รายงานการวิจัยฉบับสมบูรณ์

ผลของการออกกำลังกายต่อการป้องกันการสูญเสียหน้าที่ของหลอดเลือด
และเซลล์ประสาทในสมองผู้สูงวัย

(Protective effects of exercise training against vascular and
neuronal dysfunction in aging brain)

โดย

ศาสตราจารย์ ดร. สุทธิลักษณ์ ปทุมราช
ภาควิชาสรีรวิทยา คณะแพทยศาสตร์
จุฬาลงกรณ์มหาวิทยาลัย

กรกฎาคม 2562

กิตติกรรมประกาศ

บทคัดย่อ

การลดลงของจำนวนหลอดเลือดขนาดเล็กในสมองพบได้ในช่วงสูงวัย ซึ่งสามารถนำไปสู่ภาวะสมองได้รับเลือดไม่เพียงพอได้ โดยมีหลักฐานจำนวนมากยืนยันว่าการลดลงของหลอดเลือดขนาดเล็กในสมองช่วงสูงวัยมีความสัมพันธ์กับการเกิดภาวะเครียดออกซิเดชั่น ซึ่งภายในเซลล์มีกลไกการป้องกันภาวะเครียดออกซิเดชั่นโดยผ่านการทำงานของทรานคริปชันแฟคเตอร์ที่สำคัญชื่อว่าเอ็นอาร์เอฟทู นอกจากนี้ปัจจุบันเป็นที่ทราบกันดีว่าการฝึกออกกำลังกายอย่างสม่ำเสมอส่งผลที่เป็นประโยชน์ต่อการทำงานของสมองในช่วงสูงวัย ได้แก่ การเพิ่มอัตราการไหลเวียนเลือด และการกระตุ้นการสร้างหลอดเลือดใหม่ อย่างไรก็ตามกลไกที่อธิบายผลของการฝึกออกกำลังกายอย่างสม่ำเสมอต่อการเปลี่ยนแปลงของหลอดเลือดขนาดเล็กในสมองช่วงสูงวัยยังไม่ทราบแน่ชัด โครงการวิจัยนี้จึงมีจุดประสงค์เพื่อทำการศึกษากลไกของการฝึกออกกำลังกายต่อการเปลี่ยนแปลงของจำนวนหลอดเลือดขนาดเล็กร่วมกับวิถีพีไอทรีเค/เอเคที/เอ็นอาร์เอฟทูในสมองของหนูแรทช่วงสูงวัย โดยในการศึกษาวิจัยนี้ใช้หนูแรทเพศผู้สายพันธุ์วิสตาร์ โดยแบ่งออกเป็น 3 กลุ่ม คือ 1) กลุ่มหนูวัยเจริญพันธุ์ 2) กลุ่มหนูแก่ไม่ได้ออกกำลังกาย และ 3) กลุ่มหนูแก่ได้รับการฝึกออกกำลังกายด้วยการว่ายน้ำ 5 วันต่อสัปดาห์ เป็นเวลา 8 สัปดาห์ โดยทำการศึกษาการแสดงออกของซีดี 31 (ดัชนีความหนาแน่นของหลอดเลือดขนาดเล็ก) และการแสดงออกของเอ็นอาร์เอฟทูในชิ้นเนื้อสองด้วยวิธีอิมมูโนฮิสโตเคมิสทรี นอกจากนี้ได้ทำการแยกสกัดหลอดเลือดขนาดเล็กจากเนื้อสมองเพื่อนำมาตรวจหาการระดับการทำงานของเอ็นอาร์ทู ปริมาณของพีไอทรีเคและเอเคที ด้วยวิธีการวัดปฏิกิริยาแอนติบอดี-แอนติเจน ผลการวิจัยพบว่าสมองของหนูแก่ที่ไม่ได้ออกกำลังกายมีความหนาแน่นของหลอดเลือดขนาดเล็ก การแสดงออกและการทำงานของเอ็นอาร์เอฟทู และปริมาณของพีไอทรีเคและเอเคทีลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับหนูวัยเจริญพันธุ์ อย่างไรก็ตามหนูแก่ที่ได้รับการฝึกออกกำลังกายเป็นเวลา 8 สัปดาห์ มีการกระตุ้นการสร้างหลอดเลือดขนาดเล็กที่สมอง รวมถึงมีการเพิ่มขึ้นของการแสดงออกและการทำงานของเอ็นอาร์เอฟทู และปริมาณของพีไอทรีเคและเอเคทีอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับหนูแก่ที่ไม่ได้ออกกำลังกาย ผลการวิจัยจึงสรุปได้ว่าการฝึกออกกำลังกายสามารถกระตุ้นการสร้างหลอดเลือดขนาดเล็กที่สมองของหนูแก่ได้ โดยสัมพันธ์กับวิถีพีไอทรีเค/เอเคที/เอ็นอาร์เอฟทู

Abstract

During advancing age, reduction of microvessels in the brain contributes insufficiency tissue perfusion. Mounting evidence indicates that microvascular deterioration in aged brain relates to oxidative stress. Nuclear factor erythroid-related factor 2 (Nrf2) plays an important role in cellular antioxidant defense. Regular physical exercise is well known to have beneficial effect to brain health, including promoted blood flow and augmented angiogenesis, in aging individuals. However, the underlying mechanism of regular physical exercise in improvement of brain microvascular density during advancing age has not been fully elucidated. This study aimed to investigate the underlying mechanism of exercise training in improvement of microvascular density associated with PI3K/Akt/Nrf2 pathway in aged rat brain. Male Wistar rats were divided into three groups; sedentary-young (SY), sedentary-age (SA) and trained-age (TA). Exercise program included swimming exercise for eight weeks. Expression of CD31 (as indicator of microvascular density) and Nrf2 were evaluated by immunohistochemistry staining. Activity of Nrf2, protein levels of phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K) and phosphorylated-protein kinase B (p-Akt) in isolated brain microvessels were assessed by immunoassay. Aging (SA) induced significant reduction of brain microvascular density and expression of Nrf2, PI3K and p-Akt proteins, as well as Nrf2 activity, comparing to those of SY group. The eight-week exercise training significantly improved brain microvascular density and upregulated Nrf2, PI3K and p-Akt proteins as well as activated Nrf2 activity, than that of the age group without exercise (SA). In conclusion, exercise training can improve brain microvascular deterioration associated with PI3K/Akt/Nrf2 pathway in aging rats.

สารบัญ

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คำสัญญาลักษณ์และคำย่อ

Nrf2	=	Nuclear factor erythroid 2-related factors 2
CD31	=	Cluster of differentiation 31 หรือ Platelet endothelial cell adhesion molecule-1 (PECAM-1)
Keap1	=	Kelch-like erythroid cell-derived protein with cap 'n' collar homology associated protein 1
ARE	=	Antioxidant response element
PI3K	=	Phosphatidyl inositol 3-kinase
Akt	=	Protein kinase B
VEGF	=	Vascular growth factor
PBS	=	Phosphate-buffered saline
S.E.M.	=	Standard error of mean
MAP	=	Mean arterial blood pressure
%	=	Percent
P	=	p-value
DAB	=	Diaminobenzidine chromogen
ELISA	=	Enzyme-linked immunosorbent assay

Introduction

At present, Thailand is confronting with a rapid growth in number of elderly. A report from United Nations revealed that proportion of older persons progressively increase from 8 percent in 1950 to 11 percent in 2007, and is expected to reach 22 percent in 2050. Thailand's National Economic Social and Development Board Report in 2007 indicated that tendency for Thai elder population rapidly rise between 2000 to 2030, and is expected to establish one fourth of Thai population. Great extent of global aging population has profound impact on many aspects of health.

Abnormalities of cerebral vasculature are a crucial factor which leads to cerebrovascular diseases and neurodegenerative diseases in elders (Grammas, Martinez, & Miller, 2011). In addition, cerebrovascular accident is an important cause of death in global aging population. The Bureau of Policy and Strategy, Ministry of Public Health reported that mortality rate of cerebrovascular disease increased from 54.7 per 100,000 populations in 1996 to 110.9 per 100,000 populations in 2006.

