Effects of Exercise Training on PPARβ/δ Expression in Skeletal Muscle of Rats with Spontaneous Hypertension

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ABSTRACT

YANG, M., Y. PAN, K. LI, X. CHEN, M. LI, J. LIN, M. LI, and C. LIN. Effects of Exercise Training on PPARβ/δ Expression in Skeletal Muscle of Rats with Spontaneous Hypertension. Med. Sci. Sports Exerc., Vol. 54, No. 8, pp. 1309–1316, 2022. Purpose: This study aimed to identify the relationship and mechanism between skeletal muscle peroxisome proliferator-activated receptor β/δ (PPARβ/δ) and spontaneous hypertension. Methods: Rats were divided into four groups (n = 10): spontaneous hypertensive rats exercise group (SHR-E), spontaneous hypertensive rats sedentary group (SHR-S), Wistar-Kyoto control rats exercise group (WKY-E), and Wistar-Kyoto control rats sedentary group (WKY-S). Although the sedentary groups were placed on the treadmill without moving during the training sessions, the exercise groups were forced to run on a treadmill for 8 wk, 1 h·d−1, 5 d·wk−1. After training, the density and area of gastrocnemius microvessels were observed. PPARβ/δ, vascular endothelial growth factor A (VEGFA), superoxide dismutase 2 (SOD-2), and nitric oxide synthase in gastrocnemius were measured by real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot. Results: Except the sixth week of age, the systolic blood pressure of SHR-S was significantly higher than that of WKY-S at all time periods. Exercise significantly reduced systolic blood pressure in SHR rats. Compared with the SHR-S group, the WKY-S group had significantly higher PPARβ/δ protein level and density of skeletal muscle microvessels. Eight weeks of exercise increased the PPARβ/δ, SOD-2, VEGFA, and microvessel density and area in the skeletal muscle of SHR. Conclusions: Exercise training promoted PPARβ/δ mRNA and protein-level expression of PPARβ/δ, SOD-2 and VEGFA in skeletal muscle, thus increasing the density and area of skeletal muscle blood vessels. These regulations contribute to the reduction of peripheral vascular resistance. This may be a potential mechanism of exercise to reduce blood pressure. Key Words: EXERCISE TRAINING, SPONTANEOUS HYPERTENSION, SKELETAL MUSCLE, PPARβ/δ

Cardiovascular diseases cause 17 million deaths annually worldwide, of which hypertension is responsible for 9.4 million and a 7% burden of disease (1–3). Globally, it is estimated that more than one-fourth of people will have hypertension by 2025 (4).

The pathophysiological mechanisms underlying the development of hypertension include increased cardiac output, aortic atherosclerosis, and increased peripheral resistance to blood flow due to the thickening of the vessel wall and the narrowing of the lumen of the remodeled small arteries. Hypertension can eventually lead to myocardial infarction, stroke, and other fatal or disabling diseases (5). Continuous aerobic exercise is effective in promoting health by regulating systolic and diastolic blood pressure (6,7). There is growing evidence that aerobic exercise has a positive impact on hypertension, especially in risk factors control, hemodynamics, and autonomic function. Meanwhile, aerobic exercise has become the primary exercise recommendation for blood pressure control in prehypertensive and hypertensive patients (8,9). The molecular and cellular responses to exercise in antihypertension have been investigated and suggested various potential mechanisms, such as decrease of sympathetic activity, amelioration insulin resistance, enhancement of calcium sensitivity, inhibition of apoptosis, and so on (10). However, the underlying mechanisms of aerobic exercise in lowering blood pressure remain unclear (11).

It has been shown that the increase bioavailability of vaso-dilatory substances, such as vascular endothelial growth factor (VEGFA), endothelial-type nitric oxide synthase (eNOS), superoxide dismutase 2 (SOD-2), and nitric oxide (NO), is effective in...
reducing peripheral vascular resistance (12). VEGFA is a potent angiogenic factor that promotes endothelial cell proliferation, migration, and survival and stimulates the production of eNOS and SOD-2. The endogenous NO generated by eNOS promotes vasodilation and inhibits platelet adhesion and aggregation to reduce arterial stiffness (13,14). SOD-2 inhibits the catabolism and consumption of NO, enhancing the effect of NO (15).

