Treadmill exercise improves brain energy metabolism, motor, and cognitive functions in Hypoxic Ischemic Encephalopathy mice model

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Research

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Abstract

More evidence shows that the brain energy demands are enormous. This study aimed to examine the metabolism changes in the brain of hypoxic ischemic encephalopathy (HIE) mice model and to evaluate how treadmill exercise enhances brain metabolism. We used unilateral carotid common artery ligation mice model to simulate the clinical HIE patients. Several behavior tests were used to evaluate the motor and cognitive function impairment, western blot and immunofluorescence were used to assess the metabolism related protein changes in the different groups. Meanwhile, the HIE mice models were randomly divided into exercise training group (HIE-T) which were subjected to four weeks of treadmill exercise training and non-exercise training group (HIE-NT). The results revealed that there was decreased expression of glucose transporters GLUTs in HIE and an enhanced expression of monocarboxylate transporters MCTs, which is an important part of energy metabolism adaptation. After the period of treadmill exercise training, the expression of GLUT1 as well as MCT1 increased in the motor cortex of HIE mice model. Moreover, the mitochondrial cristae and edges were clear and intact under Transmission electron microscope compared with HIE-NT group. This suggests that increased brain energy attributed to treadmill exercise training shows promising therapeutic potential for HIE.

Introduction

Hypoxic Ischemic Encephalopathy, referred to as HIE, is a type of neonatal brain injury disease which can cause high mortality and neurodisability [1]. Typically, the infants appear hypomyotonia and unconsciousness in the early stage, even gradually manifest motor and cognitive disorders such as movement limitations and intellectual disabilities during growth phase [2, 3]. At present, the global incidence of HIE is 1.25‰ to 8‰, especially in developing countries, which seriously affects patient's quality of life and living standards [4]. Existing studies have shown that the occurrence of HIE is closely related to intrauterine hypoxia, the abnormal labor process (preterm labor or dystocia) or the asphyxia. Though there is no effectively treatment method cures HIE [5], it is known that a low Apgar score at birth increases the incidence of HIE in the developmental stage of children from the clinical data reported in recent years [6].

The brain is an important organ in the body, and its physiological activities are inseparable from energy metabolism [7]. Although it occupies only 2% of the body weight, it consumes 20% of the energy totally. Such a high consumption ratio reveals the sensitivity of the brain to energy supply [8]. Hence, the brain's responses are most vividly reflected in the energy metabolism alternation to various diseases [9]. Glucose is the main energy source of the brain. When the body's oxygen supply is sufficient, aerobic metabolism is the main method. Despite glycolysis has limited production capacity compared with aerobic metabolism, its role cannot be ignored. Meanwhile, Glucose cannot directly enter the cell, and requires the glucose transporters GLUTs to participate in intracellular energy metabolism through the sodium-mediated independent transport across the cell membrane. Among them, GLUT1 mainly exists in the endothelial cells that transports glucose across the blood-brain barrier, and helps glucose to pass through the glial cell membrane, and participates in the energy supply to glial cells [10, 11]. GLUT3 mainly helps glucose to

pass through the cell membrane of neurons and participates in the energy supply of neurons. Some studies have shown that the expression of GLUT3 is significantly decreased and GLUT1 is significantly increased during ischemic stroke, suggesting some nerve disease may indicate the energy metabolism abnormality [12, 13].

In addition, lactic acid, considered to be the product of anaerobic glycolysis, has been found in recent years participating the energy metabolism process. It can be indicated that glucose is not only the energy substrate for the brain cells, but also lactic acid take response to a state of excitement. Lactate transportation also requires the medium, Monocarboxylate transporters (MCTs). At present, monocarboxylic acid transporters are widely used in tumor research, and it has been confirmed that the growth of tumor cells can be effectively inhibited by using drugs that inhibit MCTs [14]. Therefore, for cellular metabolism, MCTs may be targeted regulatory proteins that promote cell survival. Moreover, MCTs are closely related with the effect of hypoxia [15]. The expression level distributed in the cell membrane stabilizes the level of energy metabolism in the brain under stress conditions and prevents excessive cell death due to energy depletion. Previous studies have not focused on the expression of MCTs in brain cells, so MCTs may be important target proteins for the potential treatment of brain injury caused by ischemia and hypoxia [16].

Existing HIE models mainly focus on ischemic and hypoxic brain injury. By severing unilateral or bilateral common carotid arteries, the normal blood supply to the brain is affected. Whether there is a connection between brain injury and energy metabolism and whether it can improve the energy supply of brain cells by promoting the utilization of lactic acid in the brain need to be confirmed in this experiment [17, 18]. Based on the above assumptions, we hypothesized that the normal glucose energy metabolism is blocked, and more lactic acid is used as the energy substrate in the HIE mice model. Exercise training can enhance the utilization of lactic acid in the brain, promote energy supply to the brain which effectively alleviates the brain damage caused by the abnormal energy metabolism, and plays a key role in neuroprotection

Material And Methods

Experimental Animal and group

C57BL/6J pregnant mice were purchased from Beijing Weitong Lihua Experimental Technology Co., Ltd., kept in cages in an SPF animal room, and provided a standardized 12-hour cycle of light and dark. The room temperature was kept at 21–23º c, and the humidity controlled at 40–70%. The animals had free access to food and water. When the pups were used to establish hypoxic ischemic encephalopathy (HIE) model, they were randomly divided into sham operation group (sham group, n = 20) and surgical operation group (HIE group, n = 30). After one month, the mice were further divided into groups to be subjected to treadmill exercise training (Sham-T and HIE-T) and sedentary groups (Sham-NT and HIE-NT). Sham-T (n = 8), Sham-NT (n = 8), HIE-T (n = 12), and HIE-NT (n = 12).

The establishment of HIE model

Postnatal day 7 pups were anesthetized by inhalation of isoflurane and placed on a surgical drape in supine position. The ventral neck region was disinfected with povidone iodine, and a 1cm longitudinal incision made in the ventral midline of the anterior neck. A stereo microscope was used to locate the left common carotid artery which was cauterized using an electrocoagulation hemostat pen to block blood supply to the brain, the skin incision was sutured and the blood around the wound was cleaned with normal saline. The entire surgical procedure was performed on a thermostatic pad to prevent hypothermia. After 1h the pups were placed in a constant temperature hypoxic chamber set at 6.5% oxygen and 93.5% nitrogen for 90 minutes. After the hypoxic exposure, the pups were put back on the thermostatic pad for 30 minutes to recover, and then returned to the dam in the cage. The sham- group was treated in the same way without cauterizing the common carotid artery and exposure to the hypoxic chamber.

Regular Treadmile Exercise

After 1month of life, Sham-T and HIE-T were subjected to treadmill exercise training. For the first three days the mice were subjected to low-intensity exercise training. After the third day of exercise training, we set the treadmill provide moderate-intensity exercise training. The animals were subjected to 90 minutes of treadmill exercise training every day for 6 days a week, and were allowed a day of rest for 4 weeks.

