Enhancement of Vasorelaxation in Hypertension following High-Intensity Exercise

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Abstract

Exercise can ameliorate vascular dysfunction in hypertension, but its underlying mechanism has not been explored thoroughly. We aimed to investigate whether the high-intensity exercise could enhance vasorelaxation mediated by insulin and insulin-like growth factor-1 (IGF-1) in hypertension. Sixteen-week-old spontaneously hypertensive rats were randomly divided into non-exercise sedentary (SHR) and high-intensity exercise (SHR+Ex) groups conducted by treadmill running at a speed of 30 m/min until exhaustion. Age-matched Wistar-Kyoto rats (WKY) were used as the normotensive control group. Immediately after exercise, the agonist-induced vasorelaxation of aortas was evaluated in organ baths with or without endothelial denudation. Selective inhibitors were used to examine the roles of nitric oxide synthase (NOS) and phosphatidylinositol-3 kinase (PI3K) in the vasorelaxation. By adding superoxide dismutase (SOD), a superoxide scavenger, the role of superoxide production in the vasorelaxation was also clarified. We found that, [1] the high-intensity exercise significantly \( P < 0.05 \) induced higher vasorelaxant responses to insulin and IGF-1 in the SHR+Ex group than that in the SHR group; [2] after endothelial denudation and pre-treatment of the PI3K inhibitor, NOS inhibitor, or SOD, vasorelaxant responses to insulin and IGF-1 became similar among three groups; [3] the protein expression of insulin receptor, IGF-1 receptor, and endothelial NOS (eNOS) was significantly \( P < 0.05 \) increased in the SHR+Ex group compared with the SHR group; [4] the relaxation to sodium nitroprusside, a NO donor, was not different among three groups. Our findings suggested that the high-intensity exercise ameliorated the insulin- and IGF-1-mediated vasorelaxation through the endothelium-dependent pathway, which was associated with the reduced level of superoxide production.

Key Words: exercise, vasorelaxation, aorta, nitric oxide, superoxide

Introduction

Insulin and insulin-like growth factor-1 (IGF-1) play important roles in the regulation of vascular tone via producing endothelium-derived nitric oxide (EDNO). They both modulate vasorelaxation by activating phosphatidylinositol-3 kinase (PI3K) to phosphorylate nitric oxide synthase (NOS) and subsequently inducing NO production (26, 28, 34). One previous study indicated that insulin and IGF-1 caused similar decreases in vessel tension of porcine coronary arteries after the pre-contraction with endothelin-1 (ET-1). After incubating with the endothelial NOS (eNOS) inhibitor, i.e. L-NMMA, the vasorelaxant responses to insulin and IGF-1 were blunted in the vessels (13). Many evidences demonstrated that insulin and IGF-1 caused the decreases in the phenylephrine-induced vasoconstriction and induced vasorelaxation in thoracic aortas of normal rats. After incubating with the PI3K inhibitor or...
eNOS inhibitor, the insulin- and IGF-1-induced vasorelaxant effects were abolished (34, 38, 39). Until now, limited studies indicated that insulin and IGF-1 regulating vascular function was dysfunctional in some of cardiovascular disorders, such as hypertension and obesity (17, 32, 36). The roles of insulin and IGF-1 contributing to the vascular dysfunction in hypertension and underlying mechanisms have not been fully investigated.

Exercise is well known to reduce cardiovascular risk factors, such as high blood pressure (21). The low- and moderate-intensity exercise can decrease the elevated blood pressure and delay the onset of hypertension (7, 9, 21). Moreover, some researchers emphasized the significant effects of the high-intensity exercise on cardiopulmonary function in normal and diseased subjects (18, 19, 29). One clinical study showed that the high-intensity exercise elicited greater improvements in maximal oxygen consumption (VO$_{2\text{max}}$) and reduced blood lipids in healthy subjects compared with the moderate-intensity exercise (19). In addition, the high-intensity exercise is more effective in ameliorating cardiopulmonary function than the moderate-intensity exercise in patients with coronary artery disease (1, 18, 23). However, the effects of high-intensity exercise on insulin- and IGF-1-mediated vascular function in hypertension remain unclear. Recently, we have found that acute and chronic moderate-intensity exercise can enhance insulin- and IGF-1-induced vasorelaxation in normal animals (38, 39). In the present study, we aimed to investigate the effects of one single bout of high-intensity exercise on insulin- and IGF-1-induced vasorelaxation in spontaneously hypertensive rats. Two selective inhibitors, i.e. wortmannin (an inhibitor of PI3K) and N$^\omega$-nitro-L-arginine methyl ester (L-NAME; an inhibitor of NOS), were used to evaluate the roles of PI3K and NOS in the NO-dependent vasorelaxant pathway. The denuded vessels (the removal of vascular endothelium) were also prepared to examine the insulin- and IGF-1-induced vasorelaxation via the endothelium-dependent pathway. Furthermore, the excess superoxide production may cause the decreased NO bioavailability and vascular dysfunction in hypertension (12, 14, 22). For further investigating if the high-intensity exercise could improve vasorelaxant dysfunction by decreasing the superoxide level, we evaluated insulin- and IGF-1-induced vasorelaxation with or without superoxide dismutase (SOD, a superoxide scavenger). Also, the protein expression of insulin receptor, IGF-1 receptor, and eNOS in aortas of hypertensive rats after the high-intensity exercise were examined. Finally, the endothelium-independent vasorelaxant response to sodium nitroprusside (SNP, a NO donor) was assessed after the exercise intervention.

