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EXERCISE INDUCES MITOCHONDRIAL BIOGENESIS AFTER BRAIN ISCHEMIA IN RATS

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Abstract—Stroke is a major cause of death worldwide. Previous studies have suggested both exercise and mitochondrial biogenesis contribute to improved post-ischemic recovery of brain function. However, the exact mechanism underlying this effect is unclear. On the other hand, the benefit of exercise-induced mitochondrial biogenesis in brain has been confirmed. In this study, we attempted to determine whether treadmill exercise induces functional improvement through regulation of mitochondrial biogenesis after brain ischemia. We subjected adult male rats to ischemia, followed by either treadmill exercise or non-exercise and analyzed the effect of exercise on the amount of mitochondrial DNA (mtDNA), expression of mitochondrial biogenesis factors, and mitochondrial protein. In the ischemia-exercise group, only peroxisome proliferator activated receptor coactivator-1 (PGC-1) expression was increased significantly after 3 days of treadmill training. However, after 7 days of training, the levels of mtDNA, nuclear respiratory factor 1, NRF-1, mitochondrial transcription factor A, TFAM, and the mitochondrial protein cytochrome C oxidase subunit IV (COXIV) and heat shock protein-60 (HSP60) also increased above levels observed in non-exercised ischemic animals. These changes followed with significant changes in behavioral scores and cerebral infarct volume. The results indicate that exercise can promote mitochondrial biogenesis after ischemic injury, which may serve as a novel component of exercise-induced repair mechanisms of the brain. Understanding the molecular basis for exercise-induced neuroprotection may be beneficial in the development of therapeutic approaches for brain recovery from the ischemic injury. Based upon our findings, stimulation or enhancement of mitochondrial biogenesis may prove a novel neuroprotective strategy in the future.

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Abbreviations: COXIV, C oxidase subunit IV; HSP60, heat shock protein-60; MCAO, middle cerebral artery occlusion; mtDNA, mitochondrial DNA; NRF-1, nuclear respiratory factor 1; PCR, polymerase chain reaction; PGC-1, proliferator activated receptor coactivator-1; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TFAM, mitochondrial transcription factor A.

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Stroke is a major cause of death worldwide (Chung et al., 2010). Although the number of stroke survivors is increasing, many of these suffer from chronic persistent sensory, motor, and cognitive dysfunction, which places an enormous burden on society. Therefore, there is intense interest in determining the mechanisms responsible for post-ischemic recovery that may improve the quality of life for patients.

Exercise is a well-known component of stroke rehabilitation programs. Exercise studies in ischemic rat models demonstrate that exercise can improve post-ischemic functional recovery of behavior and structural alterations in the brain (Moseley et al., 2005; Ploughman et al., 2007). Although the beneficial effects on brain function have been confirmed, the detailed mechanisms responsible for exercise-induced neuroprotection remain poorly understood.

Mitochondria produce energy through the respiratory chain and are one of the most important organelles in the cell (Garesse and Vallejo, 2001; Viña et al., 2009). Owing to the fact that mitochondria are responsible for generating energy they likely play important roles in many neuronal metabolism processes such as neurogenesis, neural proliferation, neural differentiation, neurite outgrowth, and dendritic remodeling (Cheng et al., 2010). On the other hand, experimental evidence has also shown that mitochondria-dependent damage is a key determinant in the extent of neuronal ischemic injury caused by increased production of mitochondrial-derived reactive oxygen species (ROS) (Onyango et al., 2010). Correspondingly, consistent evidence suggests that biogenesis of a larger pool of functional mitochondria maybe be helpful for neuroprotection (Cheng et al., 2010). In 2008, mitochondrial biogenesis was first confirmed in a rat model of neonatal hypoxic-ischemic brain injury. It was observed post injury that increases in the levels of mitochondrial DNA, total mitochondrial number, and the mitochondrial transcription factors proliferator activated receptor coactivator-1 (PGC-1), nuclear respiratory factor 1 (NRF-1), mitochondrial transcription factor A (TFAM) and mitochondrial protein C oxidase subunit IV (COXIV) and heat shock protein-60 (HSP60) all occurred (Yin et al., 2008). The following year, another group reported that improved mitochondrial biogenesis could help reduce ischemic cerebral injury, suggesting that mitochondrial biogenesis may be a novel endogenous neuroprotective response (Valerio et al., 2011).

Many studies have demonstrated that exercise can play a significant role in accelerating the rate of mitochon-

drial biogenesis in skeletal muscle (Hood and Saleem, 2007; Wright et al., 2007). In contrast, little is known regarding likely benefits of increased brain mitochondria in this regard. In this year, it is the first time that the study showed exercise-induced increases in brain mitochondrial biogenesis (Steiner et al., 2011). Based upon the observations derived from the studies of skeletal muscle and brain, we investigated whether exercise may also affect brain mitochondrial biogenesis using an ischemic rat model. We hypothesized that treadmill training would promote brain recovery from ischemia induced injury through regulation of mitochondrial biogenesis. We analyzed cortical mitochondrial DNA (mtDNA), expression of mitochondrial-specific transcription factors, protein levels, and histology of treadmill-trained rats after ischemic injury caused by middle cerebral artery occlusion (MCAO).

