Enhancement of Vascular Function Mediated by Insulin and Insulin-like Growth Factor-1 Following Single Exercise Session

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Abstract

Exercise is well-known in improving vascular functions, but the underlying mechanism has not been totally understood. The aim of this study was to examine whether single exercise session acutely enhances insulin-induced and insulin-like growth factor-1 (IGF-1)-induced vasorelaxation. Twenty-four male Wistar rats at age of 12 weeks were randomly divided into two groups, control (n = 12) and exercise (n = 12) group. The exercise group ran on a treadmill at a speed of 18 m/min for 60 min. Immediately after exercise, insulin-induced and IGF-1-induced vasorelaxant responses were evaluated by the isometric tension of aortic rings in the organ baths. The roles of phosphatidylinositol 3-kinase (PI3K) and nitric oxide synthase (NOS) in vasorelaxant responses were examined by treating selective inhibitors, such as wortmannin (an inhibitor of PI3K) and N^{ω}-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor). In addition, the vascular responses to sodium nitroprusside (SNP), a NO donor, were examined. We found that single exercise session significantly enhanced vasorelaxation mediated by insulin and IGF-1 in rat aortas ($P < 0.01$). Also, the exercise-enhanced vasorelaxation was abolished by wortmannin or L-NAME. There was no significant difference of SNP-induced vasorelaxation between control and exercise groups. These results indicate that single exercise session acutely enhances insulin-induced and IGF-1-induced vasorelaxation through the PI3K-NOS-dependent pathway.

Key Words: exercise, aortas, insulin, insulin-like growth factor-1, nitric oxide

Introduction

Insulin and insulin-like growth factor-1 (IGF-1) both play important roles in the cardiovascular system. They have specific vascular actions in normal or pathological conditions, including the regulation of blood flow and vascular resistance, and the induction of vasodilator or vasoconstrictor effects (10, 26, 31). Previous studies have indicated that insulin and IGF-1 have vasorelaxant effects that depend on the production of endothelium-derived nitric oxide (NO) (28, 32). Using a specific inhibitor of NO synthase (NOS), N-monomethyl-L-arginine (L-NMMA), the investigators have demonstrated that insulin-induced and IGF-1-induced vasorelaxation are highly dependent on vascular NO production. The signaling

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pathways of vascular NO formation involve phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase Akt activities (15, 16, 40). Both insulin and IGF-1 mediate vascular relaxations mainly through activating PI3K and NOS, which further modulate vascular tones (15, 20, 40). Clinically, they have potential effects on the treatment of diabetes mellitus and/or cardiovascular disorders (1, 27, 31).

The single exercise session elicits acute and transient cardiovascular responses (33). Frequent repetition of isolated exercise sessions or exercise training produces more permanent cardiovascular adaptation (30). Many studies have indicated that single exercise session and exercise training both significantly improve endothelial function and reduce insulin resistance in animal and human studies (4, 8, 11, 24). In addition, they both effectively enhance insulin sensitivity, improve glucose control, ameliorate lipid profile, and reduce the risk of developing diabetes mellitus and cardiovascular disease (12, 13, 30, 33). Our previous studies have reported that exercise training improves endothelial function by enhancing acetylcholine (ACh)-induced endothelium-dependent vasorelaxation in vessels of normal and diseased animal models (2, 3, 35, 36). Recently, we also reported that chronic exercise increases both insulin-induced and IGF-1-induced vasorelaxation in normal rats (37). The alteration of NOS gene expression and NO production were mainly involved in the training effects on these vasoactive responses (29, 34, 38, 39). However, effects of single exercise session on insulin-mediated or IGF-1-mediated vascular responses and the underlying mechanisms have not been demonstrated. In the present study, we examined whether the single exercise session acutely improves insulin-induced and IGF-1-induced vasorelaxation in the isolated thoracic aortas of normal rats. The roles of PI3K and NOS in NO-dependent vasorelaxant responses were also examined by using selective inhibitors, such as wortmannin (an inhibitor of PI3K) and N^{ω}-nitro-L-arginine methyl ester (L-NAME; an inhibitor of NOS). In addition, the vascular responses to sodium nitroprusside (SNP), a direct vasodilator of vascular smooth muscle, were evaluated after the single exercise session.