Mounting evidences indicated that morphologic and physiologic alterations of cerebral vasculature in advancing age result in pathogenesis of a variety of diseases affecting the brain (Yang, Sun, Lu, Leak, & Zhang, 2017). Aging is associated with reduction in resting cerebral blood flow (Leoni, Oliveira, Pontes-Neto, Santos, & Leite, 2017), cerebral endothelial cell dysfunction (Popa-Wagner, Buga, Turner, Rosen, & Toescu, 2012) and a decrease of microvascular density in the brain (Murugesan, Demarest, Madri, & Pachter, 2012). These alterations contribute to a decrease of brain tissue perfusion leading to an increase susceptibility of brain to ischemic injury (Yang et al., 2017).

Capillary density in the brain is necessary to several fundamental aspects of cerebrovascular functions because of its influence on regulation of blood flow to brain tissues (Murugesan et al., 2012). Brain angiogenesis is associated with phosphatidylinositol-3 kinase (PI3K)/Akt signailing pathway (Jiang & Liu, 2008). Recent evidence showed a positive correlation between brain capillary density and cerebral blood flow in aging (Izzo et al., 2018). An important mechanism underlying age-induced capillary density loss in the brain is deterioration of angiogenic process (Izzo et al., 2018).

However, the downstream intracellular signaling pathways mediating PI3K/Akt angiogenic signaling of aged brain remains to be determined.

Oxidative stress occurs during advancing age according to free radical theory of aging. Age-related oxidative stress plays an important role in impairment of brain angiogenesis, as well as, brain capillary loss is associated with an imbalance between oxidants and antioxidants in the brain (Ungvari et al., 2011). Nuclear factor erythroid 2-related factor (Nrf2) plays a crucial role in protection against cell oxidative stress. Nrf2 regulates transcription of antioxidant proteins, primarily via Keap1/Nrf2/ARE pathway (Loboda, Damulewicz, Pyza, Jozkowicz, & Dulak, 2016). Mounting evidences indicate alterations of Nrf2 in the aged brain, including downregulation of Nrf2 expression, a reduction of Nrf2 activity related to antioxidant protein transcription (Csiszar et al., 2014; Ungvari et al., 2011).

It is well known that regular physical exercise has positive impacts on cardiovascular system. Exercise training enhances tissue perfusion, including heart, brain, skeletal muscles (Olver, Ferguson, & Laughlin, 2015), partly via promoting tissue angiogenesis (Morland et al., 2017; Viboolvorakul & Patumraj, 2014). Exercise promotes antioxidant mechanisms via Nrf2 signaling in heart and skeletal muscle (Muthusamy et al., 2012; Wang, Li, Qi, Cui, & Ding, 2016). Moderate exercise training reduced cardiac oxidative stress is associated with an increase of Nrf2 activity on antioxidant transcription (Muthusamy et al., 2012). However, mechanism underlying exercise-induced brain angiogenesis related to alterations of Nrf2-relative oxidative stress during aging is not well understood. Therefore, the aim of present study was to investigate the effect of exercise training on alteration of microvascular density associated with Nrf2 signaling pathway in aged rat brain.

Material and Methods

Animal preparation

Male Wistar rats (8-week old; body weight 200-250 g) were obtained from the National Laboratory Animal Center (Narkornpathom, Thailand). The experimental procedures were conducted according to the guidelines for experimental animals by the National Research Council of Thailand, and approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University, Thailand. The animals were housed in a room with 12:12 hour light-dark cycle until they reached ages of 4-6 months and 16-18 months. All rats were allowed free access to normal chow and tap water ad libitum. The rats were divided into three groups: sedentary-young (SE-Young, aged 4-6 months) as a control age, sedentary-aged (SE-Aged, aged 16-18 months) and exercise-aged (EX-Aged, aged 16-18 months). The exercise-aged rats were exercised by swimming in a round plastic tank of water (34-36°C), 60 min/day, 5 days/week for 8 weeks (Viboolvorakul & Patumraj, 2014).

Brain tissue preparation

After 8 weeks of swimming training, individual animal was anesthetized with pentobarbital sodium (50 mg/kg body weight intraperitoneally) and a tracheotomy was performed. A common carotid artery was cannulated with a catheter for measurement of arterial blood pressure. After blood pressure measurement, the animal was transcardially perfused with ice-cold physiologic saline. The brain was harvested and freed of cerebellum and brain stem. One part of the brain was fixed in 4% paraformaldehyde for 24 h, dehydrated and cleared by graded ethanol and xylene, and finally embedded in paraffin. Each paraffin block was cut longitudinally into 5-μm sections for immunohistochemical staining. The remainder part of the brain was weighed and prepared for isolation of microvessel fraction.

Isolation of brain microvessels

Brain microvessel isolation was performed according to the method of Yamakawa et al. (Yamakawa, Jezova, Ando, & Saavedra, 2003). The animal was transcardially

perfused with 200 ml ice-cold PBS. The brain was harvested and freed of cerebellum and brain stem. All steps of microvessel isolation protocol were performed at < 4°C. The brain tissue was minced and homogenized in 3 ml of isotonic sucrose buffer (0.32 M sucrose, 3 mM HEPES, pH 7.4) using a motor-driven teflon homogenizer (Glas-Col, USA). The homogenate was centrifuged twice at 1,000 g for 10 min. The pellet was resuspended in the sucrose buffer and centrifuged twice at 100 g for 10 min. The supernatant from the last two centrifugation was pooled and centrifuged at 200 g for 2 min. The pellet was washed twice with the sucrose buffer and once with 0.1% bovine serum albumin (BSA) in PBS at 200 g for 2 min. The pellet was resuspended in 1 ml of 0.1% BSA in PBS and centrifuged at 14,000 g for 2 min. The final pellet, contained brain microvessels, was stored at -80°C. To evaluate the purity of isolated microvessel preparation, air-dried smears of isolated microvessel fractions on glass slides were stained with hematoxylin and checked by light microscopy.

Immunohistochemistry for CD31 and Nrf2

After deparaffinization, the brain sections were incubated in antigen retrieval reagent (Dako, Denmark) for 30 min. Endogenous peroxidase activity in the sections was inhibited by incubation with 3% H_2O_2 for 30 min, followed by incubation with nonspecific protein blocking reagent (Dako, Denmark) for 30 min. The sections were subsequently incubated with rabbit polyclonal primary antibody against Nrf2 (1:100; Abcam, UK) or CD31 (1:100; ThermoFisher Scientific, USA) at 4°C overnight. After being washed with wash buffer, the sections were further incubated with peroxidase-labeled polymer-horseradish peroxidase (HRP) conjugated to goat anti-rabbit immunoglobulins (EnVision Detection System Kit, Dako, Denmark) at room temperature for 30 min, and the sections were subsequently incubated with 3,3′-diaminobenzidine (DAB) (Dako, Denmark). Finally, the sections were counterstained with hematoxylin and examined under a light microscope by a blind-fashion observer.

Quantitative image analysis

Analysis of immunohistochemical-stained image in each brain tissue was obtained from two sections (8 areas/section, 5 images/area), according to modified method of Li

W. et al (Li, Suwanwela, & Patumraj, 2016). DAB positive staining (brown color) was quantified by Image-Pro Plus 6.0 (Media Cybernetics Inc., USA). Pixel of DAB-positive intensity was calculated by the software. Percentage of the positive intensity for expression of Nrf2 and CD31 was calculated using an equation;

$$\% \text{ Positive intensity} = (\text{Positive intensity}/\text{Total intensity}) \times 100$$

Immunoassay of Nrf2, PI3K and phospho-Akt

Isolated brain microvessels were homogenized in 3 μl of RIPA lysis buffer (Sigma, USA) containing protease inhibitor cocktail (Sigma, USA) and phosphatase inhibitor cocktail (Sigma, USA). After centrifugation at 1,000 g for 5 min at 4°C, the postnuclear supernatant was aliquot and stored at -80°C. The protein concentration of the postnuclear fraction was determined by using a bicinchoninic acid assay kit (23252, Pierce, USA). Activity of Nrf2 and expression of VEGF, PI3K and phosphor-Akt in the brain were determined using ELISA kits accordance with manufacturer's instructions. Kits were obtained from Abcam, USA: Nrf2, R&D Systems, USA: phosphor-Akt, and Elabscience, China: PI3K.

Statistical analysis

Data are represented as mean $\pm$ standard error of mean (SEM). Significant differences between groups were determined by using one way analysis of variance (one-way ANOVA), and the difference in pairs of means were evaluated by Tukey's post hoc test. The difference was statically significant if the statistical probability (p-value) was less than 0.05. The data were analyzed by GraphPad Prism 6.0 (GraphPad Software Inc., USA).