Gait test

In order to observe the movement posture, the HIE and Sham animal groups were placed on an open platform with sterile sheets. The gait observed by a video recording. By observing the walking gait of the animals under natural conditions and evaluating their motor function, the differences in motor coordination of the study individuals was analyzed to identify any defects. For each mouse, a new sterile sheet was used after recording the movements to exclude the influence of the smell of the previous mouse.

Climbing pole test

A plastic ball with a diameter of 2.5 cm was fixed on a wooden pole of 60 cm length and 1.5 cm diameter. The plastic ball was the starting point, and the other end of the wooden pole connected to the base was the end point. The surface of the wooden pole was wrapped with two layers of gauze, to provide a non-slippery surface. The mice were placed on the ball and the time required for the mice to climb down from the ball end to the base was recorded. Two indicators were y recorded: (1) the time it took the mice to climb down halfway the pole; (2) the time it took the mice to climb down the entire length of the pole.

Morris water maze

Morris water maze test included two experimental steps; the hidden platform experiment and space exploration experiment. When the experimental mice were put into the water for the first time to swim, the mice actively searched for a place to escape and rest. On the second day after the end of the hidden platform experiment the platform was removed. The animals searched for the location of the original platform in the existing cognitive memory, the number of times they crossed the target platform area

within a minute was recorded, and their spatial positioning and cognitive ability analyzed from the video tracks

Forelimb suspension test

The hind limbs of the mice were wrapped with a tape and the forelimbs placed on a horizontal bar. A safety pad was placed underneath to reduce the impact falling. The forelimb suspension experiment effectively demonstrated the strength of the forelimb muscle function. The time that the mice spent on the horizontal bar was a measure of the motor strength of the animals.

Western Blots

The experimental mice were anesthetized with isoflurane, quickly decapitated, and fresh tissues were taken out. The mouse brain was placed in the brain tank to isolate the cortex and placed on ice for grinding. Tissue was homogenized in RIRA's reagent according to the weight. The protein concentration was determined by the bicinchoninic acid assay, and a total of 30μg of protein was separated by electrophoresis on 15% SDS-PAGE gels. A 10-245KD rainbow spectral protein marker is located on both sides of the loading channel to indicate the approximate positions of protein bands with different protein molecular weights. Specific parameters included using 80V voltage for 30min and raising it to 120V voltage for 1h. Then the proteins were transferred onto the polyvinylidene fluoride membranes (PVDF). The membranes were blocked with 10% bovine serum albumin (BSA) at the room temperature. These were incubated with the following primary antibodies from Santa Cruz (Dallas, TX, USA): MCT1 (sc-366501, 1:1000), MCT2(sc-166925, 1:1000); Cell signaling technology (Danvers, MA, USA): SDH (11998S, 1:1000), LDHA (3582S, 1:1000), GAPDH (5174S, 1:1000); Affinity Bioscience (Cincinnati, OH, USA) :GLUT1 (AF5426, 1:500), GLUT3 (AF5463, 1:500), Beta-tubulin (T0023, 1:10000) at 4°C overnight followed by HRP-conjugated secondary antibody from Affinity Bioscience (Goat Anti-Rabbit IgG S0001,1: 5000, Goat Anti-Mouse IgG S0002, 1:5000) incubated for 2h at room temperature. The protein bands were visualized with an enhanced chemiluminescence kit (femtogram, KF8003) by Affinity Biosciences and imaged with the Bio-Image Analysis System (BIO-RAD, California, USA). The ratios of the protein band intensities to Beta-tubulin or GAPDH as internal references were determined using ImageJ.

Immunofluorescence Staining

The 20μm thickness Brain slices were obtained using a frozen microtome (Leica, Germany). Using 24-well pore plate to hold the slices for immunofluorescence. Each well rinsed 3 times with PBS buffer, lasting 5 minutes. PBS buffer containing 10% BSA (#A8020, Solarbio Life Sciences, Beijing, China) and 0.3% Triton X-100, (Amresco 0694, Biosharp, Estonia) was used as blocking solution, added to block those slices at room temperature for 2 h. Then PBS buffer containing 5% BSA and 0.3% Triton X-100 was used as the antibody diluent, adding the corresponding primary antibody stock solution in proportion, and mixed thoroughly. These primary antibodies include MCT1 (Santa Cruz, sc-366501, 1:300) and GLUT1 (Affinity Bioscience, AF5426, 1:200).150μl of antibody solution was added to each well, and incubated at 4°C overnight. After that the primary antibodies were recovered and the samples washed for 6 times with PBS buffer, each lasting 5 minutes. The fluorescent secondary antibodies (ab97035 and ab97075 from

Abcam, Cambridge Biomedical Campus, Cambridge, UK) were used according to the source of the primary antibody. DDH2O was the secondary antibody diluent, to it was added the fluorescent secondary antibody solution and mixed thoroughly. Identically 150μl of secondary antibody solution were added to it and incubated at room temperature for 2 h in the dark. Rinsing 6 times, which lasting 5minutes with PBS buffer is also needed. Finally the brain slices were mounted on glass slides, DAPI (C0065 from Solarbio Life Sciences, Beijing, China; 1:100) was added for 5 min and poured off. Washed in PBS for 3 times, dried, and an anti-fluorescence quencher (P0126, Beyotime Biotechnology, Shanghai, China) was added dropwise. Glass slip were fixed on the slides, and sample were stored at -20°C for all night to take the microscopic imaging. The brain slice images were captured by a confocal fluorescence microscope (Ni-U942877, Nikon, Tokyo, Japan), and the images representing the protein at the motor cortex were quantified by ImageJ as described in.

Electron microscopy

Brain slices which were approximately 50mm thick were obtained by an ultra-thin microtome for the following-up experiment. The slices were dyed with 2% uranyl acetate for 30min in the dark environment. Wash them 5 times quickly and then the slices were placed on the filter paper to dry. This process lasted 10 minutes. After that, use 0.3% lead citrate to dye these slices for 5 mins and wash quickly. The section were observed by an HT7700 electron microscope (HITACHI, Japan). Photographs of the cortex were obtained at random.

Data analysis

GraphPad Prism version 9.0.0 for windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and the results were expressed as Mean ± standard deviation (Mean ± SD). The difference between the HIE model group and the Sham group was analyzed using the paired T test, One-way ANOVA or Two-way ANOVA followed by Tukey's post-hoc test to compare the differences between groups. Statistically significance was considered at $P < 0.05$ values

Results

Establishment and behavioral identification of HIE model mice

The HIE mice models were established by ligating the unilateral common carotid artery and incubating in a hypoxic chamber with 6.5% oxygen concentration for 1.5 hours. It was observed that, compared with the sham group, the HIE group exhibited slower walking speed, asymmetrical and uncoordinated motor behavior [19], a large separation angle of the hind limbs, and abnormal posture (Fig. 1a). Recording the body weight of the mice at different time periods, it was observed that the weight of the HIE mice was lower [20, 21] than that of the sham-operated group at postoperative day14, and it was more obvious at postoperative day 21 (Fig. 1b), indicating that the HIE mice had obvious developmental abnormalities.

