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Exercise Training Could Reduce Age-Induced Microvascular Impairment Related to Its Anti-Oxidant Potential

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Research Article

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ABSTRACT

Objective: During aging, an ineffective perfusion of tissues/organs is a major risk factor for several diseases. Age-induced oxidative stress has been proposed to correlate with this age-related microvascular dysfunction including angiogenesis impairment. It has been demonstrated that exercise training could ameliorate oxidative damage, as well as, enhance angiogenesis in various organs. Therefore, the present study aims to investigate whether exercise training can prevent alterations of capillary vascularity in brain and bone during aging.
Design and method: Male Wistar rats were divided into three groups: sedentary-young (aged 4-6 months), sedentary-aged (aged 20-22 months) and train-aged (aged 20-22 months). The exercise program included swimming training 5 days/week for 8 weeks. We directly observed microvasculature of brain and bone by using a laser scanning confocal microscopic system. The microvascular networks were visualized by fluorescein isothiocyanate labeled dextran and were analyzed for capillary vascularity by image analysis software. Blood was collected to determine the level of malondialdehyde, an indicator of oxidative stress.
Results: In sedentary-aged group, the malondialdehyde level was significantly increased, whereas capillary vascularities in brain and bone were significantly decreased when

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compared to the sedentary-young group (P<0.05). In train-aged group, capillary vascularities in brain and bone were significantly higher, whereas the malondialdehyde level was significantly lower when compared to the sedentary-aged group (P<0.05). Besides, the result also showed a linear correlation between capillary vascularity and malondialdehyde level.
Conclusion: The exercise training could attenuate age-induced suppression of capillary vascularity in brain and bone, closely related to exercise-ameliorated oxidative stress during aging.

Keywords: Exercise training; aging; capillary vascularity; antioxidant; oxidative stress; laser scanning confocal microscopy;

1. INTRODUCTION

The number of elderly in the world is growing rapidly. According to the United Nations Report (United Nations, 2007), since 1950 the proportion of older persons has been rising steadily, passing from 8 percent in 1950 to 11 percent in 2007, and is expected to reach 22 percent in 2050. Thailand also experienced a rapid and extensive growth, starting in the late 1960s. The Thailand's National Economic Social and Development Board (NESDB, 2007) indicated that Thai aging population will continue at a rapid pace between 2000 and 2030 with persons aged 60 and older expected to constitute one fourth of the population by the end of that period. The rapid growth of global aging population has profound implications for many aspects of human health.

The result of aging process is a decline in body functions, and vascular system is no exception to this. Angiogenesis, the development of new microvessels from preexisting vasculature, is delayed and altered with age (Sadoun and Reed, 2003). The subsequent impairment of angiogenesis is detrimental to the revascularization of ischemic organs and to the repair of injured tissues. Moreover, the resultant pathophysiology of impaired angiogenesis may be related to the production of ROS (Benndorf et al., 2008).

Oxidative stress in a physiological setting can be defined as an excessive bioavailability of reactive oxygen species (ROS), which is the net result of an imbalance between production and destruction of ROS (with the latter being influenced by antioxidant defenses). The "oxidative stress theory" of aging is one of the prevalent theories which proposed that a progressive and irreversible accumulation of oxidative damage caused by ROS impacts on critical aspect of the aging process and contributes to impaired physiological function, increased incidence of disease, and a reduction in life span (Kregel and Zhang, 2007). ROS are the primary causal factor underlying aging-associated declines in physiological function. One of the most common types of evidence presented by investigators is the strong correlation between aging and an increase in oxidative damage to tissues throughout the body in species ranging from lower species such as *C. elegans* to more complex mammalian species (Kregel and Zhang, 2007).

