Improvement of Acetylcholine-Induced Vasodilation by Acute Exercise in Ovariectomized Hypertensive Rats

Tsung-Lin Cheng¹², Yi-Yuan Lin³, Chia-Ting Su⁴, Chun-Che Hu⁵, and Ai-Lun Yang⁵

¹Department of Physiology, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708
²Orthopaedic Research Center, College of Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 80708
³Graduate Institute of Clinical Medical Science, China Medical University, Taichung 40402
⁴Department of Occupational Therapy, College of Medicine, Fu Jen Catholic University, New Taipei City 24205
⁵Department of Sports Sciences, University of Taipei, Taipei 11153, Taiwan, Republic of China

Abstract

Postmenopause is associated with the development of cardiovascular disease, such as hypertension. However, limited information is available regarding effects of exercise on cardiovascular responses and its underlying mechanisms in the simultaneous postmenopausal and hypertensive status. We aimed to investigate whether acute exercise could enhance vasodilation mediated by acetylcholine (ACh) and sodium nitroprusside (SNP) in ovariectomized hypertensive rats. The fifteen-week-old female spontaneously hypertensive rats (SHR) were bilaterally ovariectomized, at the age of twenty-four weeks, and randomly divided into sedentary (SHR-O) and acute exercise (SHR-OE) groups. Age-matched WKY rats were used as the normotensive control group. The SHR-OE group ran on a motor-driven treadmill at a speed of 24 m/min for one hour in a moderate-intensity program. Following a single bout of exercise, rat aortas were isolated for the evaluation of the endothelium-dependent (ACh-induced) and endothelium-independent (SNP-induced) vasodilation by the organ bath system. Also, the serum levels of oxidative stress and antioxidant activities, including malondialdehyde (MDA), superoxide dismutase (SOD), and catalase, were measured after acute exercise among the three groups. We found that acute exercise significantly enhanced the ACh-induced vasodilation, but not the SNP-induced vasodilation, in ovariectomized hypertensive rats. This increased vasodilation was eliminated after the inhibition of nitric oxide synthase (NOS). Also, the activities of SOD and catalase were significantly increased after acute exercise, whereas the level of MDA was comparable among the three groups. These results indicated that acute exercise improved the endothelium-dependent vasodilating response to ACh through the NOS-related pathway in ovariectomized hypertensive rats, which might be associated with increased serum antioxidant activities.

Key Words: antioxidant activity, exercise, hypertension, ovariectomy, vasodilation
related estrogen deficiency significantly increases the risk of cardiovascular disease. After menopause, the prevalence of hypertension and hypertension-related cardiovascular complication is higher in women than men (24, 38). Specifically, systolic blood pressure (SBP) is significantly elevated in post-menopausal women, which indicates SBP as a more sensitive predictor of cardiovascular events (24). Loss of endogenous estrogens has been found to contribute to higher prevalence of hypertension in women after menopause (1, 9). One previous study indicated that surgery-induced menopause by bilateral oophorectomy in women resulted in pronounced increases in blood pressure within a few weeks (26). In addition, menopause is highly associated with the development of vascular remodeling and disease, especially the age-related endothelial dysfunction. Therefore, the loss of endogenous estrogens has been considered to interfere with a basal vasodilatory status and facilitate the risk of hypertension in women after menopause (10). Increasing evidences indicate that the pathogenesis of hypertension is strongly related to the elevated reactive oxygen species (ROS) and the reduced endogenous antioxidant mechanisms (21, 34). Also, the postmenopausal women have a higher level of oxidative markers and a lower level of antioxidant markers compared to premenopausal ones (33). However, there is little information regarding cardiovascular responses, oxidative stress, and antioxidant activity in the simultaneous post-menopausal and hypertensive status. Exercise has numerous benefits on improving the cardiovascular dysfunction and disease, especially for lowering high blood pressure and ameliorating vascular dysfunction (4, 32). A single bout of exercise (i.e. acute exercise) elicits acute and transient cardiovascular responses (16, 35). Frequent repetition of isolated exercise sessions produces more permanent cardiovascular adaptation, which could be induced by accumulative effects of several single bouts of exercise sessions (22, 32). Previous studies have shown that regular exercise improves endothelial function by enhancing the endothelium-dependent acetylcholine (ACh)-induced vasodilation and nitric oxide (NO) bioavailability in vessels of normal, atherosclerotic, or hypertensive animals (5, 11, 17, 39, 43). We also found that either the single-bout exercise or exercise training significantly improved the endothelium-dependent vasorelaxation in aortas of normal and hypertensive rats (40-44). In addition, regular exercise has been found to reduce risk factors of atherosclerosis and improve vascular regulation in postmenopausal women, which could be modulated by NO production (14, 27, 29). However, whether exercise could be effective for improving endothelial function and its underlying mechanisms in the simultaneous post-menopausal and hypertensive status remains unclear. Using an experimental model of postmenopause and hypertension, we aimed to investigate the effects of a single bout of the moderate-intensity exercise on the endothelium-dependent and endothelium-independent vasodilation in ovariectomized hypertensive rats. Furthermore, the imbalance of oxidative stress and antioxidant defense system has been suggested to be involved in the pathogenesis of cardiovascular dysfunction, including hypertension and postmenopausal status (2, 3, 18, 23, 33, 37). Exercise appears to positively modulate the pro-oxidants and antioxidant activity (7, 19, 30). Therefore, we further evaluated serum levels of oxidative stress and antioxidant enzyme activities, such as lipid peroxidation (i.e. malondialdehyde, MDA), superoxide dismutase (SOD), and catalase, in ovariectomized hypertensive rats after a single bout of exercise.