The changes in motor and cognitive functions of HIE mice were evaluated by the Morris water maze test. In the Morris water maze test, it was observed that on the fifth day of training, the HIE group looked for the platform in all the four quadrants, with the quadrant that had the hidden platform, showing negligible preference in contrast to the other three quadrants. The motion trajectories of the HIE group were all farther than those of the Sham group (Fig. 1c). At the same time, on the seventh day of the test, it was observed that the HIE group took longer to reach the platform for the first time compared to the Sham group (Fig. 1d), and the number of times the HIE group crossed the platform was less than that of the Sham group (Fig. 1e). By recording indicators of motor function during the exploratory phase, HIE mice were observed for movement speed and total distance covered were also smaller than those in the Sham group (Fig. 1f, g), suggesting that motor function was affected. This series of behavioral studies showed that the cognitive and motor functions of the HIE mice model had changed which verified the successful establishment of the HIE model.

Abnormal energy metabolism in the brain of HIE mice model

The changes in energy metabolism-related proteins in the brain observed by Western blotting. The results showed that the expression levels of monocarboxylic acid transporters, MCT1 and MCT2 were higher in the HIE model group than in the Sham group (Fig. 2a, b). And the expression levels of glucose transporters, GLUT1 and GLUT3 were significantly lower than those in the Sham group (Fig. 2c, d), this suggested a change of the energy substrate in the HIE brain. Additionally, by observing the levels of succinate dehydrogenase (SDH), a key enzyme in the tricarboxylic acid cycle, and lactate dehydrogenase A (LDHA), a key enzyme in lactate metabolism, it was observed that the two were differently expressed between the HIE and Sham groups (Fig. 2e, f). Meanwhile, the levels of lactic acid in the brain of the HIE model group and the sham-operated group were observed. The results showed that compared with the Sham group, the level of lactate in the cerebral cortex was higher in the HIE group due to the effect of ischemia and hypoxia (Fig. 2g). This showed that the normal energy metabolism in the brain was changed in the HIE condition as the utilization of glucose was reduced, and monocarboxylic acid was involved as backup energy supply substrate to maintain the level of energy metabolism in the brain under the stress state.

Regular Treadmill exercise improves motor function in HIE mice model

The Sham-T and the HIE-T groups were subjected to 4weeks of treadmill exercise to evaluate the effect of exercise on the motor function of HIE mice model. In the forelimb suspension experiment (Fig. 3a), the HIE-NT group had the shortest persistence time by recording the data of the persistence time of mice in different groups. But after the exercise intervention, the HIE-T group persisted longer than the HIE-NT group, and the Sham-T group persisted longer than the Sham-NT group (Fig. 3b). Similarly, in the pole climbing test (Fig. 3c), the HIE-T group with exercise intervention took the time to pass the halfway mark of the pole and the time to climb the total pole length was less than the HIE-T group (Fig. 3d). These

behavioral results indicated that treadmill exercise training enhanced the motor function of HIE mice model.

Treadmill exercise increases the expression of energy transporters in the brain of HIE mice model

The results of immunofluorescence and Western blot observation of MCT1showed that compared with Sham-NT, the MCT1-positive labeling signal of Sham-T was significantly increased in the cortex. The MCT1-positive labeling signal of HIE-T was also enhanced in the cortex compared to HIE-NT (Fig. 4). This result suggests that after treadmill exercise, the increase in lactate leads to an increase in lactate transport receptors in the brain, and the utilization of lactate is greatly enhanced, filling other energy supply methods except glucose. Similarly, the results of immunofluorescence and western blot observation of GLUT1 showed that compared with Sham-T, the level of GLUT1 in the HIE-NT group was significantly reduced, suggesting that the participation of glucose in energy supply in HIE mice was affected. Compared with HIE-NT, the GLUT1-positive labeling signal of HIE-T was significantly enhanced in the cortex (Fig. 5). This suggests that after treadmill exercise, the glucose transporters increase in the brain, glucose as the conventional metabolic substrate participates in energy metabolism activities, and the metabolism of energy in the brain is further enhanced.

Treadmill exercise improves mitochondrial morphology in the brain of HIE mice model

Electron microscopy further verified the effect of regular exercise on brain energy metabolism in HIE model mice. The results showed that in the HIE-NT group, changes in mitochondrial morphology were observed. Compared with the Sham-NT group, mitochondria were atrophied, the edges were blurred, and the internal mitochondrial cristae was also damaged. For the HIE-T group, it was observed morphologically that the damage to the mitochondrial structure was improved, the edges became clearer, and the mitochondrial cristae inside were more complete than those of the HIE-NT group (Fig. 6). These suggest that treadmill exercise can improved the structure of mitochondria in the motor cortex of HIE mice.

Discussion

In this study, we adopted the classic right unilateral common carotid artery ligation accompanied by hypoxic brain injury induced by a hypoxic environment [22, 23] to simulate the brain pathological changes of HIE. Our study found that ischemia and hypoxia are the main pathogenic causes of HIE during perinatal period [24]. Developmental abnormalities of movement and posture are often seen in HIE, together with impairments in sensory perception, communication, and behavior [25]. Through gait analysis, we observed that the HIE mice showed obvious scissor gait which formed a large angle between the hind limbs. Besides, compared to the Sham group, the walking process was slower as well as the

general motor function was significantly impaired in the HIE group. In the Morris water maze and the pole climbing test, the HIE model mice were also observed to have a decreased motor coordination, revealed by decreased memory, learning and grip strength. These observations were consistent with the movement disorders seen in the current clinical practice in children with HIE [26]. And compared with the sham operation group, the weight gain of the HIE pups were significantly less, their growth and development lagged behind the sham operation group, suggesting the development disorder in the individual condition of the HIE model. However, in clinical practice, not all HIE children show this phenomenon, as some children's physical development condition is basically the same as that of normal children. Whether there is a direct relationship between brain damage and growth retardation needs to be further verified in the future.

