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To cite this article: Fengwu Li, John T. Pendy Jr., Jessie N. Ding, Changya Peng, Xiaorong Li, Jiamei Shen, Sainan Wang & Xiaokun Geng (2017): Exercise rehabilitation immediately following ischemic stroke exacerbates inflammatory injury, Neurological Research, DOI: [10.1080/01616412.2017.1315882](https://doi.org/10.1080/01616412.2017.1315882)

To link to this article: <http://dx.doi.org/10.1080/01616412.2017.1315882>



Published online: 17 Apr 2017.



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## Exercise rehabilitation immediately following ischemic stroke exacerbates inflammatory injury

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### ABSTRACT

**Objectives:** The rehabilitative benefits of physical exercise after stroke appear to be contingent upon exercise initiation timing. The present study assessed the hypothesis that very early post-stroke exercise would amplify cellular stress and increases expression of pro-inflammatory mediators, while exercise initiated later would limit the inflammation associated with cerebral ischemia/reperfusion injury.

**Methods:** Adult rats were subjected to middle cerebral artery occlusion and subsequently assigned to one of seven groups: one sham injury control group, three stroke groups subjected to exercise initiated after 6, 24 hours, or 3 days of reperfusion, and three stroke groups not subjected to exercise. Expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-1 $\beta$  (IL-1 $\beta$ ) were examined 3 and 24 hours after completion of exercise regimens (and at corresponding time points in non-exercise controls). Heat shock protein-70 (Hsp70) and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) expression levels were also compared between exercise and non-exercise groups.

**Results:** Early post-stroke exercise was associated with increased expression of pro-inflammatory mediators (ICAM-1, VCAM-1, TNF- $\alpha$ , and IL-1 $\beta$ ) and increased expression of cell stress markers (Hsp70 and HIF-1 $\alpha$ ). Exercise initiated after 3 days of reperfusion was associated with decreased expression of these molecules.

**Conclusion:** Post-stroke exercise, if too early, may result in elevated levels of cell stress and increased expression of pro-inflammatory cytokines, which may amplify the tissue damage associated with cerebral ischemia/reperfusion injury. The results shed light on the manner in which exercise initiation timing may affect post-stroke rehabilitation.

### ARTICLE HISTORY

Received 6 December 2016  
Accepted 23 March 2017

### KEYWORDS

Ischemia/reperfusion injury; rehabilitation; ICAM-1; VCAM-1 TNF- $\alpha$ ; IL-1 $\beta$ ; Hsp70; HIF-1 $\alpha$

### Introduction

Approximately 6.6 million Americans  $\geq$  20 years of age have suffered from a stroke, and each year 795,000 people will experience new or recurrent stroke [1,2]. Due to the substantial burden on the healthcare system, a vast body of literature has focused on optimal stroke rehabilitation strategies [3–5]. Of increasing interest are rehabilitation regimens involving exercise, promising avenues of stroke recovery that appear to act by minimizing post-stroke motor impairments and cognitive damage. However, the specific roles of factors such as initiation time, intensity, and type of exercise have yet to be thoroughly investigated. Indeed, in a statement to health care professionals, the American Heart Association/American Stroke Association suggested that “there remains a great need for additional experimental studies to be performed during the acute stages of stroke recovery to establish whether the use of higher doses of physical activity commenced

early after stroke slows or prevents loss of cardiorespiratory fitness and to develop detailed recommendations for frequency, intensity, time, and type of exercise to be prescribed to this population”.[6]

To date, some animal studies have suggested that exercise may have a beneficial effect on stroke recovery if initiated as early as 24 hours after stroke onset [7–10], while others have indicated that early training actually exacerbates brain damage [11–13]. These conflicting results highlight the importance of optimizing post-stroke exercise initiation timing, not only to perform higher quality mechanistic studies in animal models, but to ultimately facilitate effective stroke recovery in the clinic.

The molecular underpinnings of inflammatory injury following cerebral ischemia have been well established. Brain inflammation during reperfusion is believed to play a pivotal role in the development of secondary brain

injury by intensifying both the accumulation of inflammatory cells and microvascular dysfunction [14–17]. Pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) are at the heart of these inflammatory reactions in brain tissue, and cerebral ischemia results in increased expression of TNF- $\alpha$  and IL-1 $\beta$  in neurons, astrocytes, microglia, polymorphonuclear (PMN) leukocytes, and blood vessels [18–21].