Materials and Methods

Animals and Acute Exercise Protocols

This study was conducted in conformity with the procedures in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Female spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats were purchased from National Laboratory Animal Center (Taipei, Taiwan). They were housed in an environmentally-controlled room (25 ± 1°C; 12:12-h light-dark cycle) and fed with standard rat chow and water ad libitum at Laboratory Animal Center of University of Taipei (Taipei, Taiwan). The fifteen-week-old SHRs were randomly divided into sedentary (SHR-O) and acute exercise (SHR-OE) groups. Age-matched WKY rats were used as the normotensive control group. Rats in the SHR-OE group ran on a motor-driven treadmill (Model T510E, Diagnostic & Research Instruments Co., Taoyuan, Taiwan) at a speed of 24 m/min for one hour in a single bout of the moderate-intensity exercise session, which was similar to previous studies (20, 44). In contrast, the sedentary SHR and WKY groups were placed on the treadmill without running for the same environmental stimulation. After acute exercise session, the rats were sacrificed under anesthesia and thoracic aortas were immediately isolated for various experiments described below. Also, the uterine weight and the ratio of uterine to body weight were recorded. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of University of Taipei (No. 20110003).
Resting Blood Pressure and Heart Rate

Before acute exercise session, resting blood pressure and heart rate were measured in conscious rats by the tail-cuff methods (LE5001, Panlab, Wood Dale, IL, USA). All of parameters, including SBP, diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate, were measured for three times and their means were recorded respectively.

Vasodilating Responses

The vasodilating responses induced by the endothelium-dependent and endothelium-independent agonists (e.g., ACh) and sodium nitro-prusside (SNP) were evaluated by changes in iso-metric tension developed by aortic segments set up in organ bath system. The protocols were performed in accordance with previous studies (39, 40). The segments of thoracic aortas (3 mm long) were isolated and mounted on the force transducers (Grass Instrument, West Warwick, RI, USA), and then submerged in the organ chambers containing the Krebs-Ringer solution bubbling with 95% O₂-5% CO₂ at 37°C. At the beginning of the experiment, segments were stretched to optimal passive tension (i.e., 2 g) and equilibrated for at least 60 min. After this period, segments were pre-contracted with phenylephrine (10⁻⁷ M) and exposed to cumulative concentrations of ACh (3 × 10⁻¹⁰~3 × 10⁻⁸ M) to evoke vasodilating responses. Some segments were denuded removing vascular endothelium by gently rubbing endothelial layer with a small woodstick. These segments were used to confirm that vasodilation was endothelium-dependent and not due to a smooth muscle cell effect. In addition, the vasodilating responses to cumulative concentrations of SNP (3 × 10⁻¹⁰~3 × 10⁻⁸ M), a NO donor, were also examined to observe whether the endothelium-independent vasodilation was affected by acute exercise. All of the vasodilating responses were expressed as percentages of the precontractile force, which was induced by phenylephrine (10⁻⁷ M).