Materials and Methods

Animals and Exercise Protocol

This study was conducted in conformity with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health. Twenty-four male Wistar rats at age of 12 weeks were purchased from National Cheng Kung University Animal Center (Tainan, Taiwan). They were housed in an environmentally controlled room (25 ± 1°C; 12 h light/12 h dark cycle), and fed with a standard rat chow and water *ad libitum*. All rats were randomly divided into two groups, control and exercise group. Twelve rats in the exercise group ran on a motor-driven treadmill (Model T510E, Diagnostic & Research Instruments Co., Taoyuan, Taiwan) at the speed of 18 m/min for 60 min continuously, corresponding to the moderate exercise intensity (2). In contrast, twelve rats in the control group did not receive any exercise program until sacrificed.

After the cessation of the single exercise session, the rats were sacrificed under general anesthesia with ether inhalation. The thoracic aortas were immediately isolated for various experiments described below.

Evaluation of Vasorelaxant Responses to Insulin or IGF-1

The vasorelaxant responses were recorded using the isometric tension of aortic rings, of which complete details had previously been provided (3, 35-37). The isolated vessel rings of thoracic aortas (3 mm long) were mounted on force transducers (Grass Instrument, West Warwick RI, USA) and submerged in organ chambers containing Krebs-Ringer solution (compositions in mmol/L: 118 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24 $NaHCO_3$, 0.03 Na_2-EDTA, and 11 glucose) bubbling with 95% O_2-5% CO_2 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, Sigma Chemical, St. Louis, MO, USA), and exposed to various concentrations of insulin (3×10^{-7}-10^{-5} M, Sigma Chemical) or IGF-1 (3×10^{-9}-10^{-7} M, CytoLab, Rehovot, Israel) to evoke vasorelaxant responses. 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 in Insulin-Induced or IGF-1-Induced Vasorelaxation

The possible roles of PI3K and NOS in the insulin-induced or IGF-1-induced vasorelaxant responses were examined by no inhibitor or pre-administration of either wortmannin (3×10^{-7} M; an inhibitor of PI3K) (Sigma Chemical), or N^{ω}-nitro-L-arginine methyl ester (L-NAME; 3×10^{-7} M; a NOS inhibitor) (Sigma Chemical) into the oxygenated organ chambers 15 min before the administration of phenylephrine (10^{-7} M).

Evaluation of Vasorelaxant Responses to SNP

In some phenylephrine-precontracted vessels,

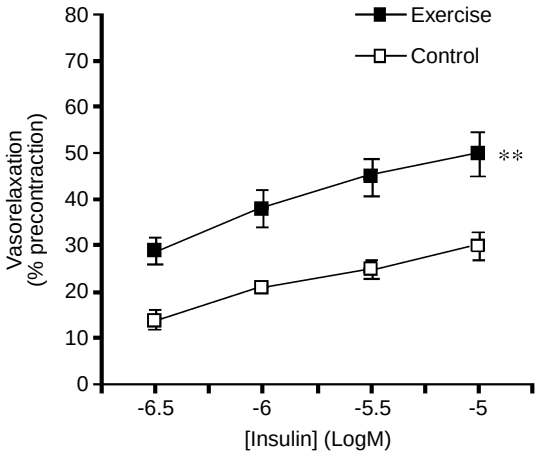

Fig. 1. Concentration-response curves for insulin (3×10^{-7}-10^{-5} M)-induced vasorelaxation in rat aortas after the single exercise session. $^{**}P < 0.01$, control *vs*. exercise (n = 12).