Results

Body weight and mean arterial blood pressure (MAP) for three groups were shown in Table 1. The body weight significantly increased in both sedentary-aged and exercise-aged rats compared to sedentary-young rats. However, the body weight of exercise-aged group was significantly lower when compared to the sedentary-aged group. The MAP was significantly higher in the sedentary-aged and exercise-aged groups compared with the sedentary-young group. Interestingly, the exercise-aged rats were significantly declined in MAP compared to the aged rats without exercise.

Table 1. Body weight and mean arterial blood pressure in sedentary-young (SE-Young), sedentary-aged (SE-Aged) and exercise-aged (EX-Age) groups.

	SE-Young	SE-Age	EX-Age
Body weight (g)	572.96 ± 11.53	743.69 ± 20.99*	665.15 ± 18.18*,#
MAP (mmHg)	97.00 ± 2.41	123.44 ± 4.07*	114.89 ± 3.63*,#

*$P<0.05$, significantly different from the SE-Young group. #$P<0.05$, significantly different from the SE-Age group.

Protein expression of CD31 and Nrf2 in the brain from three groups of animals obtained by immunohistochemistry are demonstrated in Figure 1. Quantitative immunohistochemical analysis revealed that the protein expression of CD31 and Nrf2 were significantly lower in the sedentary-aged group compared with the sedentary-young group ($P<0.05$), and was significantly higher in the exercised-aged group than in the sedentary-aged rats ($P<0.05$).

Activity of Nrf2 and expression of PI3K and phospho-Akt proteins in the brain from three groups of animals obtained by ELISA is shown in Figure 2. The activity of Nrf2 and protein levels of PI3K and phospho-Akt significantly decreased in the sedentary-aged group compared with the sedentary-young group ($P<0.01$, and $P<0.05$), however,

significantly elevated in the exercised-aged group than in the sedentary-aged rats (P<0.05).
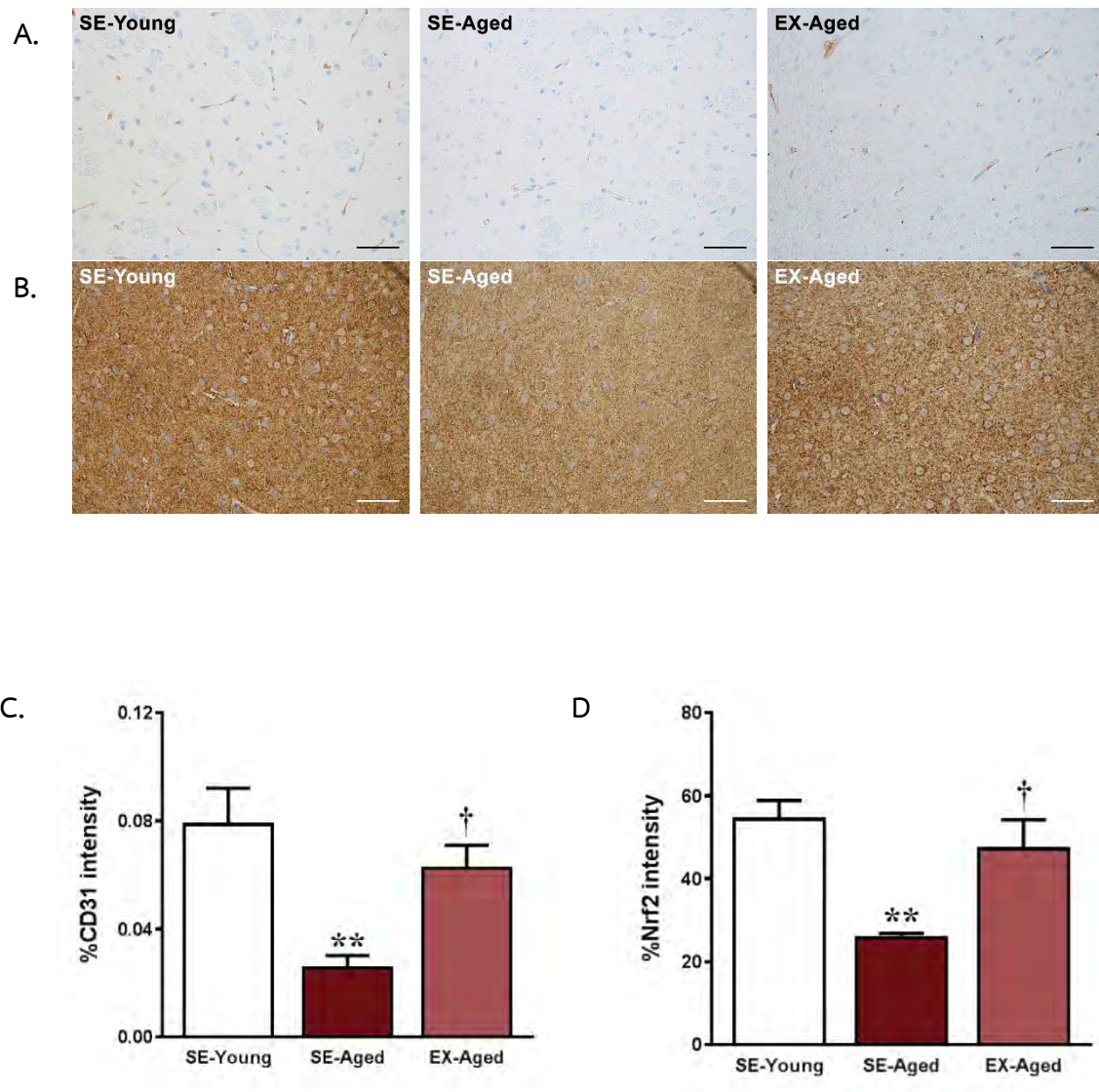
Figure 1. (A.-B.) Representative immunohistochemical photomicrograph of CD31 (A) and Nrf2 (B) expression in the brain of sedentary-young (SE-Young), sedentary-aged (SE-Aged) and exercise-aged (EX-Aged) rats (400x magnification; scale bar, 50 μm). Percent of immunohistochemical intensity of CD31 (C) and Nrf2 (D) proteins in the brain of SE-Young, SE-Aged and EX-Aged rats. **P<0.001 compared with SE-Young, and †P<0.05 compared with SE-Aged.

Figure 2. Activity of Nrf2 (A) and expression of PI3K (B) and phospho-Akt (C) by ELISA in the brain of sedentary-young (SE-Young), sedentary-aged (SE-Aged) and exercise-aged (EX-Aged) rats. *P<0.05, **P<0.001 compared with SE-Young, and †P<0.05 compared with SE-Aged.

Discussion

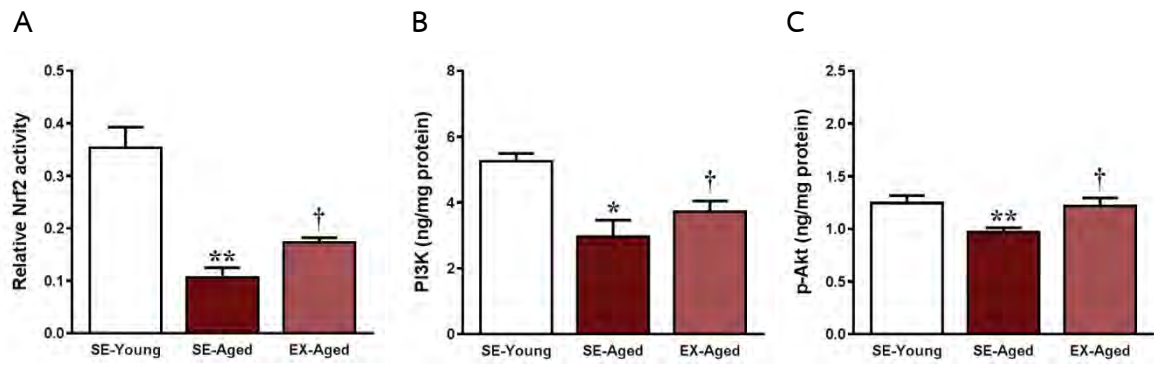In this study, we revealed that swimming exercise training significantly improved microvascular density and reversed lower Nrf2, PI3K and phospho-akt expression as well as Nrf2 activity in the brain of aged rats. Up-regulation of Nrf2 and its activity imply that exercise training ameliorated aged-brain microvascular rarefaction partly associated with PI3K/Akt signaling pathway.