Materials and Methods

Drugs

Insulin, SNP, phenylephrine, wortmannin, L-NAME, and superoxide dismutase (SOD) were purchased from Sigma Chemical, St Louis, MO, U.S.A. Insulin-like growth factor-1 (IGF-1) was purchased from CytoLab, Rehovot, Israel. Krebs-Ringer solution was composed of 118 NaCl, 4.8 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 24 NaHCO$_3$, 0.03 Na$_2$-EDTA, and 11 glucose in mM. All compositions of the solution were purchased from Merck, Darmstadt, Germany.

Experimental Animals and 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. Male spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) were purchased from National Laboratory Animal Center (Taipei, Taiwan, ROC). 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 National Cheng Kung University (Tainan, Taiwan, ROC). Sixteen-week-old spontaneously hypertensive rats were randomly divided into non-exercise sedentary (SHR) and high-intensity exercise (SHR+Ex) groups. Age-matched WKY rats were used as the normotensive control group. Rats in the SHR+Ex group ran on a motor-driven treadmill (Model T510E, Diagnostic & Research Instruments Co., Taoyuan, Taiwan, ROC) at a speed of 30 m/min until exhaustion in one single bout of the high-intensity exercise session. In contrast, non-exercise SHR and WKY groups were placed on the treadmill without running for the same environmental stimulation. After one single bout of the exercise session, the rats were sacrificed under general anesthesia with ether inhalation. The thoracic aortas were immediately isolated for various experiments described below. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of National Cheng Kung University.

Measurement of Resting Heart Rate and Blood Pressure

Resting heart rate and blood pressure were measured in conscious rats by tail-cuff pressure meter (LE5001, Panlab, Wood Dale, IL, USA). Estimated parameters, including heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP), were measured twice and their means were obtained respectively.
Assessment of Vasorelaxant Responses

The agonist-induced vasorelaxant responses were assessed by measuring the isometric tension of vessels in the organ baths. The assessment protocols were performed according to our previous studies (37, 38). The isolated vessel rings of thoracic aortas (3 mm long) were mounted on the force transducers (Grass Instrument, West Warwick RI, USA) and submerged in the organ chambers containing Krebs-Ringer solution bubbling with 95% O₂-5% CO₂ at 37°C. They were stretched to the optimal passive tension (i.e., 2 g) at which the contraction evoked by phenylephrine was maximal. The vessel rings were equilibrated for at least 90 min, precontracted with phenylephrine (10⁻⁷ M), and exposed to various concentrations of insulin (3 × 10⁻⁵-3 × 10⁻⁶ M) and IGF-1 (10⁻⁹-10⁻⁷ M) to evoke vasorelaxant responses. Some of aortic rings were denuded by gently rubbing endothelial layer of aortas with a small woodstick to examine the role of the endothelium in the vasorelaxant pathway mediated by insulin and IGF-1. In addition, the vasorelaxant responses to various concentrations of SNP (3 × 10⁻¹¹-3 × 10⁻⁹ M), a NO donor, were also examined to observe whether the endothelium-independent vasorelaxation was affected by the exercise intervention. The vasorelaxant responses (i.e., vasorelaxation), which are defined as the reduction in tension of the walls of the blood vessels, were expressed as percentages of the precontractile force induced by phenylephrine.

Examination of PI3K and NOS Roles in Vasorelaxant Responses

The possible roles of PI3K and NOS in the insulin-induced and IGF-1-induced vasorelaxant responses were examined by no inhibitor or pre-administration of either wortmannin (3 × 10⁻⁷ M; an inhibitor of PI3K), or L-NAME; 10⁻⁶ M a NOS inhibitor) into the organ chambers for 15 min before the administration of phenylephrine (10⁻⁷ M).

