there was no significant difference between the SS group and the SH group ($p>0.05$).

Conclusions: Early high-protein diet facilitates the recovery of body weights and neurological functions as well the reduction of the cerebral infarct areas of rats, thus alleviating ischemic stroke-caused oxidative stress injuries.

MeSH Keywords: **Diet • Oxidative Stress • Rats • Stroke**

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Background

Ischemic stroke includes a group of acute cerebrovascular diseases characterized by clinical manifestations, such as hemiparesis, ataxia, and sudden decrease in the level of consciousness; as well as by high incidence, morbidity, and mortality [1–3]. The main symptom of cerebral ischemic stroke is the sudden occurrence of neurological deficits, which are also characterized by deep disturbances of human body homeostasis. With the current process of clinical diagnosis and treatment, more and more stroke patients are surviving. However, the morbidity among them still exceeds 80%, leading to poor quality of life [4]. Therefore, early stroke intervention is very important for the early rehabilitation of these patients.

Currently, there are many types of rehabilitation methods for post-stroke functional limitations [5,6], including joint activity training, neurophysiological methods such as the Bobath method [7], proprioceptive neuromuscular facilitation (PNF) [8], motor relearning program (MRP) [9], and some other new therapeutic methods. But these procedures largely rely on patients' initiative and cooperation, which leads to different treatment effects. However, timely introduction of nutritional supplement is relatively simple and easy for patients to comply with [10,11]. Besides, patients with stroke often suffer from malnutrition because of chronic bed rest, which is extremely unfavorable to a patient's recovery [12]. Research has also shown that malnourished patients, or those at risk for nutritional deterioration, have poor prognosis and significantly increased mortality rates [13]. Therefore, timely and reasonable nutritional intervention in the early stage of stroke could be very important for clinical applications and basic research.

There are many studies on high-protein diets in obesity, diabetes, chronic kidney function, and so on, which have indicated that a high-protein diet can increase insulin secretion in normal individuals [14], lead to skeletal muscle insulin resistance [15], increase the sense of satiety, reduce calorie intake, and decrease the weight of obese people [16]. Thus, a high-protein diet plays a positive role in the treatment of related diseases. However, recent epidemiological findings also have shown the adverse effects of long-term high-protein diet, for instance, a regular low-carbon and high-protein diet was found to be associated with an increasing risk of cardiovascular disease [17,18], and the clinical application of high-protein diet still remains controversial. Furthermore, introducing high-protein diet intervention in the early stage of ischemic stroke reduces neurological deficit, which has not been reported in current clinical and basic research.

In order to investigate the role of early high-protein diet in rehabilitation of rats with ischemic stroke, this study used an early high-protein diet in rats with middle cerebral artery occlusion (MCAO), and obtained a series of experimental indexes. The measures were aimed at assessing the impact of an early high-protein diet on the postoperative neurological deficit, cerebral infarct area, and molecular expression of oxidative stress injuries caused by ischemic stroke, providing the theoretical foundation for early high-protein diet intervention and its clinical application in patients with stroke.

Material and Methods

Animals and groups

All Sprague Dawley (SD) rats were obtained from Shanghai Slaccas Experimental Animal Co., Ltd., and raised at the Laboratory Animal House of Fudan University on 12: 12 hour light cycle and at 23±1°C. The standard-protein and high-protein diets were purchased from Suzhou Shuangshi Animal Feed Technology Co., Ltd. Afterwards, the SD rats were randomized into four groups with 12 rats in each group: the MCAO group with high-protein diet (MH),the MCAO group with standard-protein diet (MS), the sham group with high-protein diet (SH), and the sham group with standard-protein diet (SS).

After the surgery, each rat and the food pellet in each cage were weighed every day and the neurofunctional deficit scoring as well as behavioral tests were recorded on postoperative day 1, 3, 5, and 7. On the postoperative day 7, all rats were sacrificed to detect the cerebral infarct area by triphenyl tetrazolium chloride (TTC) staining. In addition, serum and the cerebral cortex were collected for the identification of the molecular mechanisms involved. It should be noted that this study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and protocol for the animal use was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Capital Medical University.