The present study demonstrated changes in physiologic characteristics in aging rats, including greater body weight gain and elevation of arterial blood pressure, which could be reduced by the exercise training (Table 1). A large body of evidence affirms that regular aerobic exercise can reduce body weight and lower high blood pressure in the elders (Eksakulkla, Suksom, Siriviriyakul, & Patumraj, 2009; Shinoda, Latour, & Lavoie, 2002). Therefore, the physiologic alterations resulting from the present exercise program provides indication of endurance training.

Several animal and human studies reported microvascular rarefaction occurred during advancing age (Brown, Moody, Thore, Challa, & Anstrom, 2007; Murugesan et al., 2012; Villena, Vidal, Diaz, & Perez De Vargas, 2003). In the present study, CD31 expression is identified as an indicator of microvascular density in the brain, and we observed significantly decreased CD31 expression in the brain of aging rats (Figure 1). The mechanism for age-induced microvascular loss is widely believed to be associated with oxidative stress (Izzo et al., 2018). Endothelial cell is a major target for brain oxidative stress during aging (Park, Anrather, Girouard, Zhou, & Iadecola, 2007). Imbalance between oxidant marker, carbonyl content, and mitochondrial antioxidant MnSOD in the brain microcrovessls with increasing age was been reported (Tripathy et al., 2010).

Nrf2 is a transcription factor that regulates endogenous antioxidant defense and plays an important role in resistance to cellular oxidative stress. Activation of Nrf2 transcriptionally upregulates many oxidant defense pathway (Loboda et al., 2016). In vitro studies, aging induced reduction of Nrf2 activity and its target proteins expression in the cerebral endothelial cells (Csiszar et al., 2014; Ungvari et al., 2011). It is reported that oxidative stress and damage increase and activity and expression of Nrf2 decline in aging rodent (Tomobe, Shinozuka, Kuroiwa, & Nomura, 2012). The present in vivo study, we

consistently demonstrated lower both Nrf2 activity and expression in the brain tissue of aged rats (Figure 1-2).

Numerous studies have shown that the PI3K/Akt signaling participates in the activation of Nrf2 and regulates the expression of the downstream target proteins (Chen, Lu, Chen, & Cheng, 2015). Accumulation of reactive oxygen species medicates downregulation of phospho-PI3K and phospho-Akt leading to Nrf2 translocation from nucleus to cytosol and producing low level of antioxidants (Rojo, Sagarra, & Cuadrado, 2008). In aging model, activation of PI3K/Akt signaling has been shown to involve in Nrf2 activation and induction of Nrf2-mediated downstream antioxidants (Zhang et al., 2017). In present study, the results showed that PI3K and phospho-Akt downregulated in the aged brain (Figure 2.).

Regular physical exercise is extensively addressed to be a power tool for promoting brain health. Exercise training has been shown to improve memory and cognitive functions as well as enhance cerebral tissue perfusion and angiogenesis (Kleim, Cooper, & VandenBerg, 2002; Rhyu et al., 2010). Aerobic exercise, a non-pharmaceutical intervention, plays a beneficial role in prevention of age-related cognitive decline and cerebrovascular deterioration in human and animal (Paillard, 2015; Speisman, Kumar, Rani, Foster, & Ormerod, 2013). Angiogenesis-induced increased microvascular density is one mechanism through which exercise improves brain function (Ding et al., 2006; Viboolvorakul & Patumraj, 2014). Here, our result also demonstrated that effect of swimming exercise training ameliorates microvascular loss in aged brain (Figure 1).

One important role of regular physical exercise in prevention of age-related brain dysfunctions is attenuation of oxidative stress during aging (Mock, Chaudhari, Sidhu, & Sumien, 2017). Recently, Garcia-Mesa et al. found that physical exercise training normalizes brain redox homeostasis in aging mice model (Garcia-Mesa et al., 2016). The present study showed that 8-week swimming exercise training enhanced Nrf2 activity and expression in aged brain (Figure 1-2). Our results suggest that exercise prevents age-related oxidative stress in the brain partly via activation of Nrf2 mechanism. Several studies in aging animal reported that moderate-intensity exercise training attenuates oxidative stress cooperated with enhancement of Nrf2 activation in the heart and skeletal muscle (Gounder et al., 2012; Narasimhan et al., 2014) (Gounder et al., 2012;

Narasimhan et al., 2014). Interestingly, our present study is the first study showed that exercise training was capable to markedly enhance Nrf2 activation and expression, as well as IP3K and phosphor-Akt expression in aged brain of rats (Figure 1-2). Therefore, from these findings of the present study, it implied that the protective effect of exercise training on PI3K/Akt/Nrf2 mechanism represent as considerable factors contributing to the possible underlying mechanism against age-induced alteration of brain microvessels.

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ภาคผนวก

<u>ผลผลิตที่ได้จากโครงการวิจัย</u>

<u>การนำเสนอผลงานแบบโปสเตอร์ในงานประชุมวิชาการระดับชาติ</u>

1. Udomsab, R, Pamonsout, K, Patumraj, S, and Viboolvorakul, S. Effect of exercise training on microvascular density and Nrf2 expression in aged-rat brain. ในการประชุมวิชาการระดับชาติ วิทยาศาสตร์และเทคโนโลยีระหว่างสถาบัน ครั้งที่ 6 (The 6th Academic Science and Technology Conference 2018) วันพุธที่ 6 มิถุนายน 2561 ณ มหาวิทยาลัยหัวเฉลียวเฉลิมพระเกียรติ จังหวัดสมุทรปราการ

<u>การเผยแพร่ผลงานวิจัยในวารสารวิชาการ</u>

1. Chanpakdee, C, Viboolvorakul, S, Patumraj, S. Exercise training improves age-related changes in cerebral capillary vascularity through the upregulation of PI3K/Akt signaling. *Chula Med J* 2019;63(4):229-238.

2. อยู่ระหว่างดำเนินการตีพิมพ์วารสารนานาชาติเรื่อง Exercise training improves age-related cerebrovascular deterioration associated with PI3K/Akt/Nrf2 pathway.

Original article

Exercise training improves age-related changes in cerebral capillary vascularity through the upregulation of PI3K / Akt signaling

Channipa Chanpakdee[a], Sheepsumon Viboolvorakul[b], Suthiluk Patumraj[c]*

[a]*Inter-Department of Physiology, Graduate School, Chulalongkorn University, Bangkok, Thailand*
[b]*Department of Medical Science, Faculty of Science, Rangsit University, Pathum-Thani 12000, Thailand*
[c]*Center of Excellence for Microcirculation, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Background: Currently, the number of the elderly has been rising sharply worldwide. Seemingly, age-induced cerebral endothelial dysfunction may lead to vascular abnormality that can later progress to cerebrovascular and neurodegenerative diseases. Moreover, several evidences have shown that age-related oxidative stress and decline cellular function appeared in the vascular system both in humans and laboratory animals. The role of exercise training in the regulation of age-related oxidative stress and endothelial functions have been reported.
Objectives: To investigate whether exercise training can prevent age-induced cerebral endothelial dysfunction associated to PI3K/Akt signaling.
Methods: Male Wistar rats were randomly divided into 3 groups: sedentary-young group (SE-Young, 4 months), sedentary-aged group (SE-Aged, 22 - 24 months), and swimming trained-aged group (ET-Aged, 22 - 24 months), which was individually swim 1 hour/day, 5 days/week for 8 weeks. After 8 weeks of exercise period, the rats took rest for 24 hours. After the phosphate buffer saline (PBS) perfusion, brain was used for determining CD31 by immunohistochemistry. Vascular endothelial growth factor (VEGF), phospho-Akt level (p-Akt), and malondialdehyde (MDA) levels in the brain were measured by enzyme-linked immunosorbent assay.
Results: The aged rats' physiological characteristics were significant alteration when compared to the young group ($P < 0.05$). However, ET-Aged rats showed significantly reduced resting mean arterial blood pressure when compared to the young group ($P < 0.05$). This study also showed that exercise could upregulate VEGF, p-Akt level, and increase CD31 in ET-age. Furthermore, tissue MDA in ET-Aged rats was significantly reduced when compared to SE-Aged rats ($P < 0.05$).
Conclusion: Our findings imply that exercise training protected age-induced cerebral endothelial dysfunction associated with its effects on oxidative stress and PI3K/Akt signaling.