A previous study by our group has indicated that pre-ischemic exercise reduces brain inflammation in rats, a conclusion based on endothelial downregulation of intercellular adhesion molecule 1 (ICAM-1, a cell surface glycoprotein) as well as decreased numbers of infiltrating leukocytes in frontoparietal cortex and dorsolateral striatum [22]. This result suggests that pre-ischemic exercise is neuroprotective. Additionally, our work demonstrates that very early post-stroke exercise results in increased cellular apoptosis and infarct volumes [23]. However, the molecular mechanisms underlying these changes have not been well established. Thus, the aim of the present study was to examine the effect of post-stroke exercise initiation timing on expression of pro-inflammatory cytokines and markers of cell stress, such as heat shock protein 70 (Hsp70) and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), in brain tissue following a two-hour middle cerebral artery (MCA) occlusion. Hsp was reported to act as molecular chaperones with neuroprotective activities under physiological conditions [24]. More specifically, Hsp70 displays changes in expression in response to various types of stress (including hypoxia), and is associated with cellular resistance to a variety of insults in the brain [24]. Further, an interesting regulatory link exists between HIF-1 $\alpha$  and Hsp70. Thus, the present study was to determine whether post-stroke exercise leads to brain inflammation, which may provide insight into the mechanisms by exercise after stroke.

## Materials and methods

### Experimental design

A total of 104 adult male Sprague-Dawley rats (280–300 g, Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were used in this study. The protocol was approved by the Animal Care and Use Committee of the Capital Medical University, and the study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (USA). Animals were randomly divided into seven groups: one sham injury control group ( $n = 8$ ), three stroke groups subjected to exercise initiated after either 6 hours ( $n = 16$ ), 24 hours ( $n = 16$ ), or 3 days ( $n = 16$ ) of reperfusion, and three corresponding stroke groups not subjected to post-stroke exercise ( $n = 48$ ). Animals in the exercise groups were sacrificed 3 hours ( $n = 8$  per exercise time point) or 24 hours ( $n = 8$  per

exercise time point) after completion of exercise regimens, and non-exercise animals were sacrificed at corresponding time points for comparison.

### Focal cerebral ischemia

This model has been described previously by us [25]. Briefly, rats were initially anesthetized in a chamber with 1–3% isoflurane and a mixture of 70% nitrous oxide and 30% oxygen. The rats were transferred to a surgical table, and anesthesia was maintained with a facemask using 1% isoflurane delivered from a calibrated precision vaporizer. Poly-L-lysine-coated intraluminal nylon (4.0) sutures were used to yield consistent infarcts, greatly reducing inter-animal variability. During the unilateral, two-hour MCA occlusion procedure, blood pCO<sub>2</sub> and pO<sub>2</sub>, mean arterial pressure, and rectal temperature were monitored continuously. Rectal temperatures were maintained between 36.5 and 37.5 °C using a circulating heating pad and a heating lamp.

### Post-stroke exercise

The rotarod (R03-1; Xin Ruan Instruments, Inc., Shanghai, China) was used as a training platform. The rotarod is a horizontally oriented, mechanically driven cylinder upon which the animals were forced to run. The rod is 7 cm in diameter, 11 cm in length, and covered with smooth rubber. Animals performed 30 minutes of rotarod exercise (speed incrementally increased from 5 to 35 rpm over the 30 minutes period) initiated after 6, 24 hours, or 3 days of reperfusion. The apparatus delivered an electric shock (0.1 mA, 3 seconds) to animals that fell from the rotating cylinder, forcing the animals to exercise continuously for the duration of the exercise period. During the study, animals only occasionally fell from the rod and did not suffer from any additional injuries. All rats were required to perform rotarod training at a constant speed (15 rpm) for 20 minutes/day for 3 days prior to MCA occlusion. Both exercise and non-exercise animals were housed in groups of three in standard cages for equal amounts of time.