In the past, it was believed that the main source of energy in the CNS was the aerobic metabolism of glucose [27]. And the lactic acid, the end product of anaerobic glycolysis as previously known, was not involved in the energy supply in the brain. Nevertheless, recent studies have continuously confirmed that lactic acid is not only a secondary metabolite, but an essential energy supply substrate for the brain [28, 29]. Moreover, the utilization rate of lactic acid in the brain is much greater than that of glucose, especially in stress states. The physiological role of lactic acid in the brain has now attracted attention. Through the observation of lactic acid in the brains of the HIE group and the Sham group, our results showed that the content of lactic acid in the brains of HIE mice model increased, suggesting that anaerobic glycolysis was enhanced after the occurrence of HIE, lactic acid accumulated in the brain, and the utilization of lactic acid by the brain was also significant. Lactate and glucose can only participate in the physiological metabolic activities of cells when they are taken up by transporters in blood and tissues. The uptake of lactate requires the monocarboxylic acid transporters, and the uptake of glucose requires the glucose transporters. Both are membrane proteins which are widely expressed in glial cells and neuronal cells, and different subtypes of energy metabolism transporters exist on different cell membranes to mediate the transport of different metabolites. Among them, MCT1 and GLUT1 are mainly expressed on the glial cell membrane, and MCT2 and GLUT3 are expressed in neurons [30]. The existing dissertations on the involvement of lactate in energy metabolism of the brain are mainly based on the astrocyte-neuron lactate shuttling mechanism, that is, lactate is taken up or produced by astrocytes, and then passed through the monocarboxylic acid transporters [31]. It is excreted, harvested by monocarboxylic acid transporters on neurons and involved in the energy supply of neurons. In this study, Western Blot results showed that the expressions of MCT1 and MCT2 in HIE mice model were significantly increased, while the expressions of GLUT1 and GLUT3 were significantly decreased, suggesting that brain energy metabolism changes in HIE state. Glucose as an energy supply was gradually replaced by lactate, which was related to the state of ischemia and hypoxia in brain tissue [32]. Through the protein expression analysis of the key enzymes succinate dehydrogenase in the tricarboxylic acid cycle and lactate dehydrogenase in the lactate metabolism, we concluded that the aerobic metabolism of glucose decreased and the lactate metabolism increased in the HIE state, showing the metabolic pathway in the brain was powered changed. Lactate is utilized to maintain the most basic

physiological needs of the brain in the presence of insufficient glucose supply. This metabolic change could further reveal the mechanism of HIE.

Treadmill exercise is a typical aerobic exercise, which enhances the cardiopulmonary function of the organism and increases the oxygen content in the body. It can additionally provide more energy substances for the brain by increasing the whole body lactic acid. Exercise affects not only the body, but also upgrades brain function which is the focus of our attention. Previous experiments have found that exercise significantly elevated the cognitive function of mice and people [33–35]. In our study, we observed that after subjecting mice to treadmill exercise for 4 weeks, compared with the non-exercise group in the HIE mice model, the HIE-T group improved performance in the forelimb suspension test. The time was longer than that of the HIE-NT group, and the grip strength and muscle strength of the forelimbs were also much higher than those of the HIE-NT group. Meanwhile the pole-climbing experiment further confirmed that the motor function of the HIE-T group was significantly ameliorated. The HIE-T group took less time to pass the half-way mark of the pole and the whole climbing process than the HIE-NT group, which also showed that treadmill exercise greatly improved the motor coordination of HIE [36–38]. In the Sham group, by participating in treadmill exercise, the mice in the Sham-T group showed a better performance in the forelimb suspension test than the mice in the Sham-NT group, indicating that exercise intervention is not only intriguing in the HIE group but also in the other group.

Moreover, in this study, we observed through immunofluorescence analysis of the monocarboxylic acid transporters MCTs and the glucose transporters GLUTs that the HIE-T group had more MCT1 and GLUT1 in the cerebral cortex compared with the HIE-NT group [39]. The fluorescence positive signal intensity of MCT1 increased significantly on exercise training, and in the Western Blot experiment, the protein expression of MCT1 and GLUT1 also showed an upward trend, consistent with the results of immunofluorescence staining. This phenomenon revealed that after the intervention of treadmill exercise, the energy substrate transporters in different pathways increased. In other words, energy metabolism in the brain was greatly improved [39]. It is precisely this enhancement of energy metabolism that provides more abundant energy reserves for various cells in the brain [40]. From the perspective of energy metabolism, it also reveals a potential method for the treatment of HIE by improving the energy metabolism. However, how glucose and lactate are involved in the subsequent energy metabolism, and whether there are other subsequent factors involved in the intrinsic mechanism of exercise improving the motor function of HIE mice, need to be probed further. By only observing the changes in transporters does not give a full reflection of the metabolites utilization pathways, which is a limitation of this study [41, 42].

In terms of being responsible for energy metabolism and cell survival, mitochondria are even more responsible for affecting the whole body [43]. In this study, we found that compared with the Sham-NT group, the mitochondrial structure in the HIE-NT group changed abnormally; the edges of the mitochondria were blurred; some parts were severely damaged, the mitochondrial cristae also appeared swollen and broken. After a period of treadmill exercise, compared with HIE-NT, the edges of mitochondria in HIE-T group became clear and obvious, the cristae structure in mitochondria also became complete

and dense [44]. From the perspective of subcellular structure, we show that in the HIE mice model, the structure of mitochondria in the brain was greatly improved after exercise training intervention, which was due to improved energy metabolism in the brain [45]. Moreover, mitochondria are not only sites for energy metabolism, but also play a great role in regulating the physiological function of cells. Whether exercise affects the recovery of HIE by the regulation of cellular physiological activities are not reflected in this study. The complexity of mitochondrial function also limits us to assert on the basis of morphological changes whether mitochondrial function is closely related with the observed improvements [46]. Therefore, in future studies, changes in mitochondrial function may be probed to explore their role in Hypoxia Ischemia Encephalopathy.

Abbreviations

HIE
Hypoxic Ischemic encephalopathy
HIE-T
Hypoxic Ischemic encephalopathy with exercise training
HIE-NT
Hypoxic Ischemic encephalopathy without exercise training
GLUT
Glucose Transporter
MCT
Monocarboxylate Transporter
PVDF
Polyvinylidene Fluoride
BSA
Bovine Serum Albumin
GAPDH
Glyceraldehyde-3-phosphate Dehydrogenase
SDH
Succinate Dehydrogenase
LDHA
Lactate Dehydrogenase A
CNS
Central Nervous System

Declarations

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Author's contributions

Conceptualization, FP, CC; Methodology, FP, YW, HL; Data curation, FG, YW, HL; Formal analysis, SD, SC, JF; Funding acquisition, CC; Investigation, FP, YW, HL,SD; Project administration, FP, FG, SC, JF; Resources, CC; Software, HL,SD; Supervision, CC; Validation, FP, FG, YW, CC; Visualization, SD, SC, JF, CC; Writing-original draft, FP; Writing-review and editing, FG, HL, SC, CC. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Ethic approval and consent to participate

All the procedures for the care and treatment of animals were performed according to the guidelines of the committee for the Care of Research Animals of Zhengzhou University (ZZUIRB2022-32)