There is a number of evidence indicating that exercise might prevent vascular dysfunction associated with reduction in oxidative stress (Yeo and Davidge, 2001, Green et al., 2003, Haram et al., 2008). Moreover, numerous studies have demonstrated that exercise training can induce vascular angiogenesis under both physiological and pathological conditions. According to Degens (Degens, 1998), angiogenesis factors (VEGF and endothelial progenitor cells) significantly increased in exercise-patients with peripheral arterial disease, compared to sedentary individuals. Exercise represents a good tool to stimulate vascular angiogenesis in various organs (White et al., 1998, Suzuki, 2005, Ding et al., 2006, Viboolvorakul et al., 2009). Angiogenesis induced by exercise has been reported to cooperate with increasing expression of angiogenic factors. Lloyd et al. (Lloyd et al., 2003) reported that exercise training induced angiogenesis, presumably, might be associated with activation of angiopoietin and VEGF. Iemitsu et al. (Iemitsu et al., 2006) reported that the swimming exercise training ameliorated aging-induced reduction of capillary density, and a decrease in expression of VEGF and its receptors (Flt-1 and Flk-1). Therefore, the present study aimed to determine the protective effect of exercise training on capillary network in the brain and bone against aging process. Furthermore, we examined the relationship between capillary vascularities of both organs and oxidative stress biomarker, malondialdehyde (MDA).

2. MATERIAL AND METHODS

2.1 Animal Preparation

Two-month male Wistar rats (body weight: 200–250 g) were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Nakornpathom, Thailand. All experiment procedures were conducted according to the “Ethical Guidelines for the Uses of Animals” by the National Research Council of Thailand. The animals were housed in a room with 12:12 hour light–dark cycle until aged 4–6 months and 20–22 months. All rats were allowed free access to normal chow and tap water *ad libitum*. The animals were divided into three groups: sedentary young (aged 4–6 months, N = 3) as a control of age, sedentary-aged (aged 20–22 months, N = 3) and train-aged (aged 20–22 month, N = 3). The train-aged rats were exercised by swimming in a round plastic tank of water (34–36℃), 5 days/week for 8 weeks (Eksakulkla et al., 2009). The sedentary young and sedentary-aged rats were confined to their cage without swimming training for 8 weeks.

After 8 weeks of swimming training, the animals were anesthetized with pentobarbital sodium (50 mg/kg body weight, intraperitonealy) and a tracheotomy was performed. A common carotid artery was cannulated with a catheter for measurement of blood pressure.

2.2 Microvascular Imaging

The direct observation of bone microvascular network was made in the periosteal layer of femur under a microscope using a femur window chamber. By incising a 15 mm longitudinal skin and exposing the femur by blunt dissection between the flexor and extensor muscles, the femur chamber was positioned at an area for the observation of bone microvascular network (Kasiyaphat et al., 2007, 2008).

For the direct observation of microvascular network on the surface of cerebral cortex, a craniotomy was prepared over the left parietal cortex. The cranial window was suffused with artificial cerebral spinal fluid.

Fluorescein isothiocyanate-labeled dextran (FITC-dextran) was intravenously injected to visualize the intralumen of microvessels. The microvascular networks were imaged using a laser scanning confocal microscopy system and were recorded fluorescent images for further analysis of the capillary vascularity using the image analysis software, Global Lab Image/2 (Data translation Co. USA).

2.3 Determination of Capillary Vascularity

The microvascular networks images was analyzed using Global Lab Image/2 software. The software calculated the capillary vascularity in the region of interest (ROI). The RGB images will be converted into binary images in which vascular pixels and perivascular pixels were discriminable based on grayscale intensity. The capillary vascularity was defined as the number of microvessel pixels divided by the total number of pixels within the ROI. One fluorescent image (frame) and three ROIs in each rat were obtained. Accordingly, averaged CV over three ROIs was performed, and the mean CV level in each group was obtained. The mean CV level was used as an index of bone capillary density.

2.4 Determination of Malondialdehyde (MDA) Level

At the end of experiments, whole blood was collected from abdominal aorta for determining malondialdehyde (MDA) level (a common oxidative stress marker). The MDA level was determined using a commercial assay kit (Cayman Chemical, MI).

2.5 Statistical Analysis

All results are expressed as the means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was made to examine the difference of each parameter. Differences were considered significant at $P < 0.05$. The data were analyzed using the SPSS program (version 11.5) for Windows.

3. RESULTS AND DISCUSSION

Body weight, arterial blood pressure and MDA level for three groups were shown in Table 1. The body weight significantly increased in both sedentary-aged and train-aged rats compared to sedentary-young rats. However, there was no significant difference in the body weight between the sedentary-aged and the train-aged rats (*P*-value = 0.171). The mean arterial blood pressure (MAP) in the sedentary-aged group was significantly higher compared to the sedentary-young group. Interestingly, the train-aged rats were significantly reduced in MAP compared to the aged rats without exercise. The MDA level in the sedentary-aged group significantly increased compared to the sedentary-young group. However, in the train-aged group, the MDA level was significantly reduced compared to either the sedentary-young or sedentary-aged groups. These results suggest that exercise training has an effect of reduction in elevating blood pressure and attenuation in increasing MDA level (or oxidative stress) with age.