### Protein expression

Rats were sacrificed 3 and 24 hours following exercise regimens (and at equivalent time points in non-exercise controls), and Western blot analysis was used to examine protein expression at these time points in MCA-supplied tissue, as described previously [26]. Proteins extracted from rat brain were loaded onto SDS-polyacrylamide gel for electrophoresis. Upon conclusion of electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane. Membranes were incubated with primary antibody (ICAM-1 goat polyclonal IgG, VCAM-1 rabbit polyclonal IgG, TNF- $\alpha$  goat polyclonal IgG, Santa Cruz Biotechnology, IL-1 $\beta$  goat polyclonal IgG, Hif-1 $\alpha$

rabbit polyclonal IgG, Santa Cruz Biotechnology, Inc. CA, USA; and Anti-HSP70 rabbit polyclonal IgG, Enzo Life Science, Farmingdale, NY, USA) for 24 hours at 4 °C. Membranes were then washed three times with phosphate-buffered saline (6 minutes/wash), and re-incubated in secondary antibody (donkey anti-goat IgG-HRP, or goat anti-rabbit IgG-HRP, Santa Cruz Biotechnology, Inc.) for 1 hours at room temperature. An enhanced chemiluminescence system was used to detect immunoreactive bands. Western blot images for each antibody were analyzed using an image analysis program (ImageJ 1.42, National Institutes of Health, Bethesda, MD, USA) to quantify protein expression in terms of relative image density. Mean protein expression of the sham injury control group was assigned a reference value of 1.

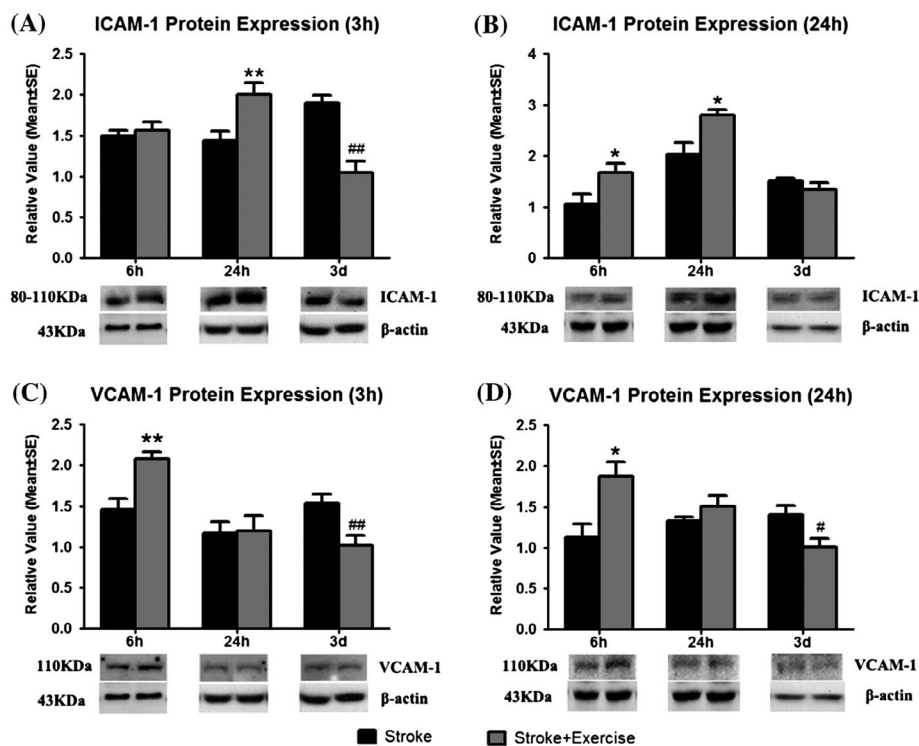
### Statistical analysis

Statistical analyses were performed with SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). Differences among groups were assessed using one-way ANOVA with a significance level of  $P < 0.05$ . Post hoc comparison among groups was performed using the least significant difference method.