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Figures

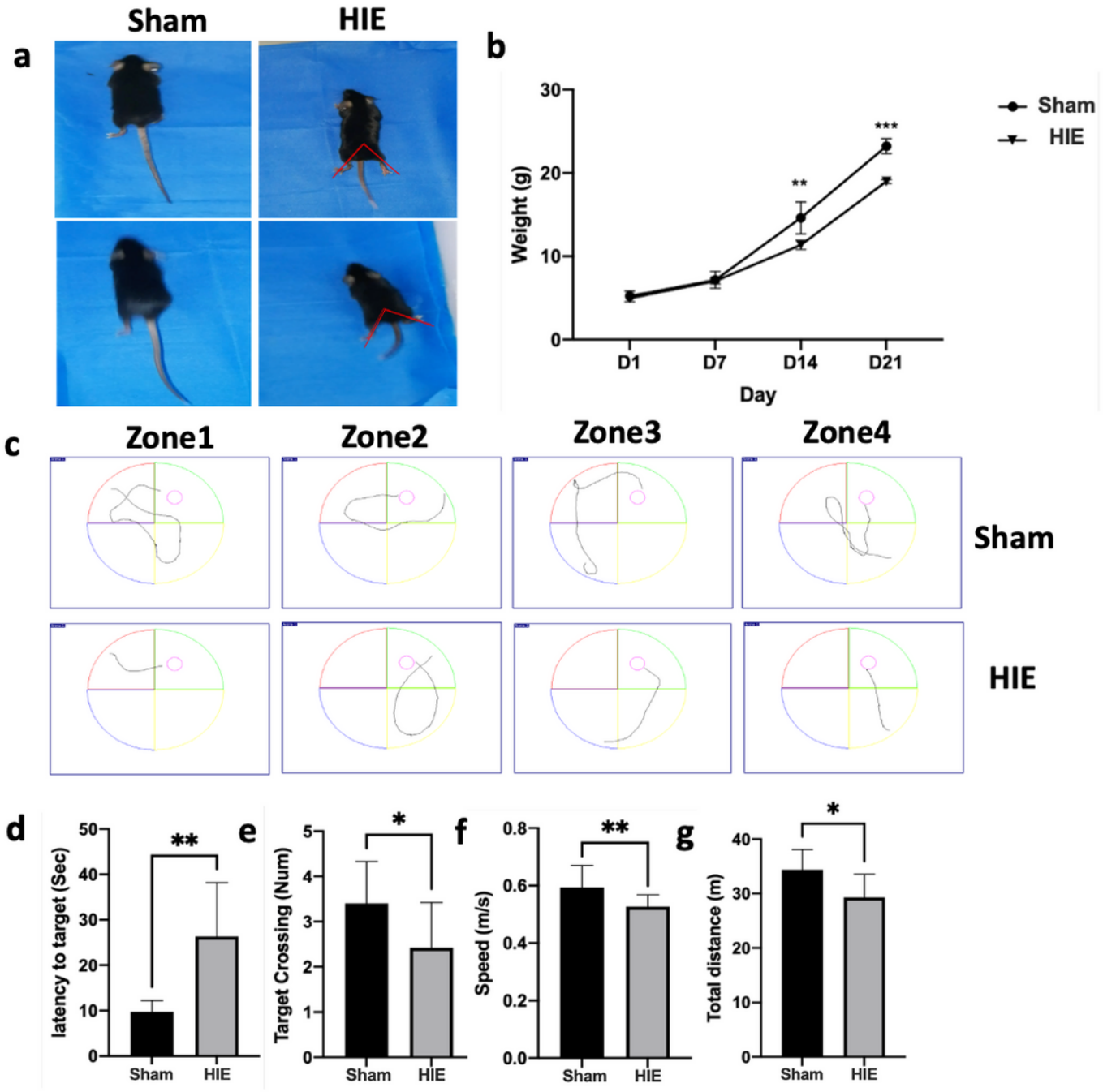

Figure 1

The HIE models represent the abnormal physical development and behavior compared with the Sham Group. **a** The HIE models exhibit marked scissors gait. **b** Weight differences at the different stages of growth. Individual data are presented as the mean ± SD for the two groups, using Two-way ANOVA for the statistical analysis (n = 25). **c** The Morris Water Maze (MWW) test's trajectory diagram in Sham and HIE group. **d** Latency to the Target in the MWW test. **e** Target Crossing times. **f** Speed in the MWW test. **g** The total distance covered in the MWW test. Individual data are presented as the mean ± SD for the two groups, using T-test for the statistical analysis (n = 12). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