 Peroxisome proliferator-activated receptors (PPAR_α, β/δ, and γ) are a transcription factor superfamily of ligand-activated nuclear receptors. Although the functions of PPAR_α and PPAR_γ have been extensively studied, PPAR_β/δ is the least studied. In recent years, the role of PPAR_β/δ in regulating cell metabolism and cell proliferation in humans has increasingly been recognized (16,17). PPAR_β/δ promotes the expressions of antioxidant genes, stimulates VEGFA generation, and inhibits the production of reactive oxygen species (ROS) in vascular endothelial cells, thus promoting angiogenesis and improving endothelial function. PPAR_β/δ agonist GW501516 can stimulate endothelial cell proliferation and induce angiogenesis through the expressions of VEGFA or other angiogenic molecules, thereby promoting capillary growth and increasing blood flow (18). It is well known that elevated blood pressure is associated with alterations in vascular structure and function. It is well known that exercise plays an important role in lowering blood pressure. Besides, elevated blood pressure is associated with alterations in vascular structure and function. Therefore, we hypothesized that aerobic exercise promotes skeletal muscle angiogenesis by affecting the expressions of PPAR_β/δ and thus promoting the expressions of VEGFA, eNOS, and SOD-2, which in turn improves vascular elasticity, dilates blood vessels, and reduces peripheral vascular resistance, ultimately achieving a blood pressure–lowering effect.

In this study, we used 6-wk-old male spontaneous hypertensive rats (SHR) and Wistar-Kyoto control rats (WKY) as the experimental animals and conducted a randomized controlled study in sedentary and exercise groups to explore whether the increased expression of PPAR_β/δ a significant component in a mechanism of lowering blood pressure.

**MATERIALS AND METHODS**

**Animal and exercise protocol.** Twenty 6-wk-old clean-grade male SHR rats and 20 WKY rats weighing 160–200 g were purchased from the Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). The animals were separately housed at ambient room temperature (20°C–25°C) and were fed ad libitum. Automatic lighting on a 12-h cycle was offered. The bedding was changed every day to keep the cage dry and stink-free. After 1 wk of acclimatization to the environment, the SHR rats were randomly divided into SHR-exercise (SHR-E) group (n = 10) and SHR-sedentary (SHR-S) group (n = 10), and the WKY rats were randomly divided into WKY-exercise (WKY-E) group (n = 10) and WKY-sedentary (WKY-S) group (n = 10). During the experiment, rats in the SHR-E and WKY-E groups were trained on a treadmill (Techman FT-200, Chengdu, China), with exercise speed gradually increasing from 10 to 20–25 m·s·−1 and exercise time increasing from 10 to 60 min·d·−1, 1 h·d·−1, 5 d·wk·−1, for 8 wk. During the training period, the sedentary rats were placed on the treadmill without moving during the training sessions. The experiment was performed at the Experimental Animal Center of Fujian Medical University. The experimental protocol was approved by the Animal Ethics Review Committee of Fujian Medical University, China.

**Blood pressure measurement.** The rat was placed in a rat manometer (Techman BP-300A, Chengdu, China) preheated to 36°C. The occluding cuff was placed at the root of the tail, and the pulse sensor was fixed in the middle of the ventral surface of the tail. Then, the tail cuff was automatically inflated for measuring the systolic blood pressure (SBP). At the start and the end of the experiment, the SBP was measured in the conscious state using a computerized rat tail cuff system. To minimize the impact of exercise on blood pressure measurement at the end of the experiment, SBP was measured 24–48 h after the end of exercise training. Measurements were performed every 2 wk, and SBP was recorded.