Examination of Superoxide in Vasorelaxant Responses

SOD is an enzyme that removes the superoxide radical. To examine the role of superoxide production in the insulin-induced and IGF-1-induced vasorelaxant responses. SOD (30 U/ml) was added by scavenging superoxide in the organ chambers for 15 min before the administration of phenylephrine (10⁻⁷ M).

Western Blot Analysis

Frozen segments of thoracic aortas were homogenized and Western blot was modified from the previous study (11). Aortic homogenates were run on a sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes (PVDF) (Pall Corporation, Port Washington, NY, USA), incubated with primary polyclonal rabbit anti-insulin receptor (1: 500; Cell Signaling, Boston, MA, USA), anti-IGF-1 receptor (1:500; Cell Signaling), and monoclonal mouse anti-eNOS (1:500; Transduction Laboratories, San Jose, CA, USA) overnight at 4°C, and followed by the correspondent peroxidase-conjugated secondary antibodies (1:5000; Cell Signaling). Actin was used as the loading control. The labeled protein was detected by enhanced chemiluminescence using ECL detection reagents in Gel Doc XR system (Bio-Rad Laboratories, Hercules, CA, USA). Then the proteins were quantitatively determined by densitometry using Quantity One Analysis Software.

Statistical Analysis

Data showed in the figures were means ± SEM. Sample sizes were indicated by “n”. Results among three groups were analyzed by ANOVA and further by Fisher’s test. Dose responses of vasorelaxation were analyzed by ANOVA with a repeated-measures design. In all cases, a difference at P < 0.05 was considered statistically significant.

Results

General Characteristics

Before the exercise intervention, body weight, resting blood pressure, and heart rate were measured in the WKY, SHR, and SHR+Ex groups, respectively. There was no significant difference in the body weight among three groups (WKY, 305 ± 3; SHR, 310 ± 4; SHR+Ex, 314 ± 3 g, P > 0.05, n = 8). Moreover, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were significantly (P < 0.05) higher in the SHR and SHR+Ex groups than those in the WKY group (SBP/DBP/MAP: WKY, 125 ± 2/96 ± 3/105 ± 2; SHR, 185 ± 3/138 ± 3/154 ± 2; SHR+Ex, 187 ± 4/136 ± 4/153 ± 4 mmHg, respectively, n = 8). Resting heart rate was also significantly (P < 0.05) increased in the SHR and SHR+Ex groups compared with that in the WKY group (WKY, 330 ± 5; SHR, 417 ± 4; SHR+Ex, 416 ± 2 bpm, n = 8).

Vasorelaxation Mediated by Insulin and IGF-1

Figs. 1 and 2 show the cumulative dose-response curves of insulin-induced and IGF-1-induced vasorelaxation in hypertensive rat aortas following the high-intensity exercise. Our results indicated that
the insulin-induced vasorelaxation was significantly \((P < 0.05)\) decreased in the SHR group, compared with that in the WKY group. Also, the high-intensity exercise significantly \((P < 0.05)\) improved the insulin-induced vasorelaxation in the SHR+Ex group. After the endothelial cells of aortic rings were denuded, the insulin-induced vasorelaxation was blunted and no significant difference was found among three groups (Fig. 1).

Similar findings were observed in the IGF-1-induced vasorelaxation (Fig. 2). The IGF-1-induced vasorelaxation was significantly \((P < 0.05)\) reduced in the SHR group, and significantly \((P < 0.05)\) improved by the exercise session in the SHR+Ex group. In the denuded aortic rings, no significant difference in the IGF-1-induced vasorelaxation existed among three groups.

**Roles of PI3K and NOS in Insulin- and IGF-1-Induced Vasorelaxation**

Fig. 3A shows that, before the pre-incubation with wortmannin or L-NAME, the vascular response to insulin was significantly \((P < 0.05)\) lower in SHR than WKY, and significantly \((P < 0.05)\) higher in SHR+Ex than SHR. When the inhibitor was added, the insulin-induced vasorelaxation was greatly diminished among three groups, and the group difference was absent. Similarly, before the inhibitor pre-treatment, the vascular response to IGF-1 was signifi-
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significantly \( (P < 0.05) \) reduced in SHR compared with WKY, and significantly \( (P < 0.05) \) elevated in SHR+Ex compared with SHR. However, the pre-treatment with wortmannin or L-NAME significantly blunted the IGF-1-induced vasorelaxation among three groups, and attenuated the group difference (Fig. 3B).