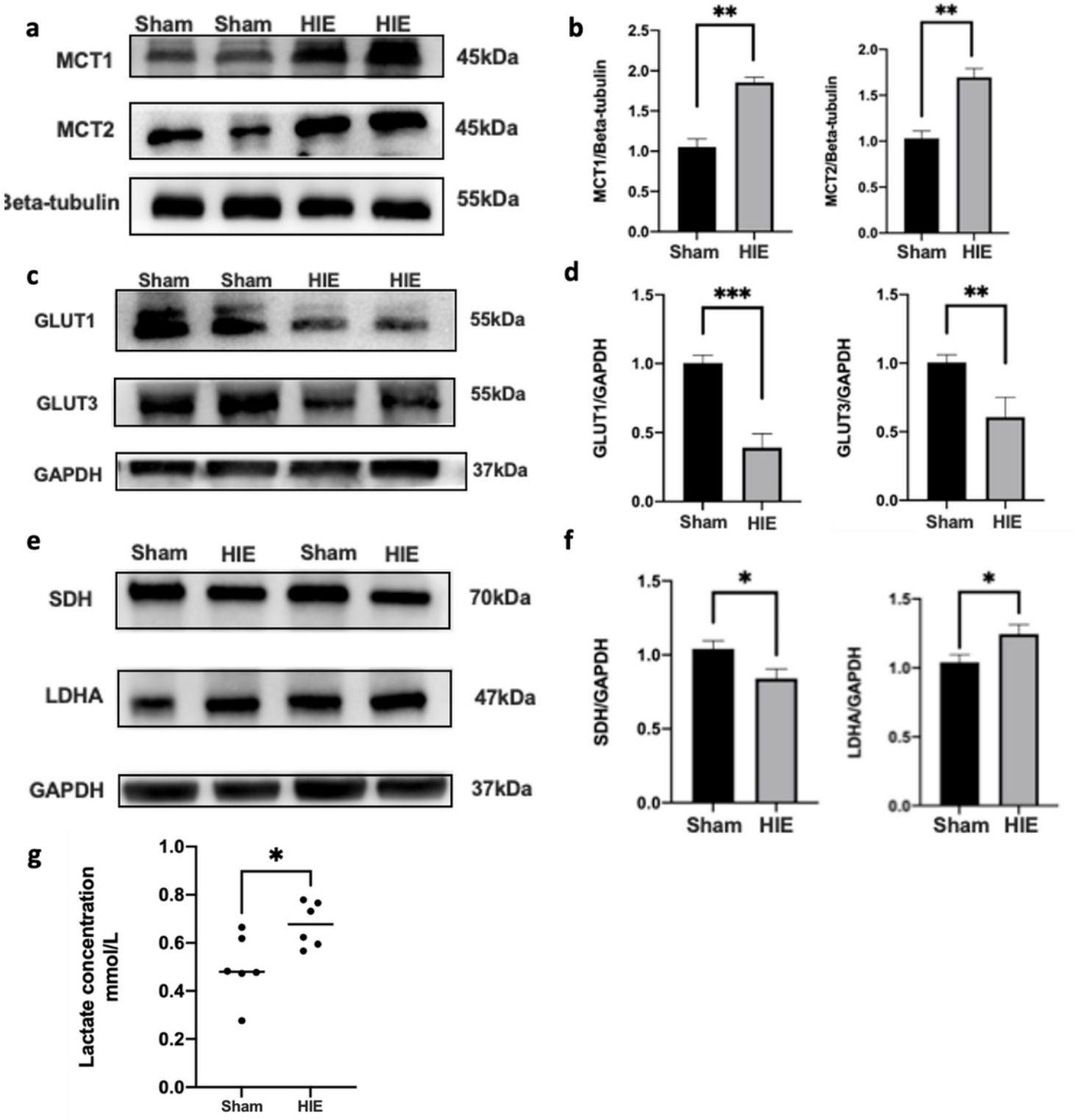

Figure 2

Changes of energy metabolism in motor cortex in HIE mice model. **a** The Immunoblots of MCT1and MCT2 in the Sham and HIE groups. Protein bands were normalized to Beta-tubulin. **b** Graphs for the Western Blot analysis of MCT1 and MCT2 in Sham and HIE groups. Data are presented as the mean ± SD (n = 4). **c** The Immunoblots of GLUT1and GLUT3 in the Sham and HIE groups. Protein bands were normalized to GAPDH. **d** Graphs for the Western Blot analysis of GLUT1and GLUT3 in Sham and HIE

groups. Data are presented as the mean ± SD (n = 4). **e** Western Blots for the SDH and LDHA proteins in the different groups. Protein bands were normalized to GAPDH. **f** Graphs for the Western Blot analysis of SDH and LDHA (n = 4). **g** The level of Lactate in the motor cortex of the Sham and HIE groups (n = 4). Individual data are presented as the mean ± SD for the two groups, using T-test for statistical analysis . $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

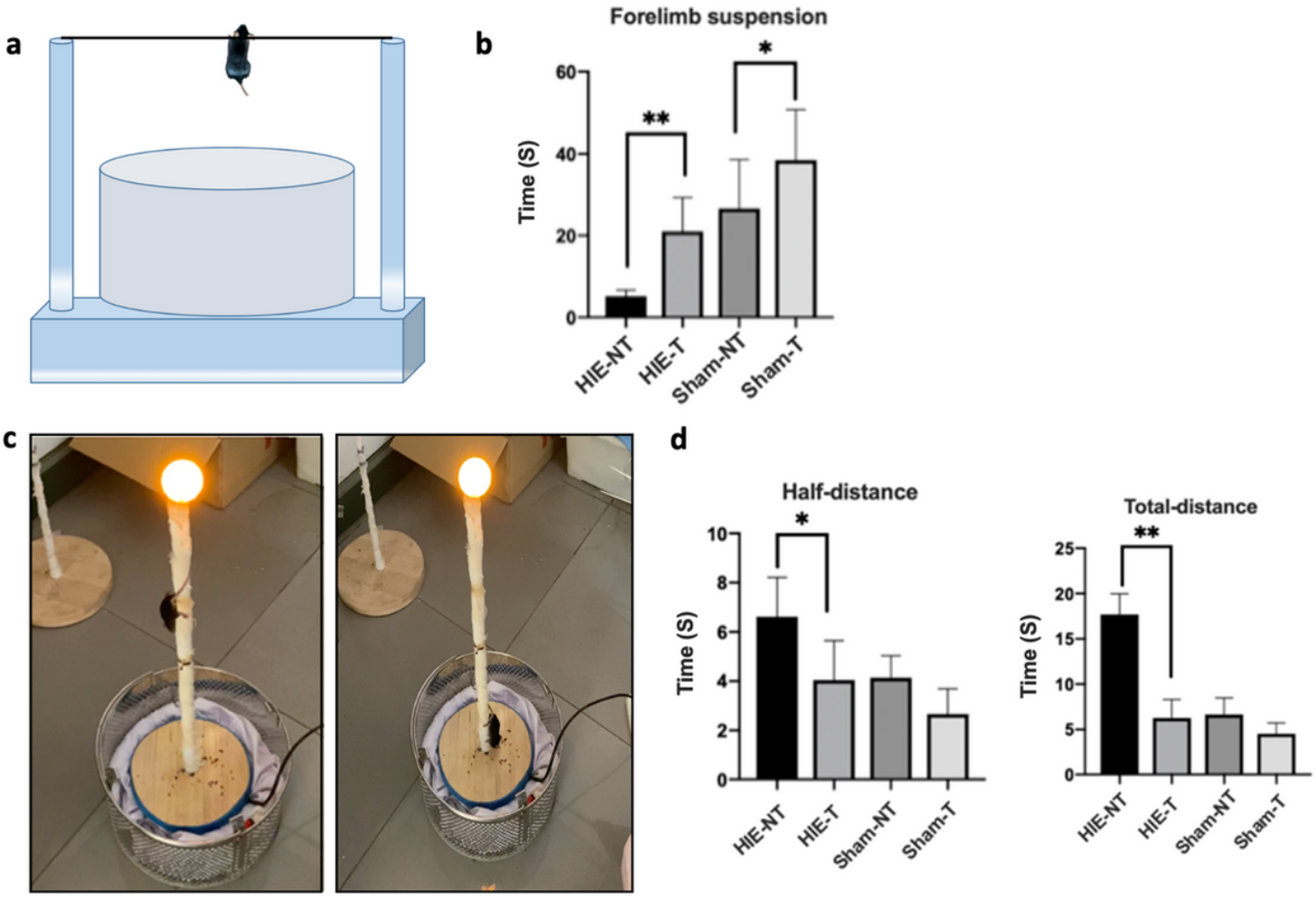

Figure 3

Regular Treadmill Exercise enhances the motor function in HIE model. **a** The forelimb suspension test. **b** Statistical graph of the persistence time of different groups of mice (n = 10). Individual data are presented as the mean ± SD for the four group, using one-way ANOVA for the statistical analysis. **c** The pole-climbing test showing the mice passing though the half-distance and total-distance. **d** Graph for the pole-climbing test in the different mice groups (n =10). Individual data are presented as the mean ± SD for the four groups, using one-way ANOVA for the statistical analysis. $^{*}P < 0.05$, $^{**}P < 0.01$.