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Table 1. Body weight, mean arterial blood pressure (MAP) and malondialdehyde (MDA) level of rats in sedentary-young, sedentary-aged and train-aged groups

	Sedentary-young	Sedentary-aged	Train-aged
Body weight (g)	485.8±8.0	641.7±33.5**	705.0±22.6**, ^{ns}
MAP (mmHg)	122.4±2.6	161.1±8.2**	126.7±3.6††
MDA (μM)	2.9±0.2	3.7±0.1*	2.8±0.1††

Data are expressed as mean±SEM.
** Significantly different from the sedentary-young group (P<0.01).*
*** Significantly different from the sedentary-young group (P<0.05).*
† Significantly different from the sedentary-aged group (P<0.01).
†† Significantly different from the sedentary-aged group (P<0.05).
^{ns} non-significantly different from the sedentary-aged group (P<0.05).

Figure 1 shows the examples of fluorescent images of femur microvasculature in sedentary-young, sedentary-aged and train-aged rats. Apparently, the sedentary-young rats exhibited clear images with rich capillaries. In aged rats without exercise, the capillary density was markedly reduced compared to that in young rats. However, in aged rats with exercise training, capillaries appeared to become greater in density, developed by exercise training.

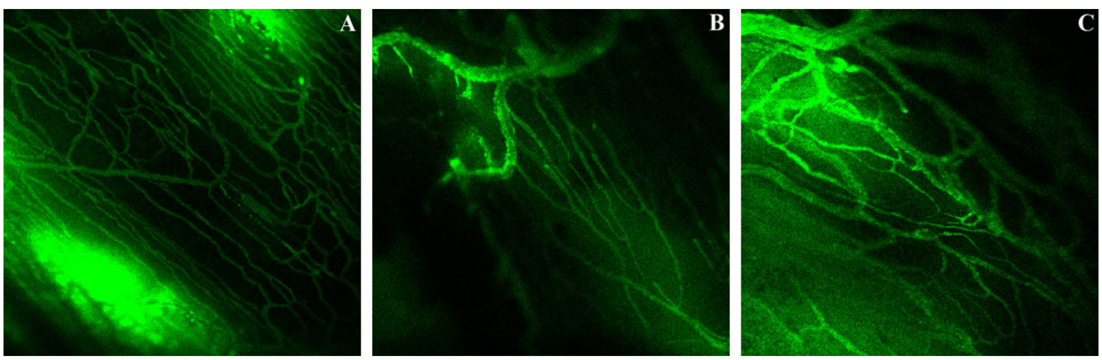

Fig. 1. Confocal laser microscope images of bone microvasculature from sedentary-young (A), sedentary-aged (B) and train-aged (C) rats.
Femur bone microvessels were visualized using FITC-fluorescence. Magnification X10.

Figure 2 shows bone capillary vascularity in three groups (sedentary-young, sedentary-aged and train-aged rats). Apparently, the bone capillary vascularity was significantly lower in the sedentary-aged rats compared with the sedentary-young rats, and was significantly higher in the train-aged compared with the sedentary-aged rats. These results show that bone capillary vascularity was reduced during aging, which could be attenuated by exercise training.

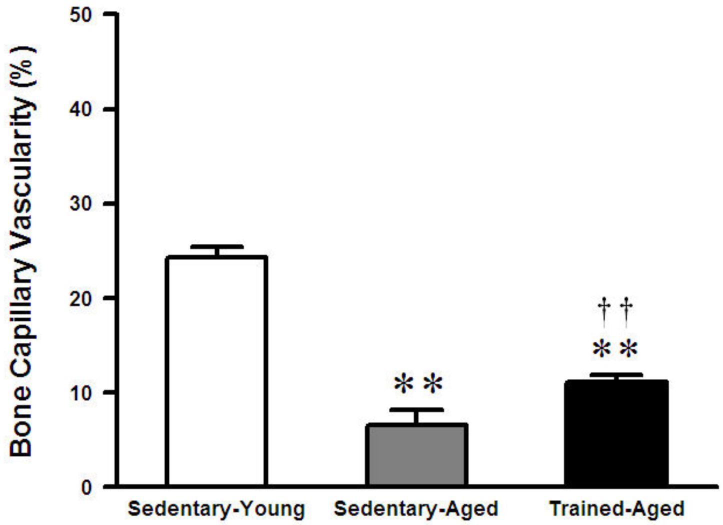

Fig. 2. Bone capillary vascularity measured from confocal laser microscopic image from sedentary-young, sedentary-aged and train-aged groups

Data are expressed as mean±SEM.