Preparation of the MCAO model

In this study, we prepared MCAO rat models to simulate cerebral ischemia and reperfusion injury. The rats were anesthetized with 10% chloral hydrate (0.3 mL/kg) and maintained in automated heat blankets (Yuyan Instruments, Shanghai, China) at 36.7±1.0°C. All groups received a midline incision in the neck and the mandibular glands, pretracheal strap along with sternomastoid muscles were retracted. In MCAO groups (Figure 1), the exposed right common carotid (CCA) was ligated at the proximal end, and the following external carotid arteries (ECA) branches were cut after electrocoagulation: the occipital, the cranial thyroid, and the ascending pharyngeal artery. A microclip was used to occlude the CCA. The right ECA was lighted and cut distally to the cranial thyroid artery.

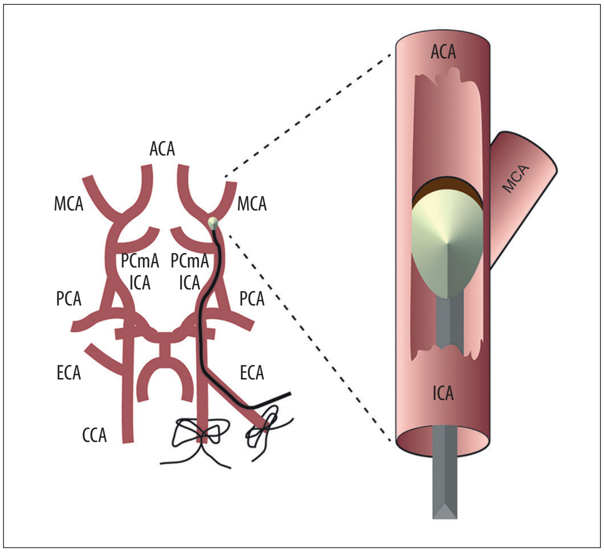

Figure 1. Illustration of the middle cerebral artery occlusion model of ischemic stroke. In the intraluminal filament model, the external carotid artery (ECA) is dissected and cauterized, and the common carotid artery (CCA) temporarily ligated. The internal carotid artery (ICA) is dissected and an arteriotomy is performed in the ECA. The ECA is then reflected so as to run into the ICA and a filament is introduced into the ICA via the ECA arteriotomy. The filament is advanced until resistance is felt where it occludes the proximal middle cerebral artery (MCA). The filament remains in place for a set period of time before withdrawal and wound closure. Confirmation of occlusion is usually via concomitant Laser Doppler Perfusion Monitor. ACA – anterior cerebral artery; PCmA – posterior communicating artery; PCA – pterygopalatine artery.

Then a silicone filament embolus of 0.40–0.42 mm in diameter was placed against the right side of the skull and gently advanced through the internal carotid artery until its tip occlude the origin of the middle cerebral artery (MCA). The cerebral blood flow changes in the sensorimotor cortex of MCAO rats were monitored with a Laser Doppler Perfusion Monitor (Moor Instruments, Axminster, UK). The microclip at the CCA was removed and silicone filament embolism was withdrawn after 60 minutes (the blood flow in the ischemic brain dropped to ~20% of the baseline) for reperfusion of the brain. At the completion of surgery, animals were kept warm during recovery with their muscles and glands replaced and wounds sutured. In addition, any MCAO rats that did not show restoration of blood flow after removal of the filament were euthanized, for this could indicate subarachnoid hemorrhage. The sham group did not have the silicone thread embolus inserted.

Behavioral assessment

In neurofunctional deficit scoring, it is assumed that the higher the score, the more serious the symptoms of the nerve function deficit appeared. For this assessment, the rat was lifted up by the tail, suspended about 1 m above the ground, and observed for forelimb flexion. Scoring criteria were 0 to 7. A score of 0 was if both forelimbs were extended to the ground and no other neurological features were present. A score of 1 was if the contralateral forelimb was bending continuously with movement ranging from mild wrist bending and shoulder joint adduction to full wrist and elbow flexion with internal rotation of the shoulder joint. In addition, the limbs of the rats showed persistent bending without other abnormal signs. A score of 2 was if the rat was pulled by its tail and was given a gentle push behind the shoulders until the forelimbs slid a few centimeters on the ground. This was repeated several times. Rats showing normal ability or mild impairment were able to resist lateral thrust similarly on both sides. But if the rats had serious impairment, the resistance to the paralyzed side thrust decreased. A score of 3 was if the rat was on the ground and pulling its tail towards the paralyzed side. A score of 4 was if the rat was on the ground and moving freely, circling towards the paralyzed side. A score of 5 was if the rat just walked by the stimulation. A score of 6 was if the rat showed no response to the stimuli, almost unconscious. A score of 7 was if the rat was dead [19].