## Results

### ICAM-1 and VCAM-1

As compared to non-exercise groups, increases in ICAM-1 expression were seen in the very early (6 hours) exercise groups at 3 hours (slight increase) and 24 hours ( $F_{(6,5)} = 5.054$ ;  $P < 0.05$ ) after completion of exercise (Figure 1(A),(B)). In the early (24 hours) exercise groups, significant increases in ICAM-1 expression were seen both 3 hours ( $F_{(6,6)} = 1.725$ ;  $P < 0.01$ ) and 24 hours ( $F_{(6,5)} = 5.054$ ;  $P < 0.05$ ) (Figure 1(A),(B)). For the late (3 days) exercise group, Western blot analysis demonstrated a significant reduction in ICAM-1 protein expression ( $F_{(6,6)} = 2.591$ ;  $P < 0.01$ ) 3 hours after exercise completion (Figure 1(A)), and a slight decrease in ICAM-1 expression 24 hours after exercise completion (Figure 1(B)). Similar to the results of ICAM-1, increases in VCAM-1 expression were seen in the very early (6 hours) exercise groups both 3 hours ( $F_{(6,6)} = 2.461$ ;  $P < 0.01$ ) and 24 hours ( $F_{(6,6)} = 1.234$ ;  $P < 0.05$ ) after completion of exercise regimens (Figure 1(C),(D)). In the early (24 hours) exercise groups, VCAM-1 expression was slightly increased both 3 and 24 hours after completion of exercise (Figure 1(C),(D)). Further, VCAM-1



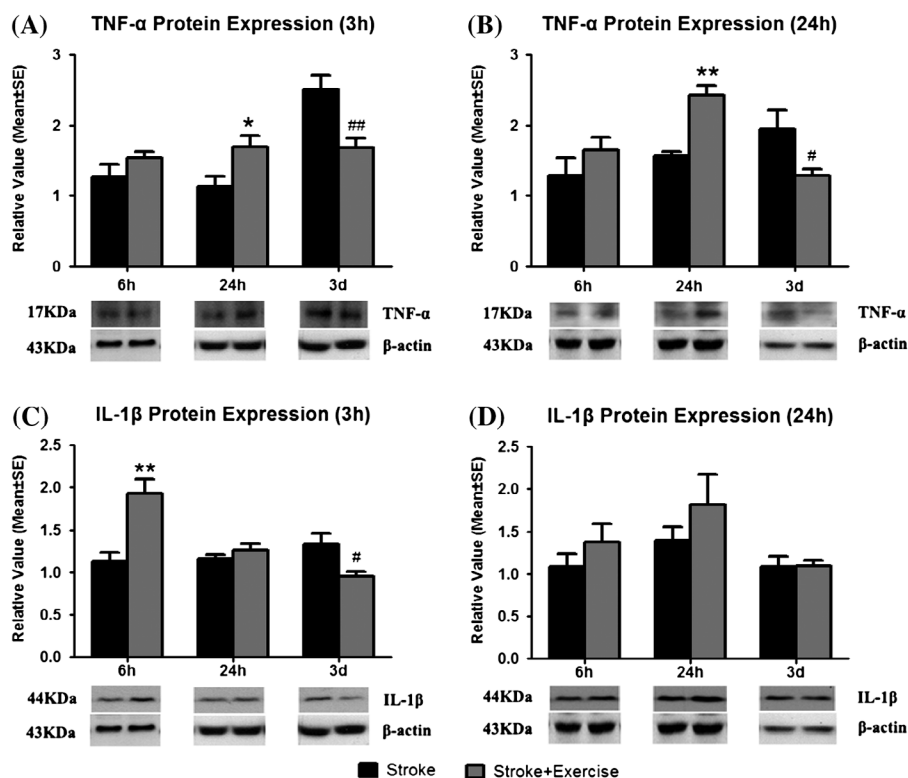
**Figure 1.** (A) ICAM-1 protein expression 3 hours after exercise completion was increased compared to non-exercise groups after 6 hours (slightly) and 24 hours (significantly,  $**P < 0.01$ ) of reperfusion. In contrast, a significant decrease ( $##P < 0.01$ ) in ICAM-1 expression was seen with exercise initiated after 3 days of reperfusion. (B) A significant increase ( $*P < 0.05$ ) in ICAM-1 protein expression 24 hours after exercise termination was observed in the very early (6 hours) and early (24 hours) exercise groups, while a slight reduction was seen in the late (3 days) exercise group. (C) VCAM-1 protein expression 3 hours after exercise completion was significantly increased ( $**P < 0.01$ ) compared to non-exercise groups after 6 hours of reperfusion, and slightly increased after 24 hours of reperfusion. In contrast, a significant decrease ( $##P < 0.01$ ) in VCAM-1 expression was seen with exercise initiated after 3 days of reperfusion. (D) In animals sacrificed 24 hours after completion of exercise, VCAM-1 protein expression was significantly increased ( $*P < 0.05$ ) in the very early (6 hours) exercise group and slightly increased in the early (24 hours) exercise group. A significant reduction ( $#P < 0.05$ ) in VCAM-1 expression was seen in the late (3 days) exercise group. Representative immunoblots are shown for all comparisons.