**Sample collection and processing.** All rats were fasted overnight. The next day, the rats were anesthetized with ether and then killed by cervical dislocation. The left gastrocnemius muscle was removed quickly, and the long head of the left gastrocnemius muscle was separated along the muscular-tract membrane with a glass minute needle. The specimens were placed in a precooled phosphate-buffered saline solution to remove residual blood, blood vessels, and connective tissues such as the myomembrane and tendon, and washed for more than three times to minimize residual blood. Then it was dried with filter paper, wrapped in tin foil and marked, put into a container of liquid nitrogen for quick freezing, and then stored in a −80°C refrigerator for testing relevant indicators.

**Real-time polymerase chain reaction for detecting mRNA expressions.** Total RNA was extracted from the gastrocnemius muscle using Trizol reagent (Takara, Japan). Reverse transcription reaction system was prepared in accordance with kit instructions. Reverse transcription was performed at 37°C for 15 min and 85°C for 5 s in a polymerase chain reaction (PCR) instrument according to the parameters of the reverse transcription reaction system. The resulting cDNA product was stored at 4°C. According to the kit instructions, 1 μL cDNA template solution was collected, and the quantitative PCR was performed in a 20-μL system (SYBR Premix Ex Tag Gc fluorescence quantitative PCR method) to determine the relative expression of PPAR_β/δ gene in the gastrocnemius muscle. A real-time fluorescence quantitative PCR instrument (ABI 2720, Waltham, MA) was used, and the reaction conditions were as follows: predenaturation at 95°C for 30 s, followed by a total of 45 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 34 s. The relative expression of each target gene was calculated using the 2^−ΔΔCt method, with β-actin selected as the internal reference. A more detailed primer sequence is shown in Table 1.

**Western blot for detecting gastrocnemius protein levels.** Protein lysates were prepared from tissues. After 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, the target proteins were transferred onto nitrocellulose membranes.
(GE Healthcare Amersham Biosciences, Oslo, Norway). Blotted membranes were incubated with the primary antibodies against PPARβ (Santa Cruz, CA), VEGFA (Abcam, Cambridge, UK), SOD-2 (Cell Signaling Technology, Boston, MA), and eNOS (Cell Signaling Technology) as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz), followed by incubation with horseradish peroxidase–coupled secondary antibodies (1:5000; TransGen Biotech, Beijing, China). The film was developed using a chemiluminescence instrument (P&Q, Shanghai, China). Measurement of the density and area of skeletal muscle microvessels. The target area of the muscle fiber tissue was selected for imaging at 400× using a photomicroscope (Nikon Eclipse Ci-L, Tokyo, Japan). After the imaging was completed, three visual fields in each slice were chosen. The number of microvessels was counted, and the area was measured using the Image-Pro Plus 6.0 software. The averages of the vessel number and microvessel area in these three visual fields were calculated.

Statistical analysis. All the data are presented as mean ± SD. Differences among more than two groups except the SBP were evaluated by two-way ANOVA, post hoc test followed by a Student–Newman–Keuls test. SBP data were analyzed by repeated-measures two-way ANOVA. A P value of less than 0.05 was considered statistically significant.

RESULTS

Exercise training reduced SBP in SHR. The SBP of the tail artery of the rat was measured before the start of the experiment, and the result was not statistically significant between the four groups. As the experiment progressed, the changes of SBP in each group are shown in Figure 1. Except the beginning of exercise (at age of 6 wk), the SBP of SHR-S was significantly higher than WKY-S for the time of study. After 8 wk period of exercise, SBP was significantly lower in SHR-E (138.1 ± 5.3 mm Hg) than in SHR-S (163.1 ± 6.2 mm Hg). The SBP of WKY rats did not change significantly during the experiment, and there was no significantly different change in SBP between the WKY-E and WKY-S groups. Thus, exercise training significantly slowed down the development and progression of hypertension in the SHR-E group; in addition, exercise training showed no significant blood pressure–lowering effect in WKY rats.