One of the most significant symptoms in the rat MCAO model is the forelimb dysfunction induced by the ischemia in cerebral hemisphere. Foot fault test was used to assess the motor coordination of rats, according to the existing experimental protocol in our laboratory, with slight modifications [20]. Scoring criteria were 0 to 6. A score of 0 was if the rats completely missed the rail and fell off without touching the rail or the body lost its normal position and balance. A score was 1 if the rats fell after touching the rail, affecting the normal walking. A score was 2 if the rats' feet were placed on the rail. When bearing the weight, they slipped but did not fall or affect the walk, and the balance and gait were maintained. A score was 3 if the feet were placed on the rail. But before bearing the weight, they quickly moved on to another rail. A score was 4 if the feet aimed at a rail, but because there was no contact with the rail, it was moved on to another rail. A score was 5 if the feet were placed on the rail with the ankle and toes, or knees as weight-bearing. A score was 6 if the rats were normal and their feet fully bore the body weight. Each animal was only evaluated on double forelimb and every step of each foot was used to calculate the average score for statistical analysis.

Beam balance test was used to assess the ability to maintain balance in rats. The scoring criteria were 1 to 7. A score was 1 if rats steadily stayed on the crossbar. A score was 2 if rats grasped the end of grab bar. A score was 3 if rats held the crossbar, but a paw fell off. A score was 4 if rats held the crossbar but two paws fell off, or rats stayed on the rail for 60 seconds. A score was 5 if rats tried to stay on the crossbar,

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succeeding in staying for more than 40 seconds. A score was 6 if rats tried to stay on the crossbar, staying for more than 20 seconds. A score was 7 if rats fell off the crossbar within 20 seconds. Each rat was evaluated five times a day, and the average scores of the runs were used for statistical analysis.

TTC staining

Briefly, each brain was sliced horizontally to obtain five slices. Then the slices were incubated in 2% TTC (2,3,5-triphenyl-2H-tetrazolium chloride, Sigma-Aldrich, St. Louis, MO, USA) for 15 minutes at 37°C and fixed by immersion in 10% neutral buffered formalin. The area of infarction on both sides of each slice was calculated by an image analyzer. And the infarct area of each slice was summed up for each brain.

Molecular analysis

Ischemic stroke leads to the increase of oxidative stress injuries, which results in cell damage and apoptosis [21]. To assess the peculiarities in the development of oxidative stress injuries, we measured the expression level of SOD (superoxide dismutase), an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide and MDA (malondialdehyde), one of the most frequently used indicators of lipid peroxidation in both serum and cerebral cortex tissue, as well as the protein expression of iNOS (inducible nitric oxide synthase), an important material to produce free radical, thus leading to injuries, in injured cerebral cortex tissues by the corresponding kit (SOD assay kit, MDA assay kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and performed in accordance with the manufacturer's instructions. The molecular level in the serum could be measured without diluting the serum. However, when measuring the molecular level in the cerebral cortex tissue, the cerebral cortex tissue homogenate should be prepared in advance: the cerebral cortex was accurately weighed before being added into 0.9% normal saline (Supply Department, Shanghai Medical College, Fudan University). After being centrifuged at 2,500–3,000 r/minute for 10 minutes, the supernatant was separated and used for the detection of protein content in cortical tissue homogenate (total protein quantitation test kit, associated standards, Coomassie brilliant blue method, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

iNOS content was detected with western blot. Briefly, proteins were separated by 10% SDS-PAGE, blotted onto nitrocellulose membranes (Hybond ECL Membrane, Amersham Biosciences, UK), and incubated with iNOS primary antibodies diluted in 5% milk TBS–Tween overnight (anti-GAPDH, Sigma-Aldrich, 1: 1,000, 42 kDa). After being washed and incubated with horseradish peroxidase conjugated secondary antibodies, labeled proteins were visualized with ECL-reagent kit according to the manufacturer's instructions (Amersham Biosciences, UK).