expression was significantly decreased in the late (3 days) exercise groups both 3 hours ( $F_{(6,5)} = 1.336$ ;  $P < 0.01$ ) and 24 hours ( $F_{(5,6)} = 1.082$ ;  $P < 0.01$ ) after exercise (Figure 1(C),(D)). Together, these results seem to suggest that very early (6 hours) and early (24 hours) post-stroke exercise promote increased expression of cell adhesion molecules, while late (3 days) exercise limits expression of these molecules.

### TNF- $\alpha$ and IL-1 $\beta$

We compared expression levels of TNF- $\alpha$  and IL-1 $\beta$ , pro-inflammatory cytokines between stroke only (non-exercise) groups and stroke + exercise groups. In the very early (6 hours) exercise group, slightly increased TNF- $\alpha$  expression was seen both 3 and 24 hours after completion of exercise regimens (Figure 2(A),(B)). In the early (24 hours) exercise group, TNF- $\alpha$  expression was significantly increased 3 hours ( $F_{(6,6)} = 1.134$ ;  $P < 0.05$ ) and 24 hours ( $F_{(6,6)} = 6.350$ ;  $P < 0.01$ ) after completion of exercise regimens

(Figure 2(A),(B)). Additionally, there was significantly reduced TNF- $\alpha$  expression in the late (3 days) exercise group 3 hours ( $F_{(6,6)} = 2.054$ ;  $P < 0.01$ ) and 24 hours ( $F_{(5,6)} = 7.876$ ;  $P < 0.05$ ) after completion of exercise (Figure 2(A),(B)). Similarly, increases in IL-1 $\beta$  expression were seen in the very early (6 hours) exercise groups both 3 hours ( $F_{(6,6)} = 2.799$ ;  $P < 0.01$ ) and 24 hours (slight increase) after exercise (Figure 2(C),(D)). In the early (24 hours) exercise groups, IL-1 $\beta$  expression was slightly increased both 3 and 24 hours after completion of exercise (Figure 2(C),(D)). Additionally, Western blot analysis demonstrated a reduced IL-1 $\beta$  protein expression ( $F_{(6,6)} = 4.407$ ;  $P < 0.05$ ) in the late (3 days) exercise group 3 hours after completion of exercise, though no significant difference was uncovered 24 hours after exercise (Figure 2(C),(D)). Together, these results seem to suggest that very early (6 hours) and early (24 hours) post-stroke exercise promote increased expression of pro-inflammatory mediators, while late (3 days) exercise limits expression of these molecules.



**Figure 2.** (A) TNF- $\alpha$  protein expression 3 hours after exercise completion was slightly increased when exercise was initiated after 6 hours of reperfusion, and was significantly increased ( $*P < 0.05$ ) when exercise was initiated after 24 hours of reperfusion. In contrast, a significant decrease ( $##P < 0.01$ ) in TNF- $\alpha$  expression was seen with exercise initiated after 3 days of reperfusion. (B) In animals sacrificed 24 hours after exercise completion, we observed a slight increase in TNF- $\alpha$  protein expression in the very early (6 hours) exercise group, and a significant increase ( $**P < 0.01$ ) in the early (24 hours) exercise group. A significant reduction ( $#P < 0.05$ ) in TNF- $\alpha$  protein expression was seen in the late (3 days) exercise group. (C) IL-1 $\beta$  protein expression 3 hours after exercise completion was increased compared to non-exercise groups after 6 hours (significantly,  $**P < 0.05$ ) and 24 hours (slightly) of reperfusion. In contrast, a significant decrease ( $#P < 0.05$ ) in IL-1 $\beta$  expression was seen with exercise initiated after 3 days of reperfusion. (D) Slight increases in IL-1 $\beta$  protein expression 24 hours after exercise completion were also observed in the very early (6 hours) and early (24 hours) exercise groups. No significant difference was seen when exercise was initiated after 3 days of reperfusion. Representative immunoblots are shown for all comparisons.