Examination of Nitric Oxide Synthase (NOS) in ACh-Induced Vasodilation

The role of NOS in ACh-induced vasodilation was examined by incubation of aortic segments with a non-specific NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 10⁻⁶ M) for 15 min prior to contraction with phenylephrine (10⁻⁷ M).

Biochemical Analysis

To analyze oxidative stress level and antioxidant enzyme activities, serum concentrations of MDA, SOD and catalase were measured in the three groups of animals studied. Serum obtained from each rat was separated into aliquots and frozen at -80°C until the assay were performed. Concentration of MDA, an index of oxidative stress, was determined using an ELISA kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to manufacturer’s instructions. Antioxidant activities of SOD and catalase were measured using selective ELISA kits (Cayman Chemical Co., Ann Arbor, MI, USA) according to manufacturer’s instructions.

Statistical Analysis

All data in the table and figures are expressed as means ± SEM. Sample sizes are indicated by “n”. Results among the three groups were analyzed by one-way ANOVA and further by Tukey post-hoc analysis. The paired t-test was used to evaluate the differences between pre-treatment and post-treatment with the inhibitor (i.e., L-NAME) in each group. Statistical analyses were performed using the SPSS software (version 18.0, SPSS, Inc.). In all cases, a difference at P < 0.05 was considered statistically significant.

Results

General Characteristics

Before a single bout of exercise, body weight was significantly (P < 0.05) increased, but uterine weight and the ratio of uterine to body weight were significantly (P < 0.05) decreased, in both of the SHR-O and SHR-OE groups compared with that in the normotensive WKY group. Moreover, resting heart rate, SBP, DBP, and MAP were significantly (P < 0.05) higher in the SHR-O and SHR-OE groups than in the WKY group. However, there was no significant difference for these parameters between SHR-O and SHR-OE groups before acute exercise (Table 1).

Measurement of ACh-Induced Vasodilation

Fig. 1 shows dose-response curves to ACh in the WKY, SHR-O, and SHR-OE following a single bout of exercise. In the intact endothelium, the ACh-induced vasodilation was significantly (P < 0.05) decreased in the SHR-O group compared with that in the WKY group. Following acute exercise, the vasodilating response to ACh was significantly (P < 0.05) enhanced in the SHR-OE group compared with that in the SHR-O group (Fig. 1A). However, in endothelium-denuded vessels, the ACh-induced vasodilation was blunted and there was no significant difference among the three groups (Fig. 1B).
The Role of NOS in ACh-Induced Vasodilation

Single dose administration of ACh (10^{-8}M) in-duced a significantly lower (P < 0.005) vasodilation in aortic segments from SHR-O than in WKY and SHR-OE groups. Pre-incubation of aortic segments with L-NAME (10^{-6} M) abolished the difference in single dose vasodilation response to ACh in the three groups of rats studied and no significant difference between groups exists (Fig. 2A).

**Measurement of SNP-Induced Vasodilation**

Fig. 2B shows dose-response SNP-induced vasodilation in aortic segments of the three groups studied following a single bout of exercise. Administration of SNP induced a dose-dependent and endothelium-independent vasodilation. However, no differences were observed in the vasodilation induced by SNP in the three groups of rats studied.

**Measurements of SOD, Catalase, and MDA Levels**

Serum activity of SOD was significantly (P < 0.05) reduced in the SHR-O group compared with the WKY group, whereas it was significantly (P < 0.05) enhanced in the SHR-OE group compared with the SHR-O group. Similarly, after a period of acute exercise, the catalase activity was significantly (P < 0.05) enhanced in the SHR-OE group compared with the WKY and SHR-O groups (Fig. 3). However, serum MDA levels showed no significant difference among the three groups of rats (Fig. 4). These results imply that higher levels in serum antioxidant activities observed in the SHR-OE group may be partly responsible for the improvement of the ACh-induced vasodilation following a single bout of the exercise session.