Statistical analysis

SPSS 20.0 (IBM, USA) was used for statistical processing. All the results were expressed as mean ±SE (standard error) and the intergroup comparisons used the one-tailed *t*-test, with $p<0.05$ as a statistical difference.

Results

Body weight changes

The changes in body weight can comprehensively reflect the recovery of sensory and other physical function in rats after stroke. As Figure 2A shows, body weights of rats in sham groups decreased until day 1 after surgery while MCAO groups until day 2 after surgery. In the following days, body weights of rats in each group increased but the growth trend of the MH and SH groups were significantly higher than that of the MS and SS groups, respectively ($p<0.01$). Overall, the early high-protein diet was able to help rats recover their body weights ($p<0.05$).

Neurofunctional deficit scoring

In this study, we used a seven-point system to score the neurofunctional deficits in MCAO rats. Apparent neurofunctional deficit symptoms were found in the MH and MS groups, especially within two days after surgery, but not in the sham-operated group (SH and SS groups). However, on the third day, the scores of neurofunctional deficit started to decrease in the MCAO groups and the scores in the MH group were significantly higher than that in the MS group, day 2 and day 4–7 in particular ($p<0.01$). Besides, the scores in the MH and MS groups were always significantly higher than that in the SH and SS groups respectively ($p<0.01$) and there was no significant difference between the SH and SS groups. In general, the early high-protein diet was able to facilitate the neurofunctional recovery ($p<0.01$, Figure 2B) in MCAO rats.

Motor coordination scoring

In this study, the foot fault test was used to score the forelimb dysfunction in all rats. The results showed that in the MH and MS groups, the level of forelimb injury was similar within the first three days after surgery. Subsequently, motor coordination ability of the MCAO groups gradually recovered in the following days and the recovery of the MH group was significantly better than the MS group on the fifth and seventh day ($p<0.01$). In addition, there appeared significant differences between the MH group and the SH group as well as

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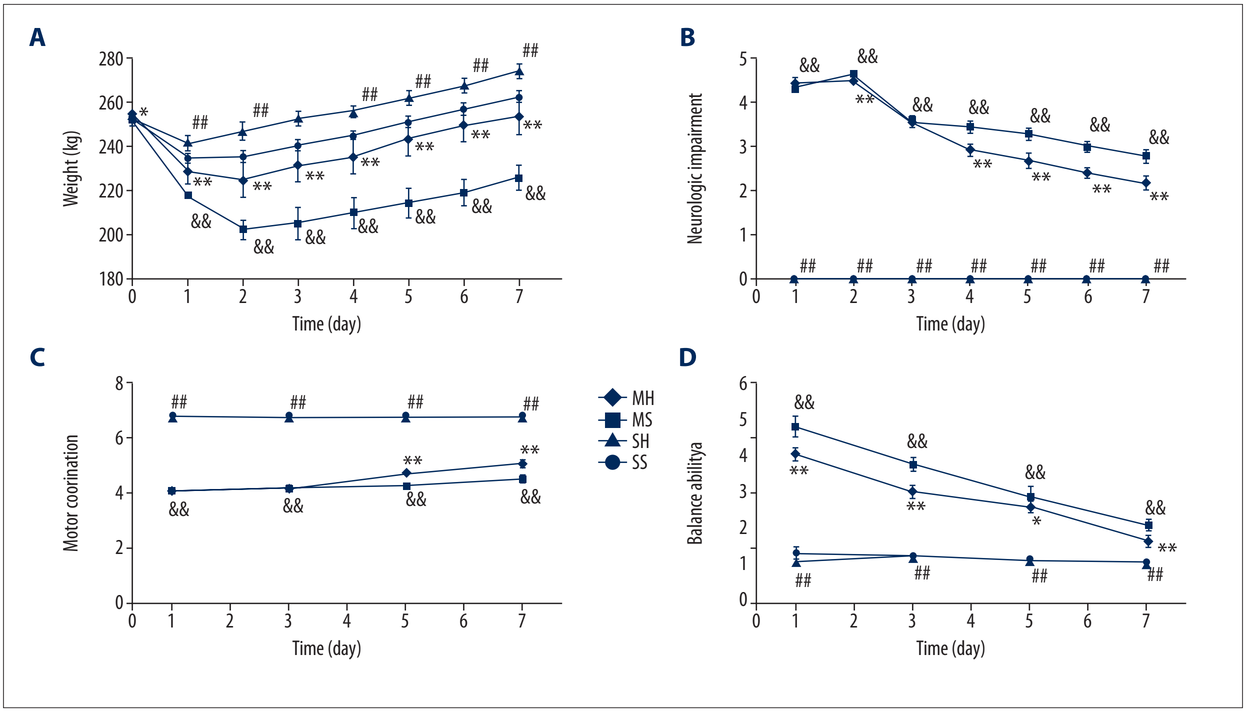