EXPERIMENT PROCEDURE

Animals and experimental groups

All procedures were performed according to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This study involved 36 healthy male Sprague–Dawley rats (weighing 250–280 g) provided by the Shanghai Laboratory Animal Center, Chinese Academy of Sciences. The rats were housed under a 12-h light–12-h dark cycle with free access to food and water. Animals were divided into three experimental groups. Animals in the ischemia-exercise group exercised on a rat treadmill (DSPT-202 Type 5-Lane treadmill; Litai Biotechnology Co., Ltd., China) from post-operation day 1 to post-operation day 7, for a total of 7 days at 30 min per day. On the first and second exercise day the treadmill velocity was 5 m/min for the first 10 min, 9 m/min for the second 10 min, 12 m/min for the last 10 min, and 12 m/min on the third and subsequent days. The tilt angle was 0°. Animals in the sham control group and ischemia-non-exercise group were housed freely in their cages.

Animal model

The experimental protocol was approved by the Animal Experimental Committee of Fudan, University at Shanghai, China. All groups were anesthetized intraperitoneally with chloral hydrate (350 mg/kg). Body temperature was maintained at 37 °C using a heating pad. The right middle cerebral artery occlusion was produced by the intraluminal suture technique as previously described (Longa et al., 1989). After anesthesia, the rat left common carotid arteries were exposed. The external carotid artery was ligated and a small incision was made. A 20-mm long surgical suture (its tip rounded by heating) was inserted into the internal carotid artery so as to occlude the middle cerebral artery, which was held to the external carotid artery with a thread. The rats in the sham group underwent the same procedure except for the occlusion of the middle cerebral artery. Cerebral ischemia through the intraluminal suture was maintained for 60 min, followed by removal and reperfusion. In addition, all measures were taken to minimize the animals' pain and distress during the experiments. Rats were scored based on a five-point scale (Longa et al., 1989). The scale ratings were as follows: 0, no neurological symptoms; 1, unable to completely extend the front jaw on the other side; 2, rotating while crawling and falling to the contralateral side; 3, unable to walk without help; and 4, unconsciousness. Rats with a score of 1–3 points were considered a successful model and included in the study. A portion of the ipsilateral cortex was collected at the indicated time and used for sample preparation.

Long fragment PCR and mtDNA quantification

Long fragment polymerase chain reaction (PCR) was used to quantify the relative abundance of intact mtDNA as previously described (Chen et al., 2001). DNA derived from collected cortex from rat brain or normal mouse brain (internal standard) was purified and linearized by digestion with the restriction enzyme SacII (Takara Bio Inc., Shiga, Japan). As an internal standard, mouse DNA was added to the PCR reaction mixture for each sample. The primers used for the amplification of 14.3 kbp mitochondrial genomes for both rat and mouse are shown in Table 1. The PCR products were digested with NcoI (Takara Bio Inc., Shiga, Japan). The rat mtDNA was 14.3 kbp. The mouse mtDNA contained an NcoI restriction site with 7.0 and 7.3 kbp products that migrated as a single band on 1% agarose gel. Band densitometry was semiquantitatively analyzed using ImageJ software.

RNA isolation and real-time RT-PCR

Total RNA was isolated from the collected frozen cortical samples using Trizol according to the manufacturer's instructions. A 2–3 μg template RNA was used to synthesize the first strand of cDNA using a reverse transcription kit purchased from Takara. Real-time PCR of cDNA was performed (ABI PRISM 7500 Sequence Detection System, Applied Biosystems) using the forward and reverse primer sequences provided in Table 1. Data were analyzed using a comparative critical threshold (Ct) method where the amount of target normalized to the amount of endogenous control and relative to the control sample was determined by the $2^{-\Delta\Delta}$Ct method.

Western blotting

Ipsilateral cortical tissue was harvested 3 and 7 days after MCAO. Protein extracts and Western blotting were performed as previously described (DiFiglia et al., 1995). Equal amounts of protein extracts were separated through 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer onto Polyvinylidene-Fluoride membranes. Polyclonal antibodies were used for the detection of TFAM (Santa Cruz Biotechnology), PGC-1 (Santa Cruz Biotechnology), NRF-1 (Abcam), COXIV (Cell Signaling Technology), HSP60 (Cell Signaling Technology), and GAPDH (Cell Signaling Technology). Quantification of band intensity (optical density) was carried out on scanned Western blot images using ImageJ software from blots of independent experiments.