Keywords: Aging, exercise training, CD31, Akt.

Nearly 47.5 million people worldwide suffer from dementia with 7.7 million more new cases every year. The general population aged 60 years and above with dementia are estimated between 5 to 8 per 100 people. The total number of people with dementia has been predicted to increase to 75.6 million in 2030 and almost 135.5 million by 2050. As people live longer, reduction in certain cognitive abilities is expected due to increasing age. Interestingly, vasculature changes have been reported regarding the close correlation with cerebrovascular disease and neurodegeneration. Dementia and Alzheimer's disease were associated with marked alterations in both cerebrovascular structure and function.

Endothelial functions play an important role in the controls of vascular tone, permeability, inflammation, remodeling, and angiogenesis. It was believed that endothelial dysfunction was a key that deterioration of aging brain. Many studies showed that endothelial dysfunction associated with aging could cause the impairment of endothelium-dependent vasodilator,

impaired modulation of vascular growth and dysregulation of vascular remodeling and then decreased capillary density in the brains. This reduced brain capillary density could cause a lowered cerebral blood flow and hypoperfusion which led to brains' lack of oxygen, glucose and nutrient supplied. Therefore, it could ultimately affect cognitive function and behavior and led to brain degeneration and age-related cognitive disorders. [1 – 3]

Interestingly, an exercise training was shown to decrease oxidative stress and improve angiogenesis through the up-regulation of vascular endothelial growth factor (VEGF) mRNA and protein in brains.[4] The researchers indicated that when exercise training increased blood vessel density, it helped to restore blood flow and to modify vascular shear stress.[4] Shear stress was known as a crucial factor for maintaining vascular tone. It played the role as the most important physiologic activator of nitric oxide (NO) production as seen from the activation of phosphoinositide 3-kinase (PI3K/Akt) pathway which was the underlying mechanism showing that shear stress stimulated NO production. [5, 6]

Therefore, the reason why exercise training could prevent cerebrovascular deterioration in aging might be able to explain through the effect of exercise training on mediated VEGF signaling cascade regulation, which improved the mechanism of the angiogenic adaptation in the brain. [7] However, there a few studies have addressed the direct effect of exercise training on aged brain capillary density associated with the VEGF and PI3k/Akt pathway. Therefore, the aim of this study was to investigate whether exercise training can prevent age-induced cerebral endothelial dysfunction associated to PI3K/Akt signaling or not.

Materials and methods
Animal preparation
Male Wistar rats (8-week-old) from the National Laboratory Animal Center of Salaya campus, Mahidol University (Nakornpathom, Thailand) were divided into three groups: sedentary-young (SE-Young, aged 4 - 6 months, n = 5), sedentary-aged (SE-Aged, 23 - 24 months, n = 8), and exercise trained-aged (ET-Aged, n = 8) rats. This study has been approved by the Ethics Committee on the Care and Use of Laboratory Animals, Faculty of Medicine, Chulalongkorn University (NO.14/2559). The present

study was conducted in accordance with the guidelines for laboratory animals established by the National Research Council of Thailand (1999).

The effects of exercise training in aging group were determined and compared between sedentary-aged (SE-Aged, placed in the swimming tanks with 5-cm water depth for 30 minutes/day and 5 days/week for 8 weeks, n = 5) and the exercise trained-aged (ET-Aged, 60 minutes/day and 5 days/week for 8 weeks, n = 8) rats. To minimize the possible stress effects associated with cold or hot water exposure, the SE-Aged group was performed according to the modified methods of exercise training program Iemitsu M, et al. and Viboolvorakul S, et al. [8, 9]

The swimming exercise protocol involved non-impact endurance exercise with moderate intensity and was modified from the methods of Iemitsu M, et al., and also from Viboolvorakul S, et al. [8, 9] Each day, the animals were transported to an exercise training room and swam individually in cylindrical tanks with a diameter and height of 50 and 65 cm, respectively, with water at a depth of 50 - 55 cm. The rats were exercised once per day between 2:00 and 4:00 p.m. for 5 days/week. The animals swam for 15 minutes/day for the first 2 days, and the swimming time was then gradually increased each week from 15 to 60 minutes/day. Thus, the trained-aged group received 8 weeks of swim training. To minimize the stress associated with exposure to hot or cold water, the water temperature was kept at 33 - 36 C. At the end of each training session, the rats were dried with a towel and hair dryer. The sedentary-young and sedentary-aged animals were transported to the same training room but remained in their cages during the training hour and were handled daily.

Malondialdehyde measurement
Malondialdehyde (MDA), a biomarker for lipid peroxidation, were estimated at the endpoint of experiment. As for the test, rats were used to measure oxidative stress in the aging brain tissue. After the rat was perfused and sacrificed under anesthesia, the data were obtained from the whole brain tissue. The supernatants of both tissue samples were used to analyze MDA levels by TBARS assay kit (Cayman Chemical Co, USA) according to the manufacturer's instruction. This protocol was adapted from study of Wang X, et al. [10] The results of MDA would be expressed in nM/mg protein unit.

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VEGF and p-Akt immunoassay

Based on the manufacturer's instructions, The supernatant aliquots were used for analyzing the tissue VEGF level, p-Akt protein contents by enzyme-linked immunosorbent assay (ELISA) (R&D system, USA; Cusabio Biotech, China).

For VEGF immunoassay, all reagents, standards and samples were prepared beforehand. Each well was filled with 50 μL of Assay Diluent RD1N. Then, 50 μL of standard and sample were added per well. The plate was covered with a plate sealer and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker, set at 500 ± 50 rpm. Then, the plate was washed by filling Wash Buffer (400 mL) to each well using a multichannel pipette. The process was repeated four times for a total of 5 washes.. After washing the plate, 100 μL of mouse VEGF conjugate was added to each well. A plate sealer was incubated for 2 hours at room temperature on the shaker. Then, the same plate washing steps were done again. 100 μL of substrate solution was added to each well afterwards. The plate was incubated for 30 minutes at room temperature in the box to protect from light. After incubation, 100 μL of stop solution was added to each well. The optical density was evaluated an automated microplate reader at 450 nm with wavelength set to 540 nm or 570 nm.

For p-Akt immunoassay, the capture antibody was immediately diluted to a working concentration of 6.0 mg/mL in phosphate buffer saline (PBS) without carrier protein before coating a 96 well microplate with 100 mL per well of the diluted capture antibody. Then, the plate was sealed and incubated overnight at room temperature.

The plate was added 100 mL of standards and samples per well and incubated for 2 hours at room temperature. To wash the plate, wash buffer (400 mL) was added to each well, repeating the process twice for a total of 3 washes. After washing the plate, 100 mL of the diluted detection antibody was added and incubating for 2 hours at room temperature. Then, the same plate washing steps were repeated. 100 mL of the diluted Streptavidin-HRP was added to each well afterwards. The plate was incubated for 20 minutes at room temperature. The plates were washed again with the same steps before adding 100 mL of substrate solution to each well and incubating for 20 minutes at room temperature in the box to protect from light. After incubation, 50 mL of stop solution was added to each well. The optical density was determined with an automated microplate reader at 450 nm with wavelength set to 540 nm or 570 nm.

CD31 Immunohistochemistry

In this study, immunohistochemistry method [11, 12] was used to detect the expression of CD31 of the aging brain. After ice-cold phosphate buffer saline (PBS) containing heparin perfusing all rats from each group through the left cardiac ventricle, the brain was removed and cut the forebrain by brain matrix, 7 mm. from the start point and cut 2 mm thick and fixed in 4% paraformaldehyde (pH 7.4) for 24 hrs. The brain sections were embedded in paraffin box and cut at 2 μm before placing on slides and put the section into 60 Celsius oven overnight. After de-paraffinization and rehydration, the slides went through antigen retrieval process (DaKo, US) by warming the slides at 100% power for 5 min and 30% power for 10 min. After blocking sections in 3% H_2O_2, nonspecific protein-blocking was performed to the sections by using diluent antibody (Dako, US). The sections were incubated in primary antibody which are rabbit polyclonal antibody CD31 (Cat: RB 10333P, 1:500 dilution, Thermo scientific) overnight at 4°C. Then, the process of incubation with secondary antibody (anti-rabbit, DAKO) for 30 min at room temperature was conducted. A light microscope (Nikon eclipse TS100, Japan) was used to photograph the immunoreactive cells number of CD31 at 40× magnification in eight areas of the brain, 4 pictures per area. The number of positive cells were analyzed by the Image-Pro plus 6.0.