Exercise training increased PPARβ/δ mRNA and protein-level expressions in skeletal muscle of SHR. The PPARβ/δ mRNA and protein-level expression were detected in the gastrocnemius muscle tissue of rats in each group at the end of the experiment (Figs. 2, 3). For the sedentary groups, the PPARβ/δ protein-level expression was much lower in the SHR-S group than in the WKY-S group, these data suggest that hypertension may have contributed to a decrease in PPARβ/δ expression in rat skeletal muscle. The PPARβ/δ mRNA and protein-level expression in the SHR-E group was much higher than that in the SHR-S group, suggesting that exercise training could increase PPARβ/δ expression in skeletal muscle.

Exercise training increased VEGFA protein levels in skeletal muscle of SHR. The VEGFA protein level in the gastrocnemius muscle tissue of rats in each group was detected at the end of the experiment (Fig. 4). For the sedentary groups, there was no significant difference in VEGFA protein between SHR-S and WKY-S. The VEGFA protein levels in SHR-E were significantly higher than those in the WKY-E group (n = 10) and SHR-S group (n = 10), and 20 WKY rats were divided into WKY-E group (n = 10) and WKY-S group (n = 10). All data are presented as mean ± SD. *P < 0.05 vs WKY-S; **P < 0.01 vs SHR-S. The SBP of WKY rats did not change significantly during the experiment. Twenty 6-wk-old SHR rats were divided into SHR-E group (n = 10) and SHR-S. After 8 wk period of exercise, SBP was significantly lower in SHR-E than in SHR-S group, suggesting that exercise training could increase PPARβ/δ expression in skeletal muscle.
the SHR-E group and the WKY-E group were higher than those in the SHR-S group and the WKY-S group.

**Exercise training increased SOD-2 protein levels in skeletal muscle of SHR.** The SOD-2 protein level in the gastrocnemius muscle tissue of rats in each group was detected at the end of the experiment (Fig. 5). For the sedentary groups, there was no significant difference between the SHR-S group and the WKY-S group in the SOD-2 protein level. For the exercise groups, SOD-2 protein level was elevated in both the SHR-E and WKY-E groups, whereas SOD-2 protein level was higher in the WKY-E group than in the SHR-E group, and the change trend was similar to that of PPARβ/δ protein level in each group. The SOD-2 protein level in the SHR-E group was higher than that in the SHR-S group.

**Exercise training failed to effectively increase eNOS protein levels in skeletal muscle of SHR.** The eNOS protein level in the gastrocnemius muscle tissue of rats in each group was detected at the end of the experiment (Fig. 6). There was no difference in eNOS protein level between the SHR-S and WKY-S group. The eNOS protein level was higher in the WKY-E group than in the WKY-S group, but it showed no significant difference between the SHR-E group and the WKY-S group, indicating that exercise training may promote eNOS protein expression in WKY rats but not in SHR rats.

**Exercise training increased microvessel density and area in skeletal muscle of SHR.** The microvessel density and area ratio in the gastrocnemius muscle tissue of rats in each group were detected at the end of the experiment (Fig. 7). The microvessel density in the WKY-S group was higher than that in the SHR-S group (17.33 ± 3.21 vs. 9.67 ± 2.08 vessels per high-power field), suggesting that vessels of skeletal muscle in SHR rats were more sparse than in WKY rats. The SHR-E group had higher microvessel density than the SHR-S group (9.67 ± 2.08 vs. 25.67 ± 1.52 vessels per HP), showing that exercise training played a significant role in inducing an increase in intraskeletal muscle microvessel density in SHR. The area of microvessel was similar between the WKY-S group and the SHR-S group and showed no significant difference.
The area of microvessels was much larger in the SHR-E group than in the SHR-S group (2690.43 ± 1433.32 vs 15719.27 ± 2886.78 pixels), suggesting that exercise training induced an increase in the area of microvessels in the skeletal muscle of SHR rats.