Table 1. Primer sequences used for PCR

Gene	Forward primer	Reverse primer
PGC-1	GTGCAGCCAAGACTCTGTATGG	GTCCAGGTCATTCACATCAAGTTC
NRF-1	TTACTCTGCTGTGGCTGATGG	CCTCTGATGCTTGCGTCGTCT
TFAM	GAAAGCACAAATCAAGAGGAG	CTGCTTTTCATCATGAGACAG
GAPDH	GGGTCAGAAGGATTCCTATG	GGTCTCAAACATGATCTGGG
mtDNA	ATATTTTCACTGCTGAGTCCCGTGG	AATTTCGGTTGGGGTGACCTCGGAG

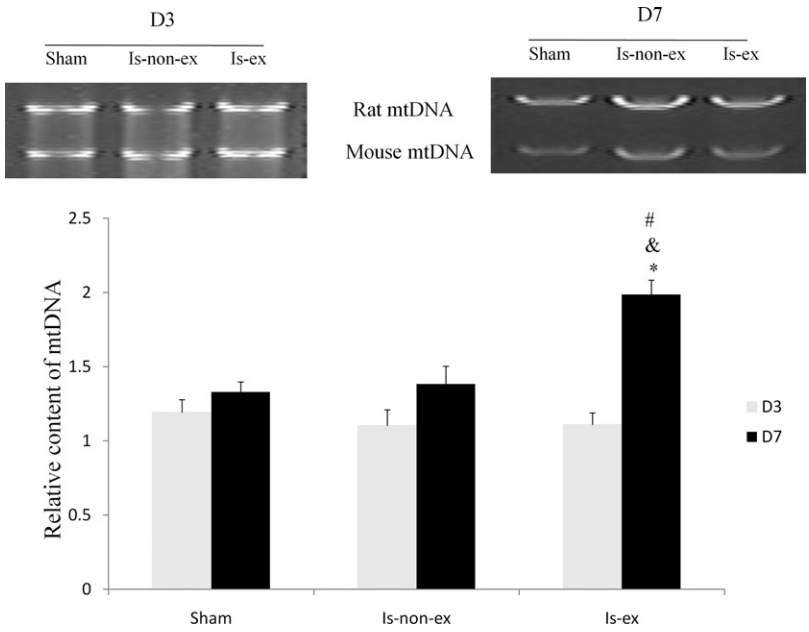

Fig. 1. Mitochondrial DNA content after exercise. The relative amount of cortical mtDNA isolated from lesioned and sham ipsilateral cortex at various training times after MCAO were measured semiquantitatively using long fragment PCR (upper). The fold change of rat mtDNA over internal controls was averaged and graphed ±SD (lower). Mouse mtDNA was used as the internal control. The mtDNA content between the ischemia-exercise group (Is-ex) and ischemia-non-exercise group (Is-non-ex) was not significantly different after 3 d of exercise (D3), but changed significantly after 7 d of exercise (D7). * $P<0.05$ vs. the respective ischemia-non-exercise group, # $P<0.05$ vs. the respective sham group, & $P<0.05$ vs. the ischemia-exercise group after 3 d of exercise, $n=6$.

Evaluation of behavioral score

Behavioral tests were performed in rats after 60 min of ischemia followed by 7 days of exercise. Each rat was scored based on a five-point scale according to a previously described method (Longa et al., 1989).

Measurement of cerebral infarction volume

After 7 days of exercise, rats were killed for infarction volume analysis. Whole brains were quickly removed and frozen at −20 °C for 30 min. The brains were cut into seven 2-mm coronal sections. The first cut was at the midpoint between the anterior pole and the optic chiasm. Slices were stained in 2% triphenyltetrazolium chloride (TTC) solution at a temperature of 37 °C for 30 min and then fixed with 40 g/L formalin for 24 h. The infarct size was determined using NIH image analyzer software (NIH, USA).

Statistical analysis

All values are reported as mean±SD. Multiple comparisons between groups were determined by using one-way ANOVA followed by Tamhane multiple comparison post hoc tests. Differences were considered statistically significant at a level of $P<0.05$.

RESULTS

Effect of exercise on the amount of mtDNA

The total amount of mtDNA is a correlate of mitochondrial biogenesis. To determine whether exercise stimulated an increase in this measure, we used long fragment PCR to measure mtDNA content in the MCAO-lesioned cerebral cortex. Mouse mtDNA was used as an internal amplification standard between samples. Rats were exercised with treadmill training beginning 24 h after MCAO, and cerebral cortices were collected after 3 and 7 days of training. There was no significant difference in amplification of the rat mtDNA between the ischemia-3-day exercise group and the ischemia-non-exercise group (Fig. 1). However, in the ischemia-7-day exercise group, mtDNA content significantly increased compared with the ischemia-non-exercise group (Fig. 1). The observed increase in relative mtDNA content suggests that exercise after MCAO may induce mitochondrial biogenesis.