Roles of Superoxide in Insulin- and IGF-1-Induced Vasorelaxation

Before the pre-treatment of SOD, the vasorelaxant responses to insulin and IGF-1 were significantly \( (P < 0.05) \) reduced in the SHR group compared with the WKY group, and significantly \( (P < 0.05) \) increased in the SHR+Ex group compared with the SHR group. However, when the SOD was added, these vasorelaxant responses became similar among three groups (Fig. 4, A and B). Those implied that the reduced superoxide level was highly related to the amelioration of vasorelaxation following the exercise intervention.

Vasorelaxation Mediated by SNP

Fig. 5 shows that the administration of SNP, a direct vasodilator of vascular smooth muscle, caused a dose-dependent manner of the vasorelaxation, but these responses were not different significantly among three groups \( (P > 0.05) \). It indicated that the endothelium-independent vasorelaxation was not affected by hypertension and exercise intervention.

Protein Expression of Insulin Receptor, IGF-1 Receptor, and eNOS in Aortas

Protein levels of aortic insulin receptor and IGF-1 receptor were significantly \( (P < 0.05) \) decreased in SHR compared with that in WKY, and after the
high-intensity exercise, they were significantly \((P < 0.05)\) elevated in SHR+Ex compared with that in SHR (Fig. 6, A and B). In addition, the aortic eNOS expression were significantly \((P < 0.05)\) lower in SHR than that in WKY, and significantly \((P < 0.05)\) higher in SHR+Ex than that in SHR (Fig. 6C).

**Discussion**

The main findings of the present study were that high-intensity exercise significantly improved insulin- and IGF-1-induced vessel relaxation in hypertensive rats through the endothelium-dependent pathway. These improvements were abrogated by PI3K blockade (wortmannin) and NOS blockade (L-NAME), and thus related to the increased PI3K and NOS activation. Also, we found that the protein expression of insulin receptor, IGF-1 receptor, and eNOS was significantly elevated following the high-intensity exercise. In addition, our findings implied that the reduced level of superoxide production could contribute to the exercise-induced amelioration of vascular dysfunction in hypertensive rats. However, there was no significant difference in the endothelium-independent SNP-induced vessel relaxation among three groups.

Insulin and IGF-1 both regulate normal physiological function of cardiovascular system and mediate vasorelaxant responses mainly through PI3K/Akt and NOS pathway (26, 28, 34). Many evidences showed that insulin-related signaling pathways in aortas and muscles were impaired in the diseased animal models, such as insulin resistance, hypertension, and obesity. \((17, 27, 36, 41)\). Moreover, one previous study indicated that insulin and IGF-1 cause the attenuation of phenylephrine- and endothelin-1-induced vasocon-
striction in normotensive but not in hypertensive rats (17). Also, the vasorelaxation evoked by IGF-1 was decreased, and after the pre-incubation of PI3K or NOS inhibitors, these vasorelaxant effects were abolished in hypertensive rats (32). In consistent with previous studies, we found that insulin- and IGF-1-induced vasorelaxation were significantly reduced in SHR compared with those in WKY. After the administration of an inhibitor of PI3K (wortmannin) or an inhibitor of NOS (L-NAME), there was no significant difference between SHR and WKY groups. Our findings suggested that the decreased insulin- and IGF-1-mediated vasorelaxation in hypertension was mainly due to the alteration of PI3K and NOS activation. Since insulin and IGF-1 stimulate NO production from vascular endothelial and smooth muscle cells (26), we denuded the endothelium to distinguish the roles of these two vascular cells involving in insulin- and IGF-1-mediated vasorelaxation. After the removal of endothelium, we demonstrated that there was no significant difference among three groups. It suggested that vasorelaxant responses mediated by insulin and IGF-1 were mainly through the endothelium-dependent pathway.

It has been known that acute exercise, an isolated exercise session, has beneficial effects on cardiovascular function. Exercise acutely reduces blood pressure and triglyceride, increases high-density lipoprotein cholesterol, lowers insulin resistance, and improves glucose control (31). In addition, not only chronic exercise but acute exercise significantly improves the endothelial function in normal and diseased animal models, such as hypertension, obesity, and atherosclerosis (5, 6, 16, 38-40). However, the most effective intensity of exercise intervention for improving cardiovascular function in different diseases remains undefined. Recent studies have indicated that higher intensity of exercise elicits greater increase in aerobic fitness (10, 29). Also, high-intensity exercise is more effective for improving VO2max and lipid profile than moderate-intensity exercise in healthy subjects and patients with coronary artery disease (10, 18, 19). The exercise intensity could be inversely associated with the prevalence of hypertension, hypercholesterolemia, and diabetes (35). In the present study, we found that high-intensity exercise significantly ameliorated insulin- and IGF-1-induced vasorelaxation to nearly normal level in hypertensive rats. It supports that the high-intensity exercise is effective in the amelioration of hypertension-induced vascular dysfunction. However, clinical staffs reported that high-intensity exercise could be related to the higher risk of cardiovascular complications and orthopedic injuries, particularly in older hypertensive patients (21). Therefore, exercise test with electrocardiogram monitoring should be warranted when patients with hypertension engage in high-intensity exercise clinically (21). More studies are encouraged to investigate whether higher intensity of exercise intervention induces more beneficial effects on cardiovascular function in patients with hypertension.