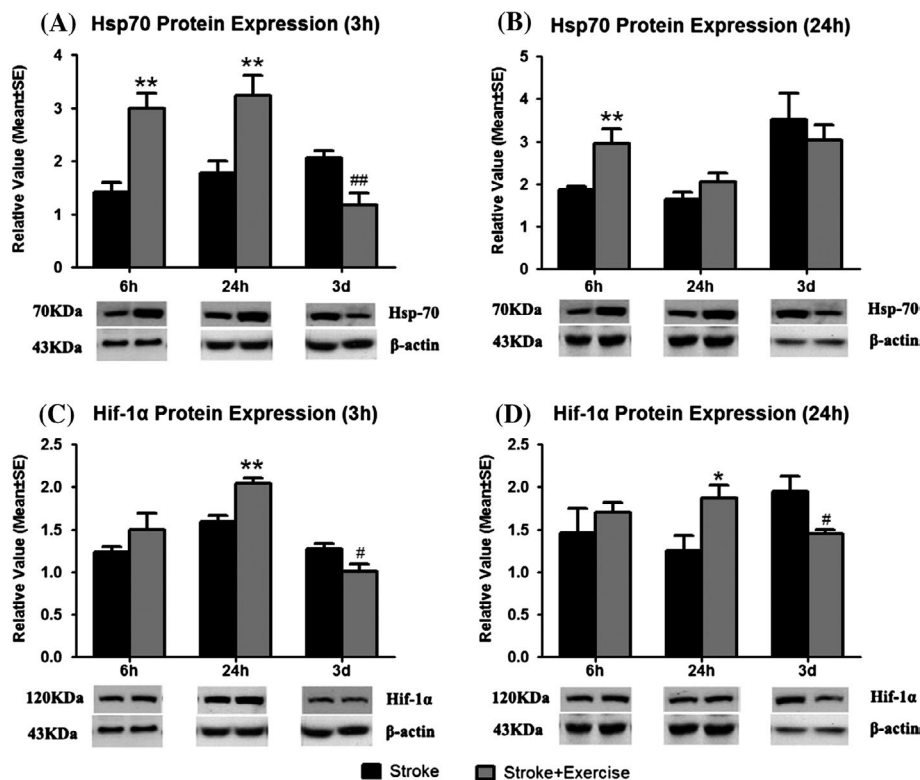
### Hsp70 and HIF-1 $\alpha$

To examine the effect of post-stroke exercise initiation timing on cell stress, we compared Hsp70 and HIF-1 $\alpha$  expression levels between stroke only (non-exercise) groups and stroke + exercise groups. Early (6 hours) exercise significantly increased Hsp70 expression at both 3 hours ( $F_{(6,6)} = 2.447$ ;  $P < 0.01$ ) and 24 hours ( $F_{(6,6)} = 18.19$ ;  $P < 0.01$ ) after completion of exercise (Figure 3(A),(B)). In the early (24 hours) exercise groups, a significant increase in Hsp70 expression was seen 3 hours after exercise completion ( $F_{(4,6)} = 2.246$ ;  $P < 0.01$ ), while a slight increase in Hsp70 expression was seen 24 hours after exercise completion (Figure 3(A),(B)). Western blot analysis also demonstrated significant decreases in Hsp70 expression in the late (3 days) exercise group 3 hours after exercise ( $F_{(5,5)} = 3.044$ ;  $P < 0.05$ ), and a slight decrease in Hsp70 expression 24 hours after exercise (Figure 3(A),(B)). Similar to the results obtained for Hsp70, slight increases in HIF-1 $\alpha$  expression were seen in the early (6 hours) exercise groups both 3 and 24 hours after exercise completion (Figure 3(C),(D)). The early (24 hours) exercise significantly increased