Expression of mitochondrial biogenesis factors

To investigate the molecular mechanisms that might be responsible for an exercise-dependent increase in mitochondrial biogenesis after MCAO, we examined mRNA levels of three transcription factors considered essential for mitochondrial gene expression in mammals. PGC-1 regulates the entire mitochondrial biogenesis program (Hood and Saleem, 2007). NRF-1, the transcriptional partner of PGC-1 controls the expression of TFAM, which is a critical transcription factor for the regulation of mitochondrial gene transcription and DNA replication (Ljubicic et al., 2010). PGC-1 mRNA expression levels were elevated above sham control animals after both 3 days and 7 days of exercise training, whereas ischemia-non-exercised animals demonstrated elevated PGC-1 mRNA levels only at day 3 (Fig. 2). Interestingly, both groups demonstrated a decrease at 7 days compared with their respective day 3 levels, although the exercise treated animals still remained above their sham controls. This suggested that the exercised treated animals may have benefitted from a partial

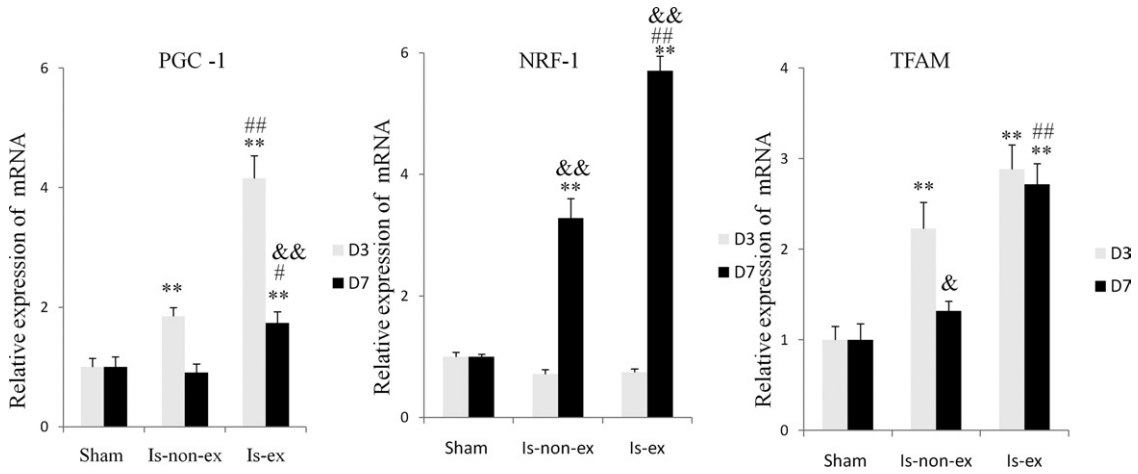

Fig. 2. mRNA expression of mitochondrial biogenesis factors in the cortex. Quantitative real-time RT-PCR was performed from RNA isolated from lesioned ipsilateral cortex. The graphs indicate average values ±SD from the sham group, ischemia-exercise group (Is-ex), and ischemia-non-exercise group (Is-non-ex) at the indicated times. D3, 3 d of exercise after ischemia; D7, 7 d of exercise after ischemia. ** $P<0.01$ vs. respective sham group, # $P<0.05$, ## $P<0.01$ vs. respective Is-non-ex group, & $P<0.05$, && $P<0.01$ vs. D3 of Is-non-ex or Is-ex group; $n=6$.

protection against a temporal decrease in the endogenous downregulation of PGC-1 mRNA after ischemia. NRF-1 mRNA levels increased in both groups only at day 7 versus sham controls although the ischemia-exercise group had significantly higher levels than the respective ischemia-non-exercise 7-day group (Fig. 2). Although TFAM mRNA expression changes were increased above sham controls in both groups at day 3, the levels in the ischemia-non-exercise group decreased back to control levels at day 7 but remained high in the ischemia-exercise group, again demonstrating protection against a temporal decrease in mRNA during the endogenous response to ischemia (Fig. 2). These results support the idea that mitochondrial biogenesis may be stimulated by exercise after MCAO. Moreover, the effects of exercise on mitochondrial biogenesis-related mRNA levels appeared to be temporally regulated in an mRNA-specific fashion.