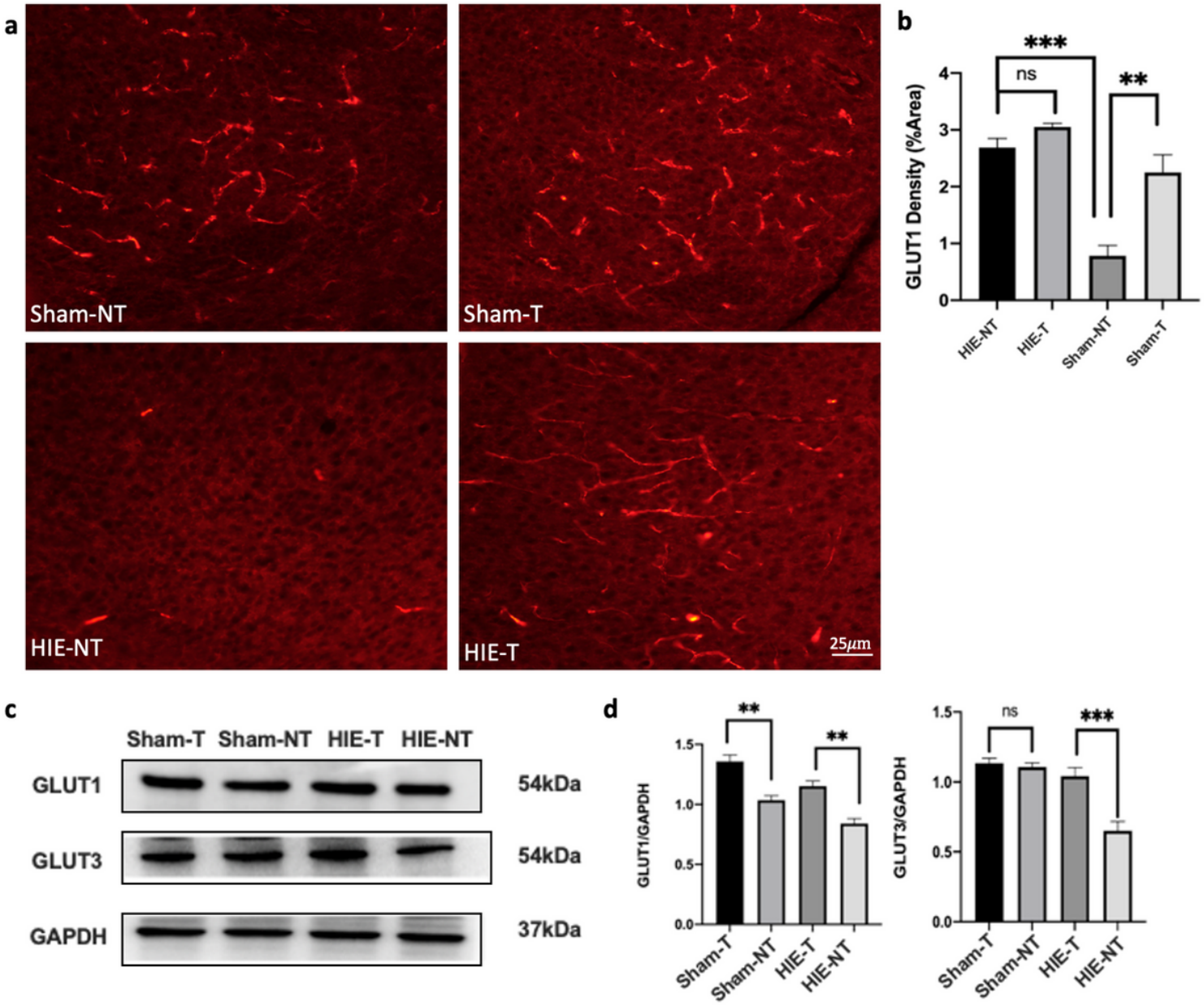

Figure 4

Regular treadmill exercise intervention increases the expression of GLUTs. **a** Representative microphotographs of GLUT1 in the motor cortex of Sham-NT mice group, Sham-T group, HIE-NT group and HIE-T group. Scale bar =25

m. **b** Quantification of GLUT1 positive signals in the different groups. **c** Representative Western Blot bands of GLUT1 and GLUT3 in the four groups. **d** Densitometric quantification of GLUT1and GLUT3. Protein immunoreactivity is normalized to GAPDH. Data are represented as mean ± SD for the four groups, using ANOVA for the statistical analysis (n = 4 in each group). $^{**}P < 0.01$, $^{***}P < 0.001$.