Figure 2. Impacts of early high-protein diet on body weight, neurofunctional deficits, motor coordination and balance performance in rats. (**A**) The weights of MCAO groups were generally lower than the sham groups. In the MCAO and sham groups, rats with early high-protein diet had higher weights. (**B**) As time went on, the neurological function of the MCAO groups recovered gradually and the MH group showed a faster recovery than the MS group. (**C**) Score changes were detected by the foot fault method on postoperative D7. (**D**) Beam balance method was applied to observe the differences of balance ability in the MH and MS groups. MH versus MS, * $p<0.05$, ** $p<0.01$; MH versus SH, ## $p<0.01$; MS versus SS, && $p<0.01$; n=6 in each group.

between the MS group and the SS group ($p<0.01$) while no difference between the SH group and the SS group was observed. All in all, the early high-protein diet was able to promote the rehabilitation of motor coordination ($p<0.05$) in the MCAO rats and facilitate the recovery of motor function in the injured forelimb (Figure 2C).

Balance ability assessment

To assess the balance abilities of rats in each group, the present study applied the balance beam test. The result showed that the recovery of balance ability in the MH group was remarkably greater than that in the MS group at 7 days after surgery ($p<0.05$). In addition, the differences between the MH group and the SH group as well as between the MS group and the SS group were always significant ($p<0.01$) while no difference between the SH group and the SS group appeared. Which means that early high-protein diet promotes the recovery of balance ability in MCAO rats (Figure 2D).

Impacts of early high-protein diet on cerebral infarct area

In order to determine whether the early high-protein diet had an effect on MCAO rehabilitation, we applied the TTC staining method to detect the cerebral infarct area in all rats at 7 days after surgery. As we can see in Figure 3, the cerebral infarct area of the MS group and the MH group was about 45% and 40%, respectively, while the area of the sham group was 1.2%. Moreover, the MH group showed a significant smaller cerebral infarct area than the MS group ($p<0.01$). We then came to the conclusion that early high-protein diet could reduce the cerebral infarct area ($p<0.01$) in MCAO rats, thus promoting brain recovery.

Molecular detection

As is shown in Figure 4A and 4C, the expression of SOD in serum and tissue were similar, with SOD expressions significantly higher in the MH group than in the MS group, in the SS group than in the MS group as well as in the SH group than the MH group ($p<0.01$) while rare differences between the SS group and the SH group. However, as to the expressions of MDA, we can see apparent differences in serum and tissue. In Figure 4B, MDA expression in serum in the MS group was significantly higher than in the MH and the SS groups ($p<0.01$) while the differences between the MH group and the SH group were relatively less significant, only slightly higher in the MH group than in the SH group ($p<0.05$). In Figure 4D, MDA expression in