***Significantly different from the sedentary-young group (P<0.05).*

††Significantly different from the sedentary-aged group (P<0.05).

Figure 3 shows the examples of fluorescent images of microvascular network on the surface of cerebral cortex in sedentary-young, sedentary-aged and train-aged rats. The sedentary-young rats exhibited clear images with rich capillaries. In aged rats without exercise, the capillary density was markedly decreased and altered in structure compared to that in young rats. However, in training aged rats, capillaries appeared to become greater in density and size.

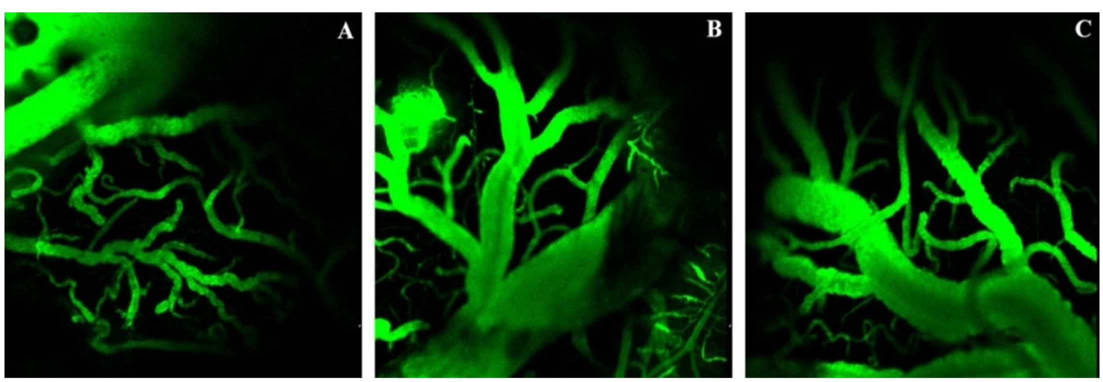

Fig. 3. Confocal laser microscope images of microvascular network on the surface of cerebral cortex from sedentary-young (A), sedentary-aged (B) and train-aged (C) rats.

Brain microvessels were visualized using FITC-fluorescence. Magnification X10.

Figure 4 shows the brain capillary vascularity in all groups. The brain capillary vascularity was significantly lower in both sedentary-aged and train-aged groups than in the sedentary-young group. However, the brain capillary vascularity in train-aged group was significantly increased compared to that in sedentary-aged. These results suggest that exercise training improves age-induced downregulation of brain capillary vascularity.

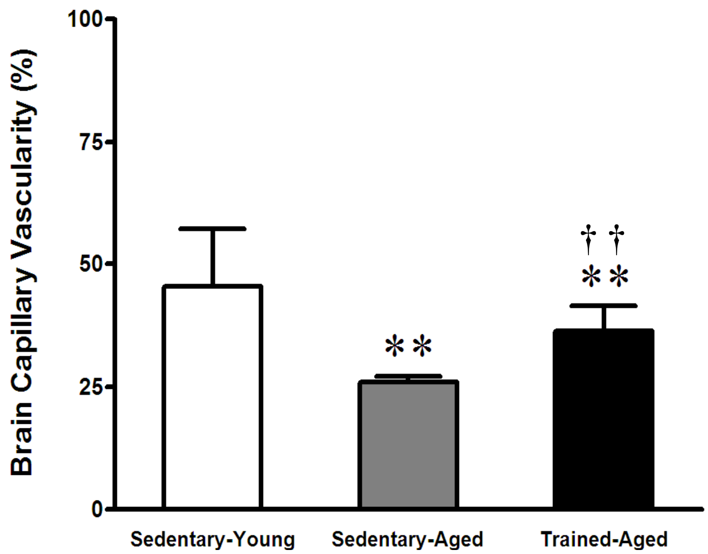

Fig. 4. Brain capillary vascularity measured from confocal laser microscopic image from sedentary-young, sedentary-aged and train-aged groups.