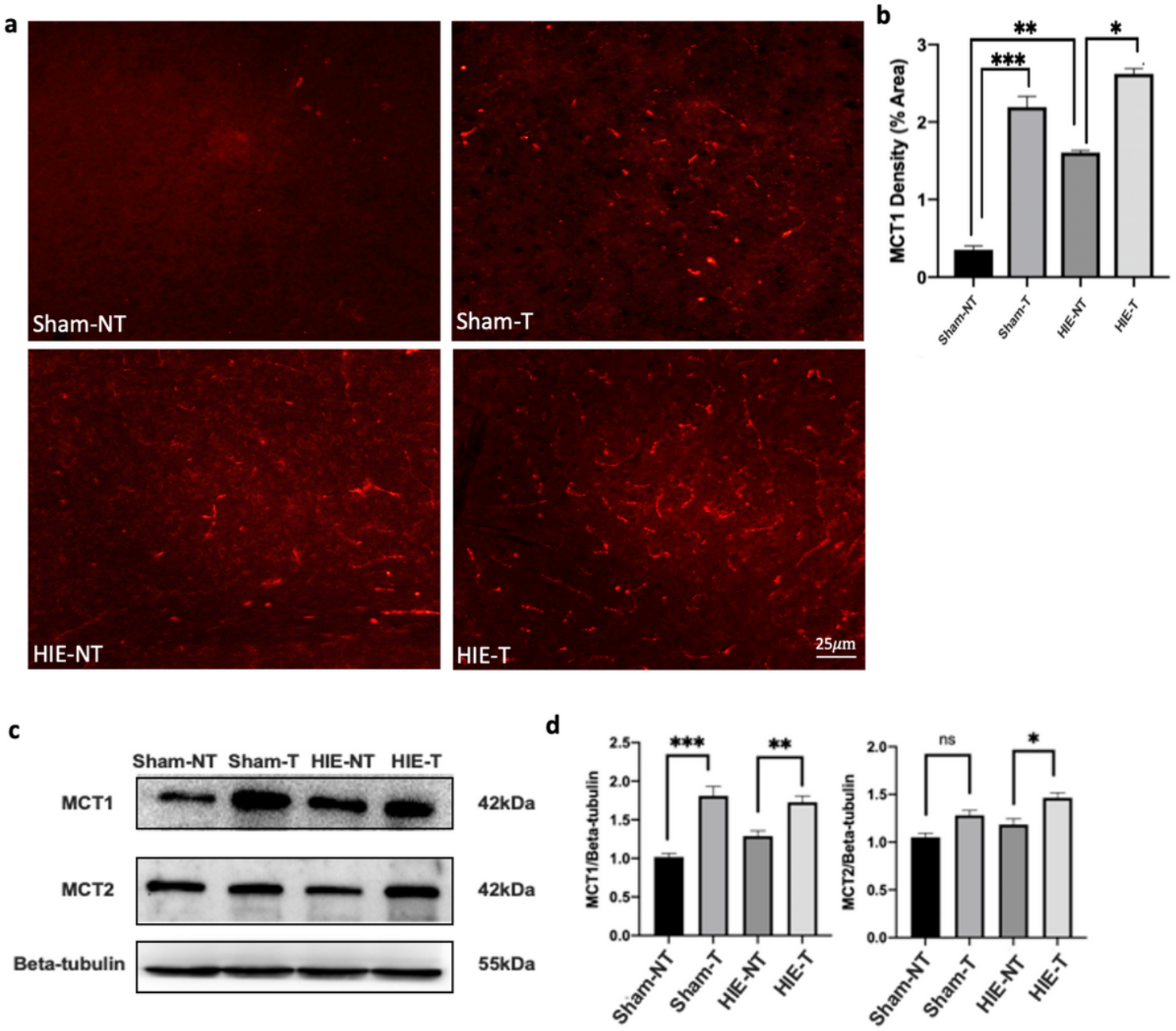

Figure 5

Regular treadmill exercise intervention increased the expression of MCTs. **a** Representative microphotographs of MCT1 in the motor cortex of Sham-NT mice group, Sham-T group, HIE-NT group and HIE-T group. Scale bar =25

m. **b** Quantification of MCT1 positive signals in the different groups. **c** Representative Western Blot bands for MCT1 and MCT2 in the four groups. **d** Densitometric quantification of MCT1 and MCT2. Protein bands were normalized to Beta-tubulin. Data are represented as mean ± SD for the four groups, using one-way ANOVA for the statistical analysis (n = 4 in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

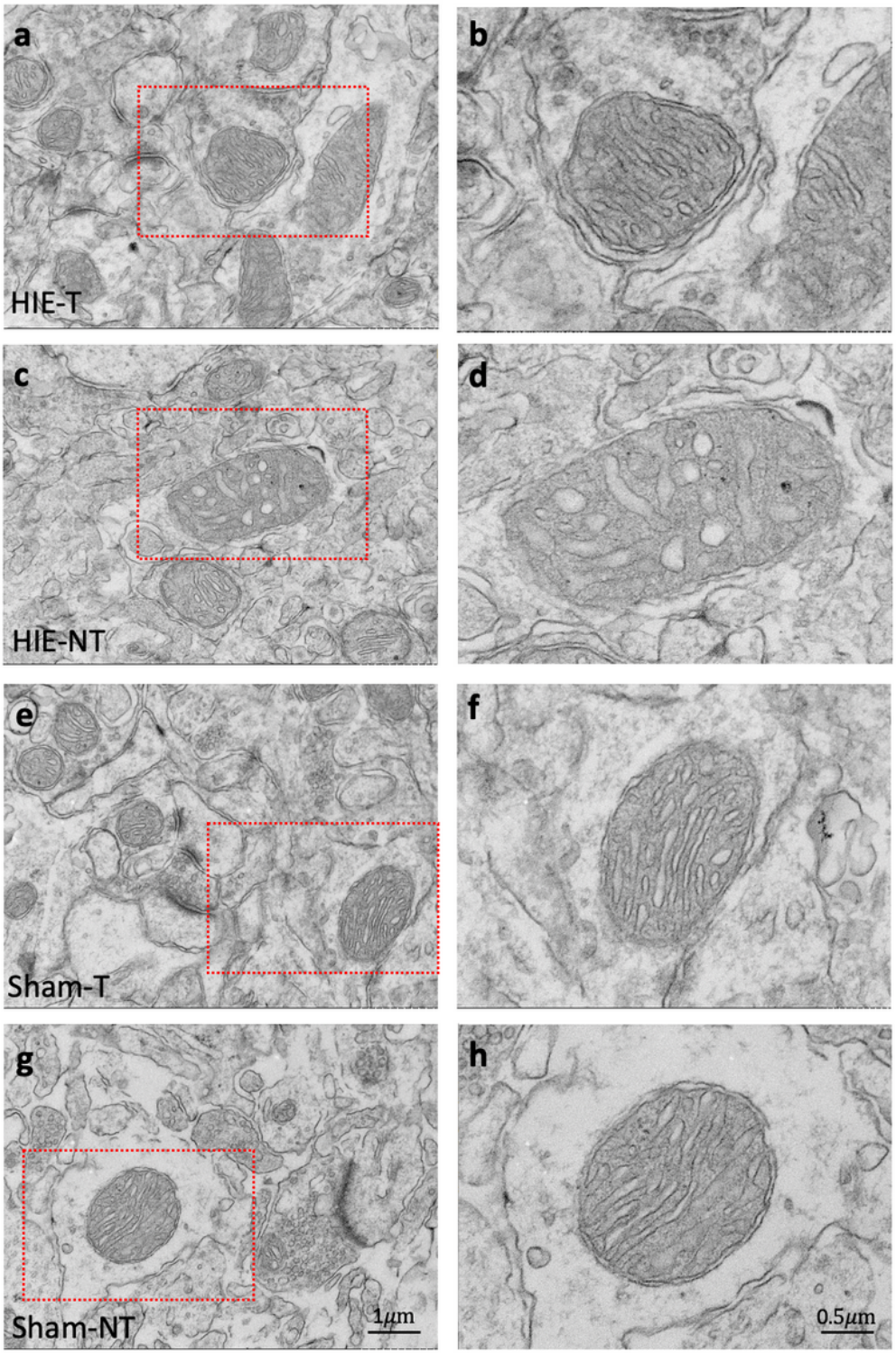

Figure 6

The morphological and structural changes of mitochondria in different groups after exercise intervention. **a** The morphological structure of mitochondria in HIE-NT group in the cortex at 1.2K magnification. Scale Bar = 1

m. **b** The morphological structure of mitochondria in HIE-NT group in the cortex at 2.5K magnification. Scale Bar = 0.5

m. **c** Mitochondria integrity in the cortex as observed in HIE-T group at 1.2K magnification. **d** Mitochondria integrity as observed in HIE-T group at 2.5K magnification. **e** The morphological structure of mitochondria in Sham-T group at 1.2K magnification. **f** The morphological structure of mitochondria in Sham-T group at 2.5K magnification. **g** Mitochondria integrity in the cortex as observed in Sham-NT group at 1.2K magnification. **h** Mitochondria integrity in the cortex as observed in Sham-T group at 2.5K magnification.