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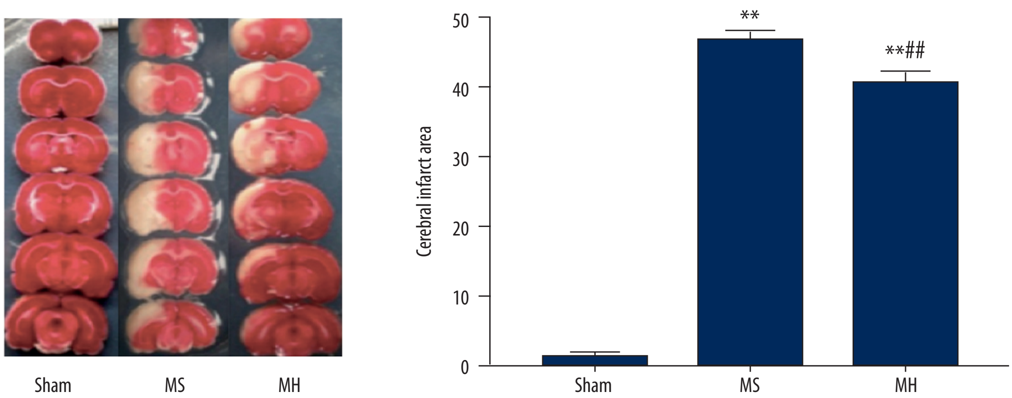

Figure 3. Impact of early high-protein diet on cerebral infarct area in rats. Representative images of TTC-stained brain slices (coronal level 1–6) after seven days of permanent MCAO. In living tissue, TTC is enzymatically reduced by dehydrogenases to 1,3,5-triphenylformazan (TPF), which is red in color, while in necrotic areas it remains white due to the absence of such enzymatic activity. Therefore, the area of infarction can be identified by its white color due to lack of conversion of TTC to TPF. The MCAO groups showed a significant larger cerebral infarct area then the sham groups and the area in the MS group was significant larger than that in the MH group. MS versus sham, ** $p<0.01$; MH versus sham, ** $p<0.01$; MS versus SS, ## $p<0.01$; n=6 in each group.

tissue was significantly higher in the MS group than in the MH group, in the MS group than in the SS group as well as in the MH group than the SH group ($p<0.01$) while small differences between the SS group and the SH group were observed. Then we focused on relative iNOS expression. In Figure 4E, we can see iNOS expression in the MS group was significantly higher than in the MH and SS groups ($p<0.01$) and the differences between the MH group and the SH group as well as between the SS group and the SH group were not significant. In summary, early high-protein diet was able to effectively regulate the expressions of SOD, MDA, and iNOS.

Discussion

Ischemic stroke is one of the most devastating diseases. The high stress level after stroke often induces the body to enter into high catabolic state, thus resulting in considerable protein loss, and leading to the increased incidence of infection, prolonged hospitalization, and increased mortality [22]. Moreover, according to Gao et al., hypokalemia is a complication of hospitalized patients and correcting serum K^+ improved the clinic outcome of ischemic stroke patients [23]. In our study, we established the MCAO models to investigate the effects of early high-protein diet on treating ischemic stroke patients.

To understand brain pathophysiology and to determine the efficacy of new therapies, numerous animal models have been used for mimicking clinical ischemic stroke [24]. Among them, mechanical, pharmacological, photothrombotic, and embolic means on mice or rats have been the most widely used models [25]. In rodents, the intraluminal filament MCAO model is widespread used because of its non-invasiveness compared to direct surgical occlusion of cerebral vessels [24]. Liu et al. substantiated that the transient MCAO model in rodents is also one of the widely utilized models in used for studying focal cerebral ischemia. [26]. Mecca et al. revealed that the endothelin-1 (ET-1)-induced MCAO model of cerebral ischemia was found to more closely mimic the temporal events of an embolic stroke [27]. Furthermore, Boltze et al. also demonstrated that permanent MCAO model was a novel large animal model for focal cerebral ischemia [28]. However, the outcome of the MCAO model is often affected by a variety of factors including the age, sex, and weight of the animals and the size of the filament, and its reproducibility varies among laboratories and species. In the study byTao et al., they applied a two hour MCAO to induce the cortical infarction [29]. However, in our study, two hour of MCAO not only induced large brain injury but also caused a high death rate, while 60 minutes of MCAO induced large brain injury but caused a low death rate. Thus, 60 minutes of ischemic duration was applied in our study. To the best our knowledge, this was the first study to identify the effects of early high-protein diet on promoting the rehabilitation of MCAO rats. According to the results of the present study, the early high-protein diet could facilitate the recovery of body weight and improve the symptoms of neurofunctional deficits. It has been reported that the higher the degree of neurofunctional deficits was, the worse the ability of nutritional support to improve the nutritional status appeared [30]. Thus, the recovery of neurofunctional function could help patients to absorb nutritional formulas, such as high-protein nutritional feeding or intravenous fluid replenishment, which