HIF-1 $\alpha$  expression at 3 hours ( $F_{(5,6)} = 1.047$ ;  $P < 0.01$ ) and 24 hours ( $F_{(5,5)} = 1.375$ ;  $P < 0.05$ ) after completion of exercise (Figure 3(C),(D)). In addition, late (3 days) exercise groups demonstrated a decreased HIF-1 $\alpha$  expression at both 3 hours ( $F_{(4,6)} = 1.179$ ;  $P < 0.05$ ) and 24 hours ( $F_{(5,6)} = 12.96$ ;  $P < 0.05$ ) after exercise (Figure 3(C),(D)). Again, these results suggested that very early (6 hours) and early (24 hours) post-stroke exercise promote increased cell stress, while late (3 days) exercise may mitigate the cell stress associated with ischemic injury.

### Discussion

The present study determined the time-sensitive molecular effects of exercise on brain inflammation after ischemic stroke by examining mediators of cell adhesion molecules, pro-inflammatory cytokines, and markers of cell stress. Overall, our results demonstrate that very early (6 hours) and early (24 hours) post-stroke exercise enhance expression of pro-inflammatory proteins (TNF- $\alpha$  and IL-1 $\beta$ ), cell adhesion molecules (ICAM-1 and VCAM-1), and cell stress markers (Hsp70 and HIF-1 $\alpha$ ), while late (3 day)



**Figure 3.** (A) Hsp70 protein expression 3 hours after exercise termination was significantly increased (\*\* $P < 0.01$ ) compared to non-exercise groups after 6 and 24 hours of reperfusion. In contrast, a significant decrease (## $P < 0.01$ ) in Hsp70 expression was seen with exercise initiated after 3 days of reperfusion. (B) A significant increase (\*\* $P < 0.01$ ) in Hsp70 protein expression 24 hours after exercise completion was seen in the very early (6 hours) exercise group, and a slight increase was seen in the early (24 hours) exercise group. A slight reduction in Hsp70 expression was seen in the late (3 days) exercise group. (C) HIF-1 $\alpha$  protein expression 3 hours after exercise termination was slightly increased in the very early (6 hours) exercise group and significantly increased (\*\* $P < 0.01$ ) in the early (24 hours) exercise group. A significant decrease (# $P < 0.05$ ) in HIF-1 $\alpha$  protein expression was seen with late (3 days) exercise. (D) Similarly, 24 hours after exercise termination, very early (6 hours) exercise resulted in a slight increase in HIF-1 $\alpha$  protein expression, while early exercise (24 hours) resulted in a significant increase (\* $P < 0.05$ ). Late (3 days) exercise resulted in a significant decrease (# $P < 0.05$ ) in HIF-1 $\alpha$  expression. Representative immunoblots are shown for all comparisons.

post-stroke exercise reduces expression of these molecules. The present data is in agreement with our recently published data, which demonstrate that neuronal apoptosis [23] and production of reactive oxygen species, lactate, and nicotinamide adenine dinucleotide phosphate oxidase (NOX) were all increased by early (6–24 hours) post-stroke exercise [13]. The present findings together suggest that early, though not late, post-stroke exercise has an injurious effect on cerebral tissue.

The complex effects of cytokine-mediated inflammatory reactions and PMN leukocyte infiltration in areas of cerebral infarction have been highlighted in previous reports. Indeed, post-stroke cytokine-induced brain inflammation is thought to exaggerate both the accumulation of inflammatory cells and microvascular dysfunction, ultimately leading to the development of secondary brain injury [14–16,27]. In the present study, very early (6 hours) and early (24 hours) post-stroke exercise were associated with elevated expression of both TNF- $\alpha$  and IL-1 $\beta$ , two potent pro-inflammatory cytokines. With respect to stroke, these molecules have individually been implicated in increasing infarct volumes [28,29]. Additionally, they act as leukocyte chemotactants in acute inflammation, and can induce the synthesis of ICAM-1 and VCAM-1 on leukocytes and/or endothelial cells after stroke [30]. In this examination, post-stroke exercise initiated at 6 and 24 hours, in addition to facilitating increased expression of TNF- $\alpha$  and IL-1 $\beta$ , promoted increased expression of ICAM-1 and VCAM-1. Endothelial up-regulation of ICAM-1 and VCAM-1 in acute stroke facilitates adhesion of leukocytes to the microvascular endothelium, congestion of the microcirculation, and infiltration of leukocytes into the parenchyma, all of which have the potential to exacerbate ischemic injury. Based on the results of our study, it appears that post-stroke exercise, if initiated too early, may exacerbate the inflammatory injury associated with acute stroke by increasing levels of TNF- $\alpha$  and IL-1 $\beta$ , thereby increasing levels of ICAM-1 and VCAM-1. On the contrary, late (3 days) post-stroke exercise was associated with decreased levels of TNF- $\alpha$ , IL-1 $\beta$ , ICAM-1, and VCAM-1, suggesting that late post-stroke exercise may reduce cerebral inflammation.