To gain additional evidence for exercise-induced generation of mitochondria after MCAO, we next investigated whether any changes occurred in the expression level of several proteins related to mitochondria including the transcription factors examined above. Consistent with the mRNA findings, protein levels of the three transcription factors displayed the same temporal dependence (Fig. 3). Specifically, PGC-1 protein levels were upregulated after 3 days of treadmill training, whereas 7 days of training was required for protein levels of NRF-1 and TFAM to increase above non-exercise MCAO controls. COXIV, a mitochondrial-specific protein, is detected at high levels in mitochondria. HSP60 is also typically enriched in mitochondria (Gupta and Knowlton, 2002). Therefore, we examined brains for changes in COXIV and HSP60 levels as an indirect assessment of mitochondrial biogenesis. Cortical COXIV and HSP60 protein levels were elevated significantly after 7 days of exercise training compared with ischemia-non-exercise controls (Fig. 4). These protein differences supported our observed temporal changes in mRNA that were induced by treadmill exercise training following MCAO. More importantly, both the mRNA and protein data indicated that PGC-1 expression changed earlier than the other proteins examined suggesting that PGC-1 activity may regulate expression of NRF-1 and TFAM.

Behavioral scores and cerebral infarct volume

To determine whether the observed increases in mitochondrial proteins and mtDNA associated with changes in behavioral recovery and lesion volume we next evaluated sham rats, ischemia-exercise and ischemia-non-exercise groups, at 7 days of exercise after ischemia. There was a significant difference in behavioral scores between the ischemia-exercise group and ischemia-non-exercise group ($P<0.05$) (Table 2). The ischemia-exercise group demonstrated fewer neurologic deficits compared with the ischemia-non-exercise group. No neurological symptoms were observed in the sham group.

After behavioral evaluation, the rats were sacrificed to compare infarct volumes with the behavioral performances. Similar to the results of behavioral score evaluation, the brain ischemic area in the ischemia-exercise group was reduced significantly relative to that observed in the ischemia-non-exercise group at day 7 (Fig. 5). However, there was a significant reduction of the ischemic area in both groups at 7 days compared with their respective 3-day time points, suggesting that exercise potentiated an endogenous temporal repair response at 7 days. None of the rats in the sham group exhibited ischemic areas.

DISCUSSION

To investigate the possible mechanisms contributing to functional improvement resulting from exercise after ischemic injury, we analyzed the relationship between exercise and mitochondrial biogenesis using a rat MCAO model. Exercise resulted in a temporal increase in mtDNA, mRNA and protein levels of mitochondrial-specific transcription