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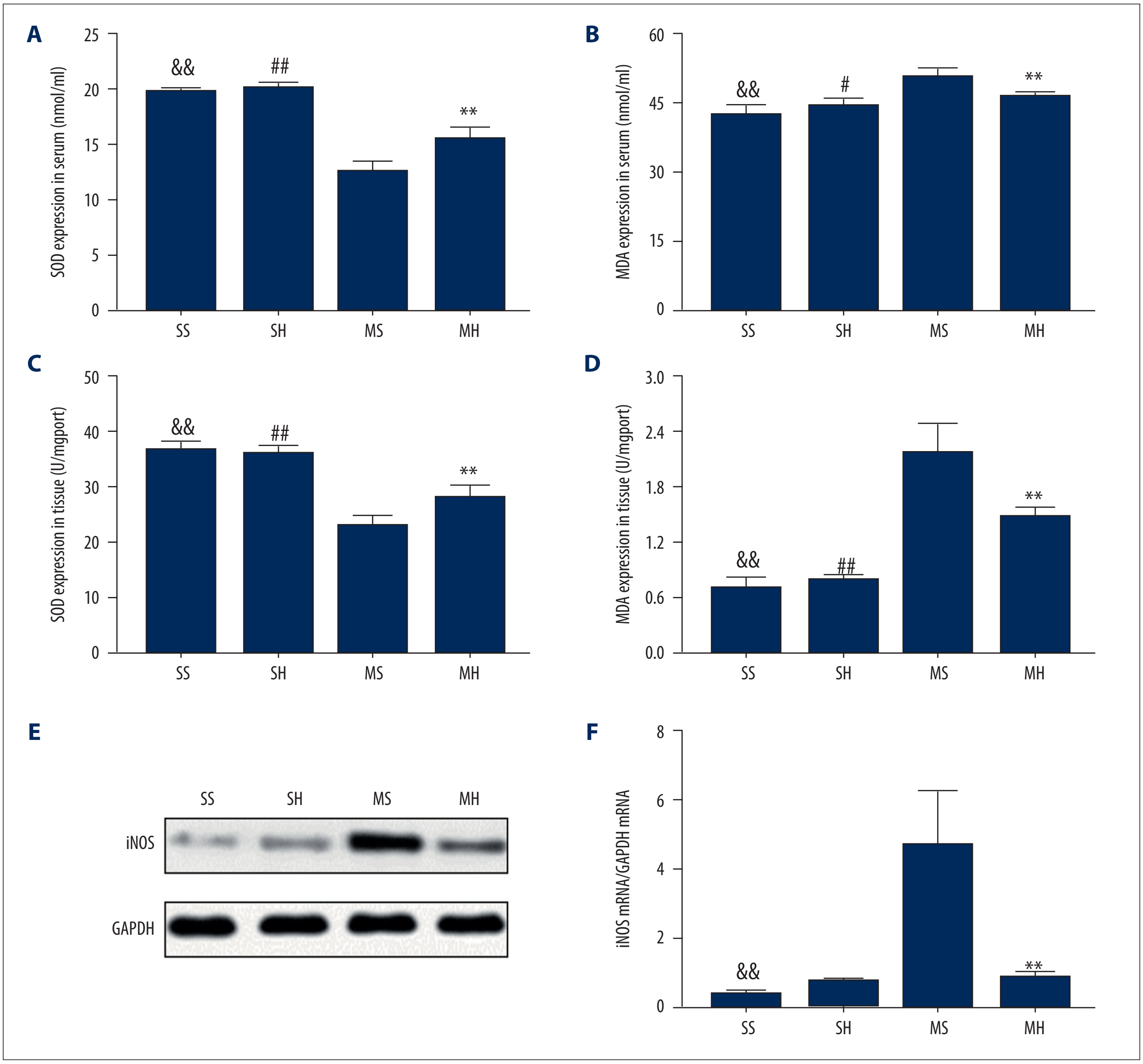