Data are expressed as mean±SEM.

[**]Significantly different from the sedentary-young group (P<0.05).

[††]Significantly different from the sedentary-aged group (P<0.05).

Data of bone capillary vascularity and MDA level for sedentary-young, sedentary-aged and train-aged groups were collected and plotted in **Figure 5**. A linear relationship existed between bone capillary vascularity and MDA level with a correlation coefficient, R^2 = 0.410. This result suggests that the reduction in bone capillary vascularity was linearly correlated with the increased in oxidative stress during aging.

Data of brain capillary vascularity and MDA level for sedentary-young, sedentary-aged and train-aged groups were collected and plotted in **Figure 6**. The brain capillary vascularity and the MDA level showed a linear relationship with a correlation coefficient, R^2 = 0.307. This result suggests that the reduction in brain capillary vascularity was linearly correlated with the increment in oxidative stress during aging.

This study focuses on the role of exercise on age-induced impairment of angiogenesis. In the present study, the swimming exercise training revealed the improvement of the age-induced reduction in bone and brain capillary vascularity. Moreover, the present study also demonstrated that exercise training improved oxidative stress closely correlated to the changes in capillary density.

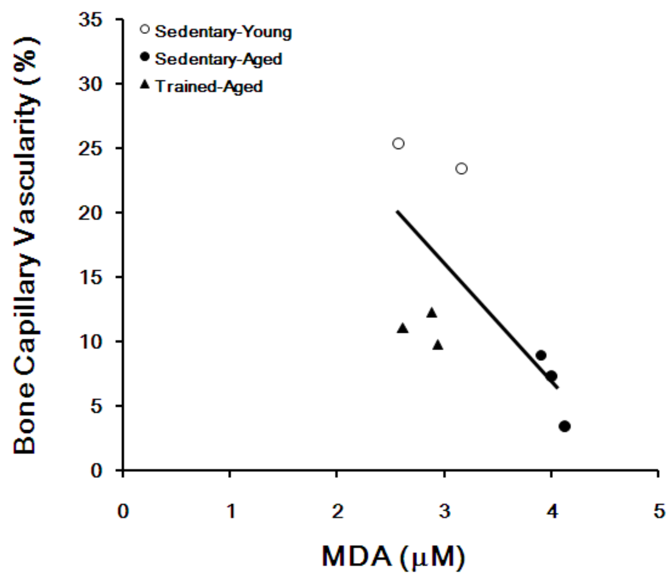

Fig. 5. Plotting of data of MDA level (x) and bone capillary vascularity (y) for three groups (sedentary-young, sedentary-aged and train-aged).

The solid line represent correlations between x and y.

The correlation is expressed $y = -7.706x + 37.94$, $R^2 = 0.410$.

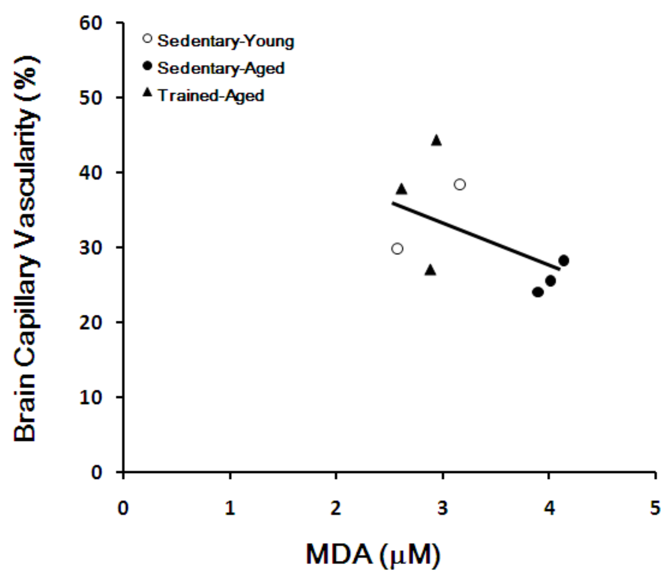

Fig. 6. Plotting of data of MDA level (x) and brain capillary vascularity (y) for three groups (sedentary-young, sedentary-aged and train-aged).