Further, this study aimed to examine the effect of post-stroke exercise initiation timing on cell stress, quantified by expression levels of Hsp70 and HIF-1 $\alpha$  proteins. Hsp70 undergoes differential expression in response to various stressors, including heat, shock, ischemia, oxidative stress, glucose deprivation, and exposure to toxins and heavy metals [24]. HIF-1 is a transcription factor that is activated in response to hypoxia [31]. A previous study has suggested that the high co-localization rate of HIF-1 $\alpha$  and Hsp70 is indicative of a strong interaction between these proteins in cells stressed by MCA occlusion [32]. Hsp70 seems to facilitate HIF-1 $\alpha$  stability via the PI3 K/Akt signaling

pathway [33], while Hsps are regulated via the HIF-1 $\alpha$  pathway under hypoxic conditions [34,35]. Our data indicate that post-stroke exercise initiated between 6 and 24 hours may lead to increased expression of Hsp70 and HIF-1 $\alpha$ , suggesting that post-stroke exercise initiated at these time points exacerbates cell stress. Along with elevated levels of pro-inflammatory cytokines and cell adhesion molecules, this increase suggests a possible mechanism by which early post-stroke exercise may exacerbate ischemia/reperfusion injury. Additionally, late (3 days) post-stroke exercise that was associated with decreased levels of Hsp70 and HIF-1 $\alpha$  may reduce cell stress. We will examine the cause-and-effect relationship between Hsp expression and inflammation in a future study by selectively blocking Hsp expression.

Several studies have demonstrated the neuroprotection by treadmill (30 minutes) in stroke [22,36,37]. In present study, rats were conditioned to run on a rotarod at a speed of 35 rpm (8 m/minutes) for 30 min, which was same to a previous modified human treadmill at a speed of 8 m/minutes for 30 minutes daily [38]. Together with our other published studies [36,39] and preliminary study, the gradually increased speed and duration of exercise (controlled by an integrated computer) were selected for exercise intensity and time. Since the exercise intensity and time we used in this study simulate the modified human treadmill, we cannot rule out the possibility that the lower exercise speed with less training time may induce a functional improvement comparable to that achieved by the present exercise. In order to optimize the exercise time and reduce the labor intensive, further experiment would be conducted.

In summary, this study demonstrated that increased expression of early exercise-induced inflammatory mediators (TNF- $\alpha$  and IL-1 $\beta$ ), cell adhesion molecules (ICAM-1 and VCAM-1), and cell stress (Hsp70 and HIF-1 $\alpha$ ) may underlie enhanced post-stroke ischemia/reperfusion injury. The data also support initiating post-stroke exercise between 24 hours and 3 days (in animal models of acute stroke) for the beneficial effects of exercise while avoiding the inflammatory injury that may hamper functional outcomes. Ultimately, the results of the present study may aid in the development of an effective stroke rehabilitation strategy for improved functional outcomes in the clinical setting.

## Contributors

FL was involved with experiment design, performance, data analysis, and writing the article. JP, JD, CP, XL, JM, and SW were involved with performance of experiments and data analysis. XG participated in experiment design, data analysis, and writing and revising the article.

## Disclosure statement

The authors report no conflicts of interest.

## Funding

This work was supported by the [American Heart Association Grant-in-Aid]; under [grant number 14GRNT20460246]; [Merit Review Award] under [grant number I01RX-001964-01]; [National Natural Science Foundation of China] under [grant number 81501141] and [Beijing NOVA program] under [grant number xx2016061].

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