**Discussion**

The present study clearly indicated that acute
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Moderate-intensity exercise significantly enhanced the endothelium-dependent (ACh-induced) vessel dilation in ovariectomized SHRs. This increased vasodilation was eliminated after the inhibition of NOS, indicating the NOS-related pathway. However, the endothelium-independent (SNP-induced) vasodilation was not affected by acute exercise in ovariectomized SHRs. In addition, serum antioxidant activities, including SOD and catalase, were significantly increased after acute exercise in ovariectomized SHRs, whereas the lipid peroxidation (i.e., MDA level) was comparable among the three groups. These results imply that this exercise-induced amelioration for the vasodilating response to ACh might be associated with increased serum antioxidant activities in ovariectomized hypertensive rats.

Numerous cardiovascular disorders, such as atherosclerosis, hypertension, postmenopausal status, diabetes mellitus, and heart failure, have been found to be associated with endothelial dysfunction, i.e., altered endothelium-dependent relaxation (10, 12). Menopause involves the development of cardiovascular disease, especially the age-related endothelial dysfunction and vascular remodeling (10). A few studies indicated that, in ovariectomized hypertensive

Fig. 2. (A) ACh (10^{-8}M)-induced vasodilation after the blockade with L-NAME in the WKY, SHR-O, and SHR-OE groups. (B) Cumulative dose-response curves for SNP-induced vasodilation in the three groups. *P < 0.05 (vs. WKY); †P < 0.05 (vs. SHR-O); ‡P < 0.05 (pre- vs. post-inhibition with L-NAME).

Fig. 3. Serum antioxidant activities of (A) SOD and (B) catalase in the WKY, SHR-O, and SHR-OE groups. *P < 0.05 (vs. WKY); †P < 0.05 (vs. SHR-O).

Fig. 4. The serum concentration of MDA in the WKY, SHR-O, and SHR-OE groups.
rats, the endothelium-dependent vasodilation and eNOS expression were attenuated, and adverse vascular remodeling was significant (15, 36). Similarly, we found that the ACh-induced vessel dilation was significantly decreased in ovariectomized SHRs compared with that in WKY. After the blockade with a NOS inhibitor (i.e., L-NAME), there was no significant difference between the SHR-O and WKY groups. Also, after the endothelium was denuded, there was no significant difference of the ACh-induced vasodilation between these two groups. These findings suggested that the ACh-induced vasodilation, which was NOS-related, was significantly impaired in ovariectomized hypertensive rats compared with normotensive WKY rats.

Exercise training is clinically effective in reducing cardiovascular dysfunction and mortality. These training effects have been suggested to be induced by the accumulative effects of several single-bouts of exercise sessions (22, 32). One possible mechanism by which exercise improves cardiovascular dysfunction is augmentation or amelioration in endothelial function. Acute exercise, as well as chronic exercise, significantly improves the endothelial function and endothelium-dependent vasodilation in human and animal models of cardiovascular dysfunction, such as hypertension, postmenopause, diabetes mellitus, and atherosclerosis (5, 6, 13, 25, 39, 40, 44, 45). Several studies demonstrate that regular exercise can reduce risk factors of atherosclerosis and improve vascular regulation in postmenopausal women, which could be modulated by NO production (14, 27, 29). However, there are limited researches discussing the effectiveness of exercise intervention on the population of simultaneous postmenopause and hypertension. In the present study, we found that a single bout of exercise significantly ameliorated the endothelium-dependent ACh-induced vessel dilation in ovariectomized hypertensive rats. By using a NOS inhibitor (i.e., L-NAME), this exercise-induced amelioration was eliminated, indicating the NOS-related pathway. It supports that the single-bout exercise is effective in the amelioration of endothelial dysfunction, such as increases in the endothelium-dependent and NOS-related vasodilation, in the status of simultaneous postmenopause and hypertension. Further studies are encouraged to investigate whether long-term exercise intervention induces accumulative and beneficial effects on cardiovascular dysfunction in the status of simultaneous postmenopause and hypertension.