**DISCUSSION**

Hypertension is among many of the modifiable risk factors in life and health, and its development is closely associated with lifestyle (19,20). Although regular use of antihypertensive medications can effectively control blood pressure, few antihypertensive drugs for prehypertension have been available (21). Exercise therapy is the cornerstone of nonpharmacological treatment for patients with prehypertension or hypertension (22–24). In the present study, the elevation of blood pressure in the SHR-E group was slightly below the SHR-S group before and after the training, whereas the blood pressure in the WKY-E group and the WKY-S group did not remarkably change. It suggested that the hypertensive effect of the exercise was significant in hypertensive rats (Fig. 1). The trend of blood pressure in SHR was similar to other researches, including large cohort studies and animal models (8,10,25).

The gastrocnemius muscle, made up of rapidly oxidized glycolytic muscle fibers, contracts more quickly than other lower limb muscles and is more resistant to fatigue, making it important during treadmill running. Previous studies have shown that regular exercise training for the SHR can improve the dilatation response to gastrocnemius blood flow (26). Through individualized endurance training, the gastrocnemius muscle of transgenic sickle cell disease mice showed an adaptive response to the balance of oxidants and antioxidants, whereas similar results were not found in soleus and plantaris muscles (27); PPAR γ gene was highly expressed in the gastrocnemius muscles of metabolic syndrome rats modeled by SHR (28). Based on the physiological characteristics of gastrocnemius muscle and the aforementioned three previous studies, we chose gastrocnemius muscle in the experimental design at that time.

PPARβ/δ is widely expressed in different cell types including endothelial cells, skeletal myocytes, keratinocytes, vascular smooth muscle cells, and monocytes–macrophages (29). Moreover, PPARβ/δ plays an important role in regulating the expressions and functions of these cells (30,31). Studies have shown that the activated PPARβ/δ promotes the proliferation of endothelial cells and endothelial progenitor cells. The activated PPARβ/δ is a vital modulator of angiomyogenesis via the paracrine effects of endothelial progenitor cells in a mouse skin punch wound model (32). PPARβ/δ regulates endothelial cell function through multiple mechanisms including oxidative damage, inflammation, coagulation, and cell proliferation and apoptosis (33). It increases the phosphorylation of NO synthase and the release of NO in endothelial cells, upregulates the expressions of antioxidant enzyme genes, reduces inflammation and apoptosis, and regulates angiogenesis (34).

The activation of PPARβ/δ can help lower the risks of cardiovascular diseases. However, the underlying mechanisms, especially in the vascular system, remain unclear (33). In the present study, skeletal muscle PPARβ/δ protein expressions were lower in the SHR-S group than in the WKY-S group after...
the completion of the experiments. PPARβ/δ mRNA and protein expression significantly increased in the SHR-E group, suggesting that exercise therapy can increase skeletal muscle PPARβ/δ mRNA and protein expression.

In our experiments, we examined the protein expressions of VEGFA, eNOS, and SOD-2 in skeletal muscles of rats. We found that the changes in VEGFA and SOD-2 had the same trend. Compared with the WKY-S group and the SHR-S group, there was no difference in expression of the two proteins, but exercise significantly increased VEGFA and SOD-2 proteins expression in SHR rats. Notably, the expressions were even higher in the WKY-E group than those in the SHR-E group. VEGFA, probably the best known and most extensively studied angiogenic growth factor, is believed to be important for exercise-induced angiogenesis in skeletal muscle (35). Research has shown that VEGFA may have the potential to modulate endothelial function, thereby accelerating or slowing down the progression of atherosclerotic disease (36). Hansen et al. found that VEGFA protein level increased after endurance training in patients with cardiovascular disease, which was consistent with our findings (37). PPARβ/δ is involved in the mediation and maintenance of endothelial cell survival by VEGFA (38). This is verified by our experimental results that VEGFA protein expression and PPARβ/δ expression had a similar changing trend.