The solid line represent correlations between x and y.

The correlation is expressed $y = -6.351x + 52.70$, $R^2 = 0.307$.

The present study shows that aging induces changing in physiological characteristics (body weight, MAP). Body weight increment occurs due to alterations in body fat content and particularly body fat distribution, and reduction of skeletal muscle mass and strength, which are common found in the elderly (Elmadfa and Meyer, 2008). However, no significant difference in body weight between the sedentary-aged and the train-aged groups. This reason may be that swimming protects hot condition, which can suppress ghrelin (appetite-stimulated hormone) (Tomasik et al., 2005). MAP also elevated in the sedentary-aged group. Remodeling of vascular wall including intima and medial thickening, increased arterial stiffness, increase oxidative stress, impaired endothelial function and reduction of cardiac output, heart rate and stroke volume all occur as part of the aging process. Many studies have shown reduced NO-dependent vasodilation in an elderly population (Lyons et al., 1997, Singh et al., 2002). NO contributes to regulation of blood pressure and impaired NO bioactivity is associated with hypertension (Hermann et al., 2006). Nevertheless, exercise training can improve MAP in aged rats as shown in the present study. As many studies shown that regular physical activity can improve endothelial function, increases in vascular eNOS protein level may play a role in exercise-induced improvements in NO bioavailability (Rush et al., 2005), leading to ameliorate MAP in aging.

Previous studies have shown that age-dependent impairment of angiogenesis is primarily due to a defect in the transcriptional regulation of VEGF (Rivard et al., 1999). Exercise training has been shown to increase capillary density with neocapillarization in various tissues. Recent data indicate that exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in the heart (Iemitsu et al., 2006). They have reported that the capillary density in rat hearts was significantly lower in sedentary aged than in hearts from sedentary young and importantly, exercise training caused a significant recovery of angiogenesis in hearts from sedentary aged. Moreover, exercise has been shown to increase VEGF serum levels and circulating endothelial progenitor cells in patients with peripheral arterial disease (Sandri et al., 2005). The present data also showed that the eight-week swimming exercise enhanced bone and brain capillary vascularity aged animals. The noted increases in vascular antioxidant enzymes suggest a role in improved NO-mediated endothelium-dependent function that accompanies exercise training-induced angiogenesis (Rush et al., 2005).

The evaluation of capillary vascularity based on the fluorescent microvascular images by selecting three areas (ROIs) covering only capillaries, the number of pixels within all capillaries as well as the total number of pixels in each ROI, and the capillary vascularity was calculated and expressed as percentage (Yoysungnoen et al., 2005). Using confocal laser microscopy, the microvasculature at either surface of femur or surface of cerebral cortex could be easily exposed and clearly viewed. The obtained images were correctly analyzed to calculate the capillary vascularity.

Laser confocal microscopic images are most useful to analyze the capillary network (Komai et al., 2005) and the fine structure of mesenteric microvessels (Nakano et al., 2007). The obtained FITC-fluorescent images of the microvasculature using laser confocal microscopy were clearer than FITC-fluorescent images using fluorescence videomicroscopy. The images demonstrated different features of the microvasculature between sedentary-young and sedentary-aged rats as showed in Figure 1 and Figure 3. Interestingly, the number of capillaries was markedly decreased in aged rats, in particular, relatively large capillaries disappearing. The present study used the capillary vascularity as an index of capillary density in the tissue.

The present results showed a linear correlation between bone capillary vascularity and MDA level, a common indicator of oxidative stress, and between brain capillary vascularity and MDA level. This indicates that aging caused organs microvascular deterioration in associated with high MDA production. A mechanism underlying microvascular changes during aging was due to increased oxidative stress (Almeida et al., 2007). The excess of free radical could destroy the vascular endothelial lining, causing suppression of blood supply to tissues. Exercise training acceptably demonstrated free radical scavenging property. The result of decreasing the MDA level in the train-aged group, compared with the sedentary-aged group, indicated that the swimming exercise program ameliorated the increasing oxidative stress with age. Moreover, the train-aged animals presented higher blood perfusion than the sedentary-aged animals. These results indicated that exercise training improved tissue perfusion and attenuated increasing oxidative stress with age.

4. CONCLUSION

The exercise training could attenuate age-induced suppression of bone and brain capillary vascularity closely related with exercise-ameliorated oxidative stress during aging.

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