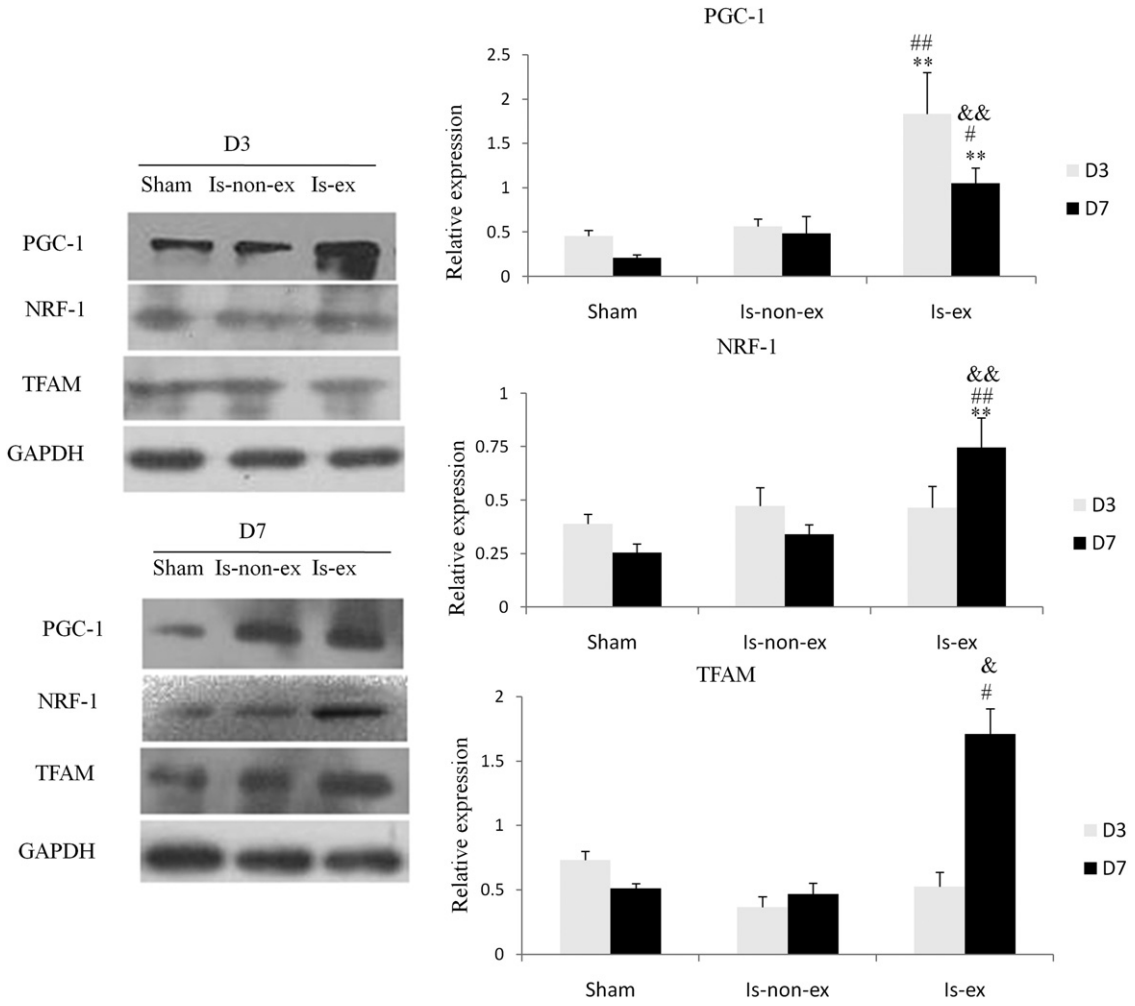

Fig. 3. Protein expression of mitochondrial biogenesis factors in the cortex. Ipsilateral cortex from sham and lesioned animals were collected for SDS-PAGE and Western blot analysis. Shown are representative Western blots for PGC-1, NRF-1, and TFAM from the sham group, ischemia-non-exercise group (Is-non-ex), and ischemia-exercise group (Is-ex) at various time points after exercise. GAPDH was used as a loading control. Optical density values normalized to their respective GAPDH loading control then averaged ±SD and graphed (relative expression) to semiquantitatively compare protein levels. D3, 3 d of exercise after ischemia; D7, 7 d of exercise after ischemia. ** $P<0.01$ vs. respective sham group, # $P<0.05$, ## $P<0.01$ vs. respective Is-non-ex group, & $P<0.05$, && $P<0.01$ vs. D3 of Is-non-ex or Is-ex group; $n=6$.

factors, and the mitochondrial proteins COXIV and HSP60. These exercise-induced changes in mitochondrial assessment followed with improved behavioral performance and reduced lesion volume compared with non-exercised ischemic controls. The results indicated that exercise might promote mitochondrial biogenesis after ischemic injury as a novel component of exercise-induced repair mechanisms in the brain.

Prior studies have indicated that exercise is particularly helpful in recovery following brain ischemia. Particularly, exercise has been shown to correlate with reduced infarct volume, improved neurologic function, and induction of neurogenesis (Pyoria et al., 2007; Langhammer et al., 2007; Gertz et al., 2006). However, the optimal time window for exercise intervention post ischemia has been debated for some time. Some studies indicate that early training after an ischemic event may be helpful in repairing damage (Ohlsson and Johansson, 1995), whereas others found that early training had a negative effect on recovery (Risedal et al., 1999). In 2003, Yang and coworkers (Yang et al., 2003) investigated the effects of early and late treadmill training after injury, and demonstrated that exercise starting 24 h after ischemia promoted functional improvements without increasing neuronal tissue loss. Based upon this prior study, we focused on exercise-induced recovery mechanisms occurring as early as 24 h following the initiation of exercise training following MCAO in our rat model. Therefore, we cannot exclude the possibility that delayed exercise training might offer improved or alternative effects on mitochondrial biogenesis and lesion volume and behavioral performance. Investigating the most beneficial time to start exercise training following post ischemia may be useful for future work. It is also important to point out that the changes we observed in mitochondrial parameters increasing after 3 or 7 days of exercise demonstrating that regardless of training initiation period there may be some critically important sequence of changes that must occur during any exercise-dependent recovery mechanism.