In addition, hydroxyl radicals are highly invasive under normal physiological conditions. Normally, superoxides are maintained at low levels because they can be detoxified by SOD to be H₂O₂, which eventually forms the water. Various antioxidants such as catalase, peroxidase, glutathione, and thioredoxin protect cells against ROS (39). It has been found that PPARβ/δ agonists inhibit cytokine-induced ROS production in endothelial cells by proactively regulating antioxidant genes (e.g., SOD-2), attenuating endoplasmic reticulum stress and NADPH oxidase activity, and eliminating ROS overproduction, which in turn improves NO bioavailability (40,41). This is consistent with our findings that SOD-2 increased with the increased expression of PPARβ/δ, which indicates that exercise can stimulate PPARβ/δ expression, which in turn promotes protein expressions of VEGFA and SOD-2.

We also observed microvascular changes in the rat gastrocnemius muscle under microscope and found that the density of microvessels was higher in the WKY-S group than in the SHR-S group, which were consistent with the different PPARβ/δ expressions in these two groups. It has been assumed that reduced vascular density, observed at the microcirculation level in hypertension, may be a result of impaired angiogenesis. The phenomenon, known as “rarefaction,” has been detected in patients with borderline hypertension (42). Rarefaction is more common than reduced small artery diameter in spontaneous hypertension (43). In the present study, there was no significant difference in microvascular density and microvascular area under a microscope in skeletal muscle between the WKY-E and WKY-S groups with or without exercise training, and exercise training did not cause microvascular sprouting or lumen enlargement in WKY rats. This is consistent with the finding that there were no significant fluctuations in blood pressure in the WKY-E and WKY-S groups before and after the experiment. A possible explanation is that the increased PPARβ/δ expression was more contributive to preventing vascular endothelial injury. In contrast, microvessel density and microscopic microvascular area were significantly increased in the SHR-E group compared with the SHR-S group. Endothelial cells play a central role in regulating blood pressure and blood flow by continuously regulating vascular tension through the balance between vasodilators and vasoconstrictors. PPARβ/δ causes the relaxation of vascular smooth muscle by stimulating NO release from endothelial cells. It was found that aerobic exercise increased endothelium-dependent vasodilation by 1.45% in hypertensive patients (P = 0.001) (44). These findings showed that there were beneficial changes in vascular structure and function beyond the activated vascular bed (45,46). The present study revealed a less increase in blood pressure in the SHR-E group than in the SHR-S group with the exercise training, suggesting that increased PPARβ/δ expression contributed to the massive formation of vascular collateral circulation and enlargement of the vascular lumen in skeletal muscle, which in turn reduced peripheral vascular resistance and lowered cardiac load in rats, greatly delaying/decreasing blood pressure elevation.

In patients with spontaneous hypertension, exercise training has been extensively associated with cardiovascular, metabolic, and neuroendocrine benefits, including downregulation of the renin–angiotensin–aldosterone system and sympathetic nervous system, reduction in left ventricular hypertrophy, fibrinolytic homeostasis, decreased oxidative stress, reduced arterial stiffness, and enhanced endothelial function (47,48).

Our study had some limitations. First, we did not fully explore the mechanisms of hypertension in terms of aortic vascular tone and atherosclerosis, ventricular hypertrophy, and sympathetic nerve activity in rats. Second, because of the short duration of the experiment, we did not observe the long-term changes in each of the study parameters. It has been found that there is a temporary association between the increase in VEGFA and the number of capillaries, and that the number of microvessels does not always increase over time (49). Thus, it is more likely that PPARβ/δ controls blood pressure by exerting its effect on endothelial diastolic function, which deserves further investigations.

CONCLUSIONS

Aerobic exercise training can promote PPARβ/δ mRNA level and expression of PPARβ/δ, SOD-2 and VEGFA in skeletal muscle, thus increasing the density and area of skeletal muscle blood vessels. Reducing peripheral vascular resistance and cardiac load may be a potential mechanism of exercise to reduce blood pressure.

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The authors declare that they have no conflicts of interest related to this study.
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