One previous study demonstrated that insulin treatment improved IGF-1-induced vasorelaxation by inducing the protein expression of IGF-1 receptor and NOS in aortas of diabetes rats (15). Cheng et al. reported that exhaustive exercise acutely enhanced the endothelium-dependent vasorelaxation, and these improvements were associated with the receptor upregulation in normal rats (6). Similar with the previous study, our study showed that protein levels of insulin receptors, IGF-1 receptors, and eNOS were elevated in the SHR+Ex groups compared with those in the SHR group. It implied that the exercise-induced improvements in vasorelaxant responses to insulin and IGF-1 could be due to the increased expression of their receptors and eNOS in aortas of hypertensive rats.

Some researchers indicate that autonomic imbalance (increased sympathetic tone accompanied by reduced parasympathetic tone) plays important roles in the pathogenesis and progression of hypertension (2, 8). It is highly associated with the metabolic, hemodynamic, and thrombotic abnormalities which may cause increased cardiovascular morbidity and mortality (2, 8). Before the exercise intervention, we found that resting heart rate and BP were significantly higher in hypertensive rats than normotensive WKY rats. The increased heart rate and BP could be resulted from the imbalance of sympathetic and parasympathetic nervous activities. However, whether the high-intensity exercise ameliorates the autonomic imbalance and underlying mechanisms in hypertension needs to be further investigated.

In some pathological conditions, such as hypertension, diabetes, and atherosclerosis, vascular endothelial cells release cyclooxygenase-derived endothelium-dependent contracting factors and reactive oxygen species (ROS). These two factors counteract vessel relaxing function which was mediated by the NO production (30). It has been found that excess superoxide production is related to the decreased NO bioavailability and vascular dysfunction in hypertension (12, 14, 22). Also, treatments of several antioxidants, including glutathione, vitamin C, and SOD, can improve BP control and vascular function in hypertension (3, 24). In our study, we examined the effects of SOD on vascular responses to insulin and IGF-1. After the pre-treatment of SOD, there was no significant difference among three groups. It implied that the less superoxide production could contribute to the exercise-induced
improvements of vascular function in hypertensive rats. However, the protein expression and activity of SOD were not measured in the present study. Chen et al. suggested that the impaired vasorelaxation was associated with the decreased protein expression of SOD (4). Also, the antioxidant supplementation was found to improve insulin signaling and insulin sensitivity in high-fat-fed rats, such as increasing insulin receptor substrate-1 tyrosine phosphorylation (33). In addition, the antioxidant could up-regulate the IGF-1 receptor in human dermal fibroblasts (25). However, whether a short period of SOD stimulation could induce insulin/IGF-1 receptors and eNOS expression in hypertensive rats remains unknown. It should be investigated to further clarify the roles of superoxide and antioxidant in hypertension and exercise intervention.

The recent study has showed that the SNP-induced vasorelaxation is lower in mesenteric arteries of hypertensive rats than normotensive rats (20). Moreover, some researchers indicated that exercise intervention would not affect the vasorelaxant responses to SNP in normal and hypertensive animal models. (5, 38, 39). In the present study, we found that the SNP-induced vasorelaxation was similar in thoracic aortas among three groups, indicating the endothelium-independent vasorelaxant pathway was not affected by hypertension and exercise intervention. We speculated that different findings of the vascular responses to SNP might be due to different vascular beds, disease progression, and exercise intervention.

In conclusion, our study demonstrated that the high-intensity exercise acutely enhanced vasorelaxant responses to insulin and IGF-1 in the endothelium-dependent manner, which was associated with the less superoxide production. Our findings provided parts of theoretical base for the improvements of hypertension-induced vascular impairments through the exercise intervention. Clinically, the high intensity of exercise intervention might be suggested and considered as one of therapeutic agents to ameliorate cardiovascular function in patients with hypertension.

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