Statistical analysis

The data were expressed as the mean ± standard error of mean (SEM). Any significant differences between groups were determined using one-way analysis of variance (one-way ANOVA), and differences between pairs of means were evaluated by the least significant difference test. To evaluate the difference between the SE-Aged and ET-Aged groups, Student's t - test for unpaired values was used. Differences were statistically significant if P-value was less than 0.05. The data were analyzed using SPSS 16.0 for Windows (SPSS Inc., USA).

Results

All physical adaptations including body weight, mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure of sedentary-young group (SE-Young), a sedentary-aged group (SE-Aged) and a trained-aged group (ET-Aged) are summarized in Table 1.

Table 1. Body weight (g), mean arterial blood pressure (mmHg), systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and brain tissue malondialdehyde (nM/mg protein) in the SE-Young, SE-Aged, and ET-Aged groups.

	SE-Young	SE-Aged	ET-Aged
Body weight (g)	500.67 ± 9.25	$711 \pm 22.98^{*}$	729.33 ± 15.45
	(5)	(8)	(8)
Mean arterial blood pressure (mmHg)	102.12 ± 4.51	$125.54 \pm 5.28^{*}$	107.32 ± 1.96
	(5)	(8)	(8)
Systolic blood pressure (mmHg)	114.17 ± 5.85	$141.33 \pm 4.29^{*}$	$120.67 \pm 2.27^{\#}$
	(5)	(8)	(8)
Diastolic blood pressure (mmHg)	96.11 ± 3.91	$117.67 \pm 5.98^{*}$	$100.67 \pm 1.87^{\#}$
	(5)	(8)	(8)
Plasma malondialdehyde (nM/mg protein)	2.46 ± 0.11	$3.41 \pm 0.31^{*}$	$2.65 \pm 0.26^{\#}$
	(5)	(8)	(8)

Values are expressed as the mean ± SEM, and the number of rats is shown in parentheses.
$^{*}P < 0.05$; significantly different from the SE-Young group
$^{\#}P < 0.05$; significantly different from the SE-Aged group

Mean arterial blood pressure

Resting blood pressure was measured with pressure transducer (Statham, USA) which connected to polygraph system (Nihon Koden, Japan). In Table 1, the systolic blood pressure of the sedentary-aged group was significantly higher than that of the sedentary-young group while the systolic blood pressure of the trained-aged group was significantly lower than that of the sedentary-aged group. Because of an 8-week exercise training program, the diastolic blood pressure of the trained-aged group significantly decreased when compared to the sedentary-aged group ($P < 0.05$). It also showed significantly higher mean arterial blood pressure (MAP) in the sedentary-aged group than in the sedentary-young group. The MAP of the trained-aged group significantly decreased when compared to the sedentary-aged group ($P < 0.05$).

Malondialdehyde (MDA)

MDA level which is the indicator of oxidative stress in the brain tissue of the sedentary-young rats, the sedentary-aged rats, and the trained-aged rats are summarized in Table 1. In this study, the MDA level was significantly elevated in the sedentary-aged group when compared to sedentary-young group. In contrasted, MDA level was significantly reduced in the trained-aged group compared to the sedentary-aged group ($P < 0.05$).

Vascular endothelial growth factor (VEGF)

VEGF protein levels in brain tissue homogenate of rats in the sedentary-young group, the sedentary-aged group, and the trained-aged group are summarized in Figure 1. It could be seen that VEGF level in the sedentary-aged group was significantly lower than that is the sedentary-young group while the effect of the exercise training significantly increased VEGF level in the trained-aged rats compared to the sedentary-aged group ($P < 0.05$).

p-Akt level

As shown in Figure 2, p-Akt protein level in the sedentary-aged group was significantly lower than the sedentary-young group. The exercise training caused the p-Akt level in the trained-aged group to significantly elevate when compared to the sedentary-aged group ($P < 0.05$).

CD31

The expression of CD31 was detected by immunohistochemistry. According to Figure 3A, the white arrows pointed at the positive CD31 which was stained in brown color in 8 brain areas of each group. As in Figure 3B, the results were represented by the percentage of positive cell/ total area. The positive stained in the whole cortex in the sedentary-aged group was significantly decreased when compared to the sedentary-young group ($P < 0.05$) while the effect of the exercise training in the trained-aged rats significantly improved the number of CD31 positive staining cells expression when compared to the sedentary-aged group ($P < 0.05$). Image-Pro plus 6.0 software was used to confirm the results of CD31 expression.
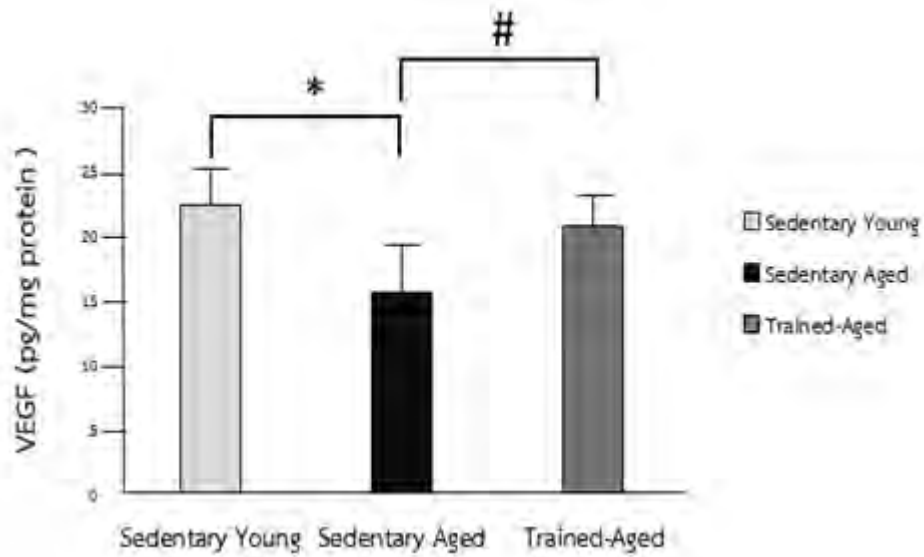
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Figure 1. The effect of exercise training on vascular endothelial growth factor protein (VEGF) levels in the sedentary-young group (SE-Young), the sedentary-aged group (SE-Aged), and the trained-aged group (ET-Aged).
*$P < 0.05$; significant differences from sedentary-young group
#$P < 0.05$; significant differences from sedentary-aged group