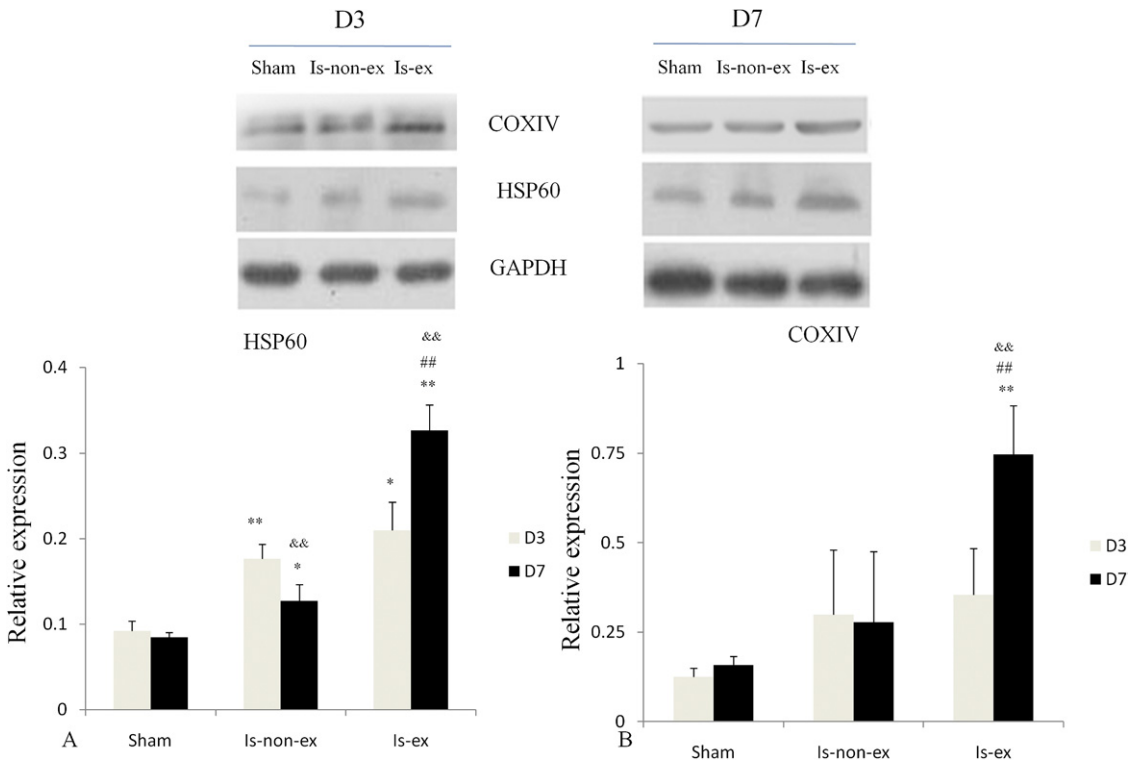

Fig. 4. Mitochondria protein expression in the cortex. Ipsilateral sham and lesioned cortex were collected for SDS-PAGE and Western blot analysis. A representative Western blot for mitochondria protein COXIV and HSP60 from the different groups is shown. Optical density values normalized to their respective GAPDH loading control were averaged ±SD and graphed (relative expression). Semiquantitative analysis demonstrated increased COXIV and HSP60 levels after 7 d of exercise. D3, 3 d of exercise after ischemia; D7, 7 d of exercise after ischemia. * $P<0.05$, ** $P<0.01$ vs. respective sham group, ## $P<0.01$ vs. respective Is-non-ex group, && $P<0.01$ vs. D3 of Is-non-ex or Is-ex group; $n=6$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

The mitochondria are key organelles responsible for producing ATP for normal function and also serve a role in mediating specific cell death pathways (Onyango et al., 2010). Thus, there is a rationale for improving or preserving mitochondrial function following a variety of insult or degenerative paradigms. One such possibility is mitochondrial biogenesis, which is defined simply as the growth and division of mitochondria. Mitochondrial biogenic programs have been found to augment tolerance to cardiac ischemia and have been suggested as new targets for therapeutic interventions to treat ischemic heart disease (McLeod et al., 2005). In addition, numerous studies support the hypothesis that disruption of mitochondrial function plays a central role in the pathophysiology of many neurological diseases, and adaptive mitochondrial biogenesis has been investigated in the nervous system (Sims and Anderson, 2002; Fiskum et al., 2003; Vosler et al., 2009). Stroke also induces decreased mitochondrial capacity for respiratory activity, which results in impairment of energy metabolism (Sims and Anderson, 2002). However, mitochondrial biogenesis can likely minimize the effects of ischemia-induced damage to mitochondria through increasing the

Table 2. Behavioral scores

Scores	Ischemia-non-exercise group	Ischemia-exercise group
D3	3.17±0.75	2.83±0.76
D7	3.00±0.63	2.17±0.41*

Behavioral scores were determined and the averaged ±SD. D3, 3 days of exercise after ischemia; D7, 7 days of exercise after ischemia. * $P<0.05$ vs. Is-non-ex group of D7; $n=6$.

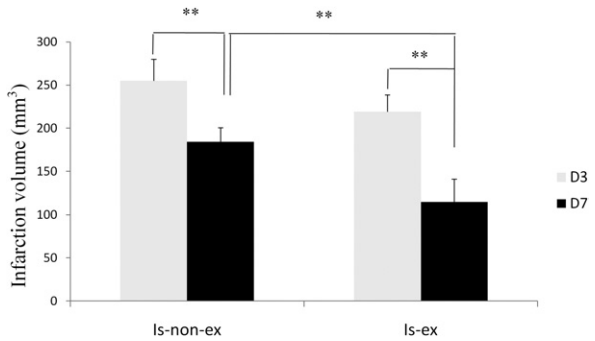

Fig. 5. Cerebral infarct volume. After 3 or 7 d with or without exercise following MCAO, animals were killed for infarction volume analysis. Brains were sectioned and stained with 2% triphenyltetrazolium chloride to quantify lesion volume. Optical density of infarct volume from serial sections from each group was determined and the averaged ±SD were graphed. The infarct volume of the sham group was zero (not shown). The ischemia-exercise group (Is-ex) demonstrated significantly lower infarct volume compared to the ischemia-non-exercise group (Is-non-ex). ** $P<0.01$, $n=6$.