Figure 4. Impacts of early high-protein diet on the SOD, MDA, and iNOS contents in the serum and tissues of the rats. (**A**) Expressions of SOD in the serum. (**B**) Expressions of MDA serum. (**C**) Expressions of SOD in the injured cerebral cortex tissue. (**D**) Expressions of MDA in the injured cerebral cortex tissue. (**E**) Relative iNOS expression in injured cerebral cortex tissues (in the vertical bar). MH versus MS, ** p<0.01; MH versus SH, $^{\#}$ p<0.05, $^{\#\#}$ p<0.01; MS versus SS, $^{\&\&}$ p<0.01; n=6 in each group. SOD – superoxide dismutase; MDA – malondialdehyde; iNOS – inducible nitric oxide synthase.

could subsequently promote the recovery of sensory and other body functions in the rats.

The early high-protein diet has also been found to help restore motor coordination. Residual motor dysfunction is often observed in stroke survivors and patients' lives are adversely affected. The affected limb functions in post-stroke hemiplegia can be gradually improved by training and drug treatment [31,32]. Nowadays, hospitals use exercise therapies, such as teaching healthy side posture, relearning of movement patterns, and elbow flexor and extensor training. Although they can help the patients to restore their full limb motion, the effects tend to be minimal for severely disabled patients and those unwilling to cooperate. The early high-protein diet may facilitate patients' cooperation, in consequence lead to the improvement of patients' motor coordination. In our study, the early high-protein diet significantly reduced the cerebral infarct area in the rat MCAO models and enhanced motor coordination. This showed that the limb motor dysfunction in stroke patients may be related to the infarct size. The study by Jung et al. suggested that sensory motor functional recovery after brain injury might be a consequence of the reorganization

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of the neural network as a process of neuroplasticity [33]. This may explain why the decrease of infarct area was positively correlated with the increase motor coordination.

The early high-protein diet has also been verified to exert regulatory effects at the molecular level. The reduced oxygen supply after stroke can affect the oxidative stress, which plays an important role in cell damage and death in stroke. When cerebral ischemia occurs, the oxidative stress level increases, and the excess oxygen-free radicals can thus cause lipid peroxidation and produce peroxide and other products among which MDA can cause the most severe damage [34]. SOD is an important antioxidant that can directly eliminate free radicals. *In vivo*, the activity of SOD and MDA could indirectly reflect the body's ability to eliminate oxygen free radicals and the severity of the free radical attack respectively, therefore, the combined detection of these could initially detect the oxidative stress status. Ischemic stroke -caused hypoxia, ischemia, etc., could induce the production of iNOS [35]. As one of important inflammatory mediators produced by macrophages, the production of NO and iNOS expression are major signs reflecting macrophages' responses towards pro-inflammatory stimuli, and play an important role in the formation of atherosclerosis [36]. Once activated, iNOS will continue to produce high levels of NO, and caused oxidative stress injuries and increase the pathology [37]. In our study, SOD expression was lower in the MCAO groups than in sham groups and MDA expression was higher in the MCAO groups than in the sham groups. However, the early high-protein diet helped to increase the expression of SOD and inhibit the expressions of MDA in the MCAO groups. The early high-protein diet was able to restore iNOS expression in MCAO rats as well, indicating that the early high-protein diet could inhibit oxidative stress injuries, thus helping the body to eliminate free radicals, reduce inflammation, and promote the post-stroke recovery.

This study was subject to several limitations that should be taken into account. First, the purpose of this study was to investigate the effects of the early high-protein diet on the rehabilitation of MCAO rats; however, the long-term effect of high-protein diet seems to be of interest to be investigated as well. Second, our study was carried out on rats; further studies with human samples might yield more reliable results on the effects of early high-protein diet. Third, the side effect of the early high-protein diet should be taken into consideration, such as obesity, osteoporosis, and others side effects.

Conclusions

The early high-protein diet could promote the rehabilitation of MCAO rats, and this could provide a theoretical basis for the clinical applications of the early high-protein diet in patients with stroke. Indeed, there are various factors affecting patients with stroke, and patients' individual differences should not be ignored; therefore, the roles of the early high-protein diet in the rehabilitation of patients with stroke still needs to be investigated clinically.

Conflict of interest

None.

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