Figure 2. The effect of exercise training on p-Akt in the sedentary-young group (SE-Young), the sedentary-aged group (SE-Aged), and the trained-aged group (ET-Aged).
*$P < 0.05$; significant differences from sedentary-young group
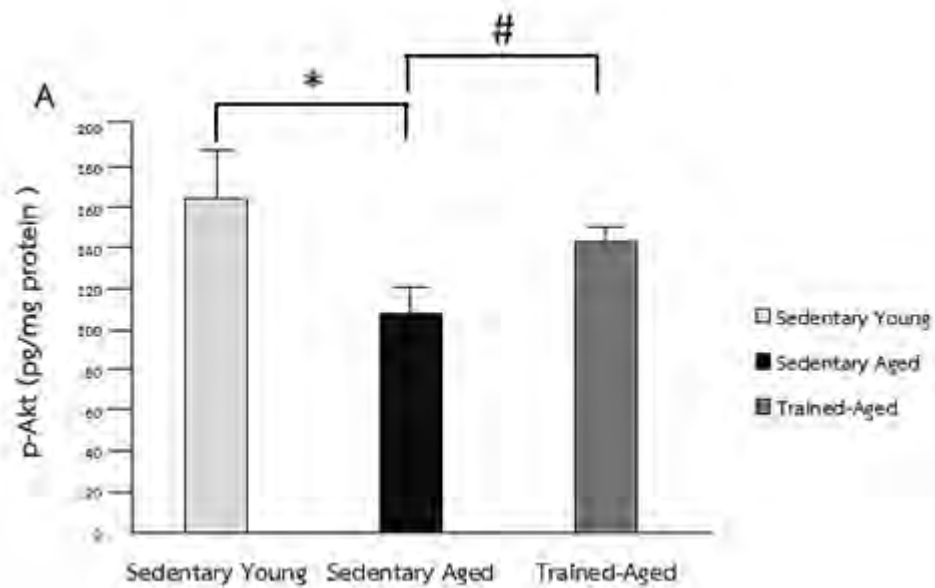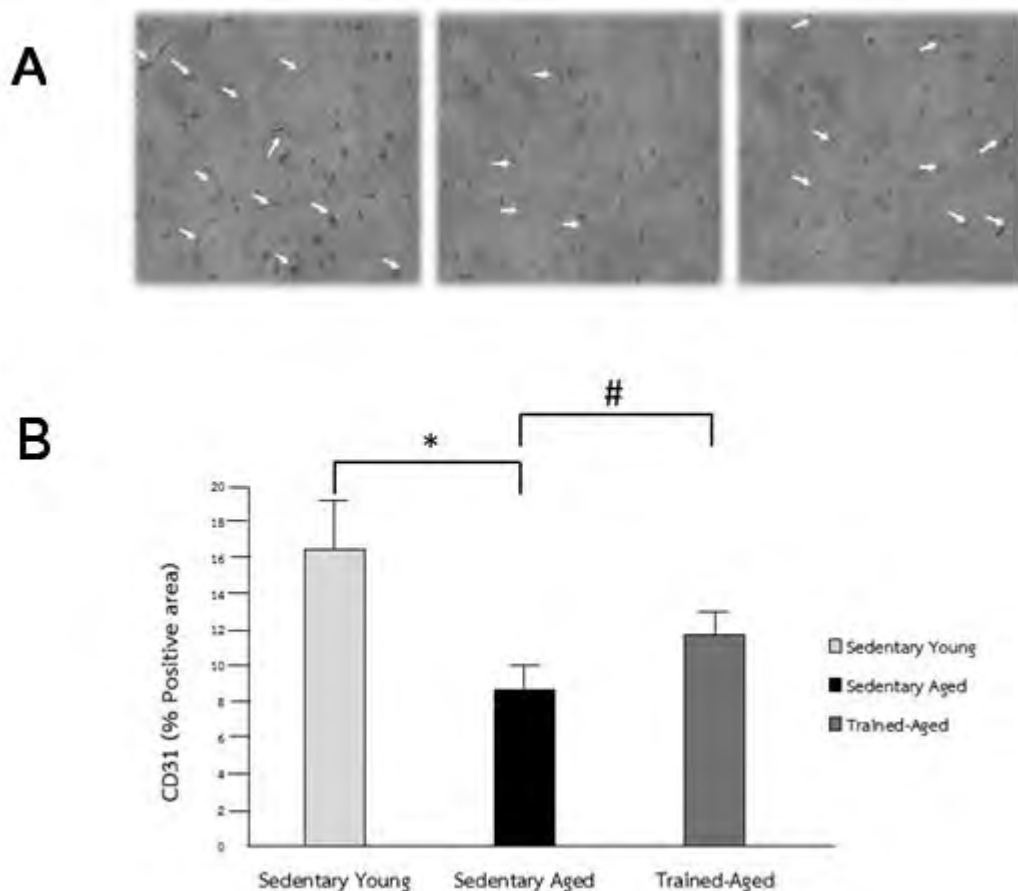#$P < 0.05$; significant differences from sedentary-aged group

Figure 3. Effect of exercise training on CD31 expression (A) using an immunohistochemistry assay and observed under a light microscope with $40 \times$ magnification as indicated by white arrows. (B) The result represented by percentage of positive cell/ total area of sedentary-young group (SE-Young, $n = 5$), the sedentary-aged group (SE-Aged, $n = 8$), and the trained-aged group (ET-Aged, $n = 8$).
$*P < 0.05$; significant differences from sedentary-young group
$\#P < 0.05$; significant differences from sedentary-aged group

Discussion

In the present study, the protective effects of moderate exercise training on brain microvessels against age-induced microvascular changes were revealed. It was shown that the preventing mechanism might be associated with stabilized level of VEGF, p-Akt expression. Moreover, tissue MDA was reduced, indicating the decrease in oxidative damage.

The results show that the changes in physiological characteristics (Table 1) including body weight, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure were age-related. These negative alterations could be improved by 8-week swimming exercise training protocol used in the study.

The sedentary-aged rat had a markedly higher body weight than the sedentary-young rat. Aging was generally associated with increases in total adiposity

from middle age until old ages. Higher body fat were the most relevant changes leading to an increased body weight and a reduction of the basal metabolic rate,[14, 15] a sedentary life-style was also a major risk for weight gain. [16] Thus, it could be concluded that the increased weight in aging was due to less physical activity. This may perpetuate the decline in muscle strength leading to muscle atrophy that attenuates resting metabolic rates to promote weight gain. [17, 18] However, in the present study, exercise training did not change the body weight between the trained-aged rats and the sedentary-aged rats (Table 1). The sedentary-aged group still had a markedly higher body weight than the trained-aged group at 24 months of age. The result above was similar to the research of Caponi PW, et al. [20] According to Caponi, aerobic exercise training did not change the body weight in

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hypertensive rats, but it increased the insulin sensitivity. Viboolvorakul S, *et al*. and Kusunoki M, *et al*. [9, 19] also found that exercise training could reduce body fat, plasma total cholesterol, and triglycerides, and improve high density lipoprotein (HDL) cholesterol in aging rats. These effects may be related to the fact that exercise increase muscle strength, lrading to higher basal and exercise metabolic rate.

Aging and hypertension were associated with an increased incidence of coronary and cerebrovascular disease. [21, 22] Cardiovascular complications were mostly related to changes in both vascular structure and function including endothelial dysfunction, increased oxidative stress, vascular remodeling, and decreased compliance. [22 - 24] Alternations of the endothelium and smooth muscle cells occurred by structural and functional abnormalities in the vasculature. When the interaction between these important two cells layer of the vascular wall changed, it influenced modulate arterial stiffness. [24] Many findings show that dysregulated vascular tone happened because age-associated endothelial dysfunction decreased NO bioavailability but raised endothelin-1 production. This increased arterial stiffness which further contributed to higher SBP. [25, 26]

Similarly, the result of this study shows that the systolic blood pressure of the sedentary-aged group was higher than the sedentary-young group. This may be because of large artery stiffening is associated with systolic blood pressure in aging. High systolic blood pressure could damage small vessels in the heart and brain and caused abnormal remodeling and rarefaction in those small vessels. These changes raised the resistance to mean arterial blood pressure. [27, 29] In addition, diastolic blood pressure increased in the sedentary-aged group compared to the sedentary-young group suggesting that this alteration of arterial was linked to the idea of endothelial dysfunction as mention above.

The present study also showed that after 8-week exercise training, MAP was significantly decreased in trained-aged group compared to the sedentary-aged group. According to animal studies, it was showed that attenuation of vascular oxidative stress associated with aerobic exercise induced antioxidant defenses by downregulation superoxide dismutase (SOD). [30 - 32] Moreover, exercise training was found to increase shear stress that restored NO bioactivity and reduce blood pressure by increasing arterial

distensibility, arterial compliance and decrease sympathetic tone. [33]

PI3K/Akt signaling was confirmed to be decreased in brains of 24-month-old rats when compared to 6-month-old rats. The decrease of PI3K/AKT signaling was also observed in various organs of older mice and aged mouse such as skeletal muscle. [34 - 35] Age-associated impairment of Akt phosphorylation in primary rat hepatocytes and cardiac muscles were remediated by alpha-lipoic acid through PI3 kinase, PTEN, and PP2. [36 - 37] Age-related death-survival balance in myocardium: an immunohistochemical and biochemical study were reported in pancreatic tissues of mouse and human and in kidney, lung and liver of mice. [38 - 39]

Tomobe K, *et al*. suggested that in 10-month-old mice, the total nuclear factor erythroid 2-like factor 2 (commonly known as Nrf2) in liver was decreased in response to a decreased AKT phosphorylation when compared to normal aged mice. [40] Similarly, the present study showed decreased phospho-Akt protein level in the sedentary-aged group in comparison to the sedentary-young group. This may be further implied that the protective effect of exercise training may be associated with the interactions between the PI3K/Akt pathway and the Nrf2-dependent antioxidant system.