number of mitochondria themselves. Mitochondrial biogenesis has been described in the context of cerebral hypoxic preconditioning (Gutsaeva et al., 2008) and neonatal hypoxia-ischemia (H-I) (Yin et al., 2008). These studies report that an increase in the number of mitochondria, levels of mitochondrial related factors, and citrate synthase activity occurred 24 h after H-I. Other recent studies indicate that preventing a reduction in mitochondrial number may be beneficial in reducing infarct size caused by MCAO (Vosler et al., 2009). Collectively, prior work as well as our current data indicate that improving or preserving mitochondrial biogenesis could help attenuate any detrimental consequence of ischemia on mitochondrial function or contribution to neuron death and serve instead as a novel component of the brain repair mechanisms.

We chose treadmill exercise as a stimulus for promoting mitochondrial biogenesis based on the fact that a positive relationship has been confirmed between exercise and mitochondrial biogenesis not only in skeletal muscle but also in brain (Lanza and Sreekumaran, 2010; Steiner et al., 2011; Bayod et al., 2011). In 2011, the effect of exercise training on brain mitochondrial biogenesis has been clearly demonstrated (Steiner et al., 2011; Bayod et al., 2011). These works suggest exercise training is a stimulus to increase markers of brain mitochondrial biogenesis. Exercise can play a significant role in accelerating the rate of mitochondrial biogenesis and likely serves to attenuate mitochondrial dysfunction. Based upon these observations, we hypothesized that a similar regulatory relationship also exists in the brain that could be exploited particularly during brain repair processes. Our data acquired using the rat MCAO model lends credence to this possibility. We found that mtDNA content changed significantly after 7 days of training and mitochondrial gene expression was increased after 3 and 7 days of training. PGC-1 levels were significantly increased in animals collected on the third day of exercise training, whereas the other changes were only significant after 7 days of training. It is interesting to note that in the case of both PGC-1 and TFAM the exercise-dependent benefits were superimposed upon an endogenous decrease in both mRNAs from days 3–7 regardless of treatment, and exercise appeared to attenuate the normal increase at 3 days followed by a decline at 7 days. In spite of these mRNA changes, protein analysis indicated that only the exercise-treated groups demonstrated significant changes from sham animals at either time point. As PGC-1 protein levels were upregulated before the other two transcription factors it is possible that initial PGC-1 activity regulates subsequent expression changes of NRF-1 and TFAM. Indeed, this conclusion correlates well with other studies demonstrating that mitochondrial synthesis is stimulated by the PGC-1–NRF-1–TFAM pathway (Viña et al., 2009; Ljubicic et al., 2010). Similar to our findings, in this response pathway PGC-1 activity is implicated as the initiating transcriptional event required to stimulate mitochondrial biogenesis. NRF-1 is an intermediate transcription factor whose activity then stimulates the synthesis of TFAM, a final effector activity that is necessary for the duplication of mitochondrial DNA. Besides the temporal changes in transcription factor expression and mtDNA, we also observed that levels of the mitochondrial-specific protein COXIV and mitochondrial-enriched protein HSP60 increased after 7 days of exercise, again supporting the idea that mitochondrial biogenesis was stimulated by training. When examining infarct volumes of the groups, we not only found ischemic area reduced in the ischemia-exercise versus the ischemia-non-exercise groups at 7 days, but also found that both groups appeared to have an endogenous decrease in ischemic area from 3 to 7 days that was potentiated in the exercise groups. This decrease in volume correlated with improvement of behavior. Based upon the fact that our evidence of exercise-associated mitochondrial biogenesis accompany well with an exercise-associated decrease in lesion volume and improved behavioral performance, we propose that biogenesis is contributing directly to improved recovery through preservation of mitochondrial function and attenuation of any detrimental contributions of mitochondria dysfunction to cell death.

In conclusion, we have demonstrated that treadmill exercise initiated early can induce a temporal increase in expression of mitochondrial factors and result in mitochondrial biogenesis after ischemic brain injury, indicating that exercise may benefit the brain recovery from ischemia-induced injury through regulation of mitochondrial biogenesis. Further efforts to understand the mechanisms regulating exercise-induced mitochondrial biogenesis will help clarify the complexities of exercise-associated neuroprotection. In addition, our data further supports the notion that stimulation or enhancement of mitochondrial biogenesis may be a novel neuroprotective strategy in the future.

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