The SNP, a NO donor, induces the endothelium-independent vasodilation directly via smooth muscle cells. Several studies have shown that exercise intervention do not significantly affect the vasodilating responses to SNP in normal and hypertensive animal models (5, 42, 44). Moreover, one previous study indicated that, in ovariectomized SHR, the endothelium-independent (SNP-induced) vasodilation was not impaired (36). In agreement with previous findings, we found that the SNP-induced vasodilation was similar in aortas from the three groups studied, indicating that the endothelium-independent vasodilating pathway was not affected by acute exercise. However, another study indicated that the SNP-induced vasodilation is significantly attenuated in mesenteric arteries of hypertensive rats compared with normotensive rats (28). In our opinion, different vascular beds, disease progression, or exercise intervention could contribute to different vasodilating reponses induced by SNP.

It has been shown that either hypertensive or postmenopausal status is strongly related to the levels of ROS and antioxidants (2, 21, 33, 34). In postmenopausal women, a higher level of oxidative markers and a lower level of antioxidant markers were observed compared to premenopausal women (33). In addition, regular exercise has been found to induce greater activities of antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase (7). One previous study indicated that postmenopausal women with higher level of fitness had higher antioxidant activities and lower oxidative stress (29). However, little information exists regarding influences of exercise intervention on levels of oxidative stress and antioxidant activities in the status of simultaneous postmenopause and hypertension. Our results showed that antioxidant activities, such as the serum SOD activity, were significantly decreased in the SHR-O group compared to the WKY group, but significantly enhanced after a single bout of exercise in the SHR-OE group. Similar findings were shown in serum catalase activity. However, serum concentration of MDA showed no significant difference among the three groups. Previous studies indicate that acute exercise can increase SOD activities in a number of tissues, such as skeletal muscle, liver, and heart (19). MDA, the by-product of lipid peroxidation, is frequently used as the marker of oxidative damage during exercise. An increase of MDA concentration is a result of subsequent lipid peroxidation. One previous study indicated that acute high-intensity exercise, but not mild- or moderate-intensity exercise, induced oxidative stress (16). Another study showed that there were negative association between oxidative stress (e.g., MDA) and the level of physical fitness (29). Some of these results were consistent with our findings. In addition, the exercise-induced lipid peroxidation, such as MDA, has been found to be reduced by chronic exercise, which induces higher levels of antioxidant enzyme activities (7). Until now, the physiological mechanisms responsible for enhancing antioxidant capacity against oxidative stress are conflicting depending on different intensity and...
period of exercise intervention. In this study, we found that acute exercise enhanced serum antioxidant activities of SOD and catalase, but not the lipid peroxidation (i.e., MDA), in ovariectomized hypertensive rats. Longer periods of exercise intervention should be investigated to examine how chronic exercise affects levels of antioxidant activities and oxidative stress in ovariectomized hypertensive status.

In hypertension and atherosclerosis, vascular endothelial cells have been shown to release cyclooxygenase-derived endothelium-dependent contracting factors (EDCF) and ROS. These two factors counteract vessel dilating response that are mediated by the production of NO (12, 34). Excess of superoxide production is related to the decreased NO bioavailability and vascular dysfunction in some pathological conditions, such as hypertension (12, 18). Many evidences have indicated that treatment with several antioxidants, including glutathione, vitamin C, and SOD, can improve vascular function in hypertension (3, 31). In our study, we found that a single bout of exercise significantly improved the ACh-induced vasodilation, accompanied by the enhancement of serum antioxidant activities, in ovariectomized hypertensive rats. It implies that the increased serum antioxidant activities could partly contribute to the exercise-induced improvements of vasodilating response in ovariectomized hypertensive rats. However, the causal relationship of antioxidant activities and vasodilating responses following acute exercise needs further studies for confirmation.

In conclusion, the single-bout exercise significantly ameliorated the endothelium-dependent (ACh-induced) vasodilation through the NOS-related pathway in ovariectomized hypertensive rats. Moreover, this exercise-induced amelioration might be associated with increased serum antioxidant activities. Our findings suggested some of the physiological mechanisms to explain the improvements of simul aneous postmenopause and hypertension-induced endothelial dysfunction through acute exercise. The beneficial effects of acute exercise on endothelial function might, at least in part, contribute to the effects of chronic exercise in the pop-ulation of simultaneous postmenopause and hypertension.

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