In the present study, endothelial dysfunction in aging was studied by staining with the immunohistochemistry of CD31. CD31 was known as a reliable marker to identify endothelial cells. Thus, its expression was widely used as a way to understand endothelial cells distribution. [41, 42] The result of this study showed that positive stained in the whole cortex in the sedentary-aged group was significantly lower in comparison with the sedentary-young group. There were many evidences suggesting that in aging, endothelial dysfunction was related to endothelium-dependent NO-mediated vasodilatation that was impaired. Increased reactive oxygen species levels could inactivate NO in aged vascular by promoting endothelial dysfunction in both aged adults [43, 44] and older laboratory animals. [45 - 46]

The results from this study reveal that VEGF level in the sedentary-aged group decreased when compared to the sedentary-young group. Similarly, there was an evidence of the reduction of VEGFR2 expression with aging in the cerebral vessels. [47] The distribution of VEGFR2 in neuron instead of blood vessels was also reported. If the VEGFR2 expression

in brain microvessels was decreased, it could be assumed that VEGF receptor may have a role in angiogenesis regulation. [48 - 49] Exercise training did not only enhance VEGF and downstream signaling through Akt to increase NO ability for angiogenesis process, but also vascular vasomotor from these processes kept endothelial functioning. This was because the percentage of positive CD31 number in the trained-aged rats was significantly improved compared to the sedentary-aged group. The adhesion molecule CD31/PECAM-1 was pointed as a detector for shear stress. [50] There was a study showed that a mechanosensory complex at endothelial cell-cell junctions which were composed of PECAM-1 and VEGFR2 was capable of fluid shear stress detection. In this complex, the role of PECAM-1 was the mechanotransducer, implicating shear stress-dependent VEGFR2 activation. This may also suggest that exercise training induced shear stress through this process. Exercise training was also reported to activate Nrf2 translocation for nucleus in order to regulate antioxidant defense system via the mechanotransducer process of shear stress through PI3K/Akt pathway signaling in endothelial cells. [51]

Increased shear stress from exercise training also stimulated an AKT1 level. Many studies showed that the physiological hypertrophy induced by exercise training and the regulation of normal cardiac growth[52] required AKT1 was for development. [53] Moreover, upstream PI3K could activate AKT1 induced by swimming exercise through phosphorylation in the LV. [54] Hence, exercise training was another way to stimulate PI3K/Akt pathway for maintaining Nrf2/ARE signaling. This led to the transcription process of antioxidant enzyme that protected cerebral endothelial in the brain from ROS. In this study, the malondialdehyde level in the trained-aged group was significantly lower than the sedentary-aged group.

Conclusion

Our findings indicate that the effective mechanisms of exercise training on age-induced brain microvascular changes are involved the up-regulation of VEGF and p-Akt expression in association with changes in the oxidant-antioxidant balance.

Acknowledgements

Conflict of interest

The authors, hereby, declare no conflict of interest.

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ผลของการฝึกออกกำลังกายต่อความหนาแน่นของหลอดเลือดจุลภาคและการแสดงออกของเอ็นอาร์เอฟ 2 ใน สมองของหนูแก่

Effect of exercise training on microvascular density and Nrf2 expression in aged-rat brain

ฤทธิชัย อุดมทรัพย์[1] กฤติน ภมรสูตร[1] สุทธิลักษณ์ ปทุมราช[2] และชีพสุมน วิบูลย์วรกุล*[1]

Rittichai Udomsab[1], Krittin Pamonsout[1], Suthiluk Patumraj[2], and Sheepsumon Viboolvorakul*[1]

[1]ภาควิชาวิทยาศาสตร์การแพทย์ คณะวิทยาศาสตร์ มหาวิทยาลัยรังสิต

[2]ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

*ผู้ประสานงานหลัก: sheepsumon.v@rsu.ac.th

บทคัดย่อ

การลดลงของหลอดเลือดจุลภาคพบได้ในสมองของผู้สูงอายุ ซึ่งการลดลงของหลอดเลือดจุลภาคในสมองช่วงสูงวัย สัมพันธ์กับการเกิดภาวะเครียดออกซิเดชัน โดยพบว่าเอ็นอาร์เอฟ 2 (Nrf2) ซึ่งมีหน้าที่สำคัญต่อการต้านภาวะเครียดออกซิเดชัน ของเซลล์นั้นมีการทำงานบกพร่องในช่วงสูงวัย การออกกำลังกายสามารถเพิ่มอัตราการไหลเวียนเลือดสมองและเพิ่มจำนวนของ หลอดเลือดจุลภาคสมอง อย่างไรก็ตามกลไกของการฝึกออกกำลังกายต่อการเปลี่ยนแปลงของหลอดเลือดจุลภาคและ Nrf2 ของ สมองช่วงสูงวัยยังไม่มีรายงาน งานวิจัยนี้จึงมีจุดประสงค์เพื่อศึกษาผลของฝึกออกกำลังกายต่อการเปลี่ยนแปลงของหลอดเลือด จุลภาคและการแสดงออกของ Nrf2 ในสมองช่วงสูงวัย โดยใช้หนูแรทแบ่งเป็น 3 กลุ่ม คือ กลุ่มหนูวัยเจริญพันธุ์, กลุ่มหนูแก่ไม่ได้ ออกกำลังกาย และกลุ่มหนูแก่ได้รับการฝึกออกกำลังกายด้วยการว่ายน้ำเป็นเวลา 8 สัปดาห์ โดยสมองของหนูทั้ง 3 กลุ่มนำไป ตรวจวิเคราะห์ความหนาแน่นของหลอดเลือดจุลภาคและการแสดงออกของ Nrf2 ด้วยวิธีอิมมูโนฮิสโตเคมิสทรี ผลการศึกษา พบว่าการฝึกออกกำลังกายสามารถป้องกันการลดลงของการแสดงออกของ Nrf2 และความหนาแน่นของหลอดเลือดจุลภาคใน สมองของหนูแก่ ดังนั้นจึงสรุปได้ว่าการฝึกออกกำลังกายสามารถป้องกันการลดลงของหลอดเลือดจุลภาคในช่วงสูงวัย ซึ่งเกี่ยวข้อง กับกลไกการต้านอนุมูลอิสระของ Nrf2

คำสำคัญ: *การฝึกออกกำลังกาย, ภาวะเครียดออกซิเดชัน, หลอดเลือดจุลภาคสมอง*

Abstract

Reduction of microvascular loss in the brain contributes tissue perfusion insufficiency with advancing age. Microvascular deterioration in aged brain relates to oxidative stress. Nrf2, a transcription factor plays an important role in cellular antioxidant defense, markedly dysfunction in aged-tissues. Exercise training has beneficial effects to brain health, including promoted blood flow and enhanced angiogenesis in elders. However, the mechanism of exercise training on Nrf2-related microvascular alterations in brain during aging has not been fully elucidated. This study aimed to investigate the effect of exercise training on Nrf2 expression and microvascular density in aged brain. Male rats were divided into 3 groups: sedentary-young, sedentary-aged, and trained-aged. Exercise program included swimming exercise for 8 weeks. The brain tissues were harvested to examine microvascular density and Nrf2 expression by immunohistochemistry. The results showed that exercise training could prevent Nrf2 downregulation and microvascular loss in aged-rat brain. These results suggest that exercise training can protect brain microvascular deterioration against aging, associated with Nrf2-dependent antioxidant defense.

Keywords: exercise training, oxidative stress, cerebral microvasculature

ประวัตินักวิจัยและคณะ

<u>หัวหน้าโครงการ</u>

ชื่อ – สกุล	นางสุทธิลักษณ์ ปทุมราช
ตำแหน่งทางวิชาการ	ศาสตราจารย์
สถานที่ทำงานปัจจุบัน	ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ความเชี่ยวชาญ	Microcirculation and endothelial function
	Computer-assisted image analysis

<u>ผู้ร่วมวิจัย</u>

ชื่อ – สกุล	นางสาวชีพสุมน วิบูลย์วรกุล
ตำแหน่งทางวิชาการ	-
สถานที่ทำงานปัจจุบัน	หมวดวิชาสรีรวิทยา ภาควิชาวิทยาศาสตร์การแพทย์ คณะวิทยาศาสตร์ มหาวิทยาลัยรังสิต
ความเชี่ยวชาญ	Microcirculation
	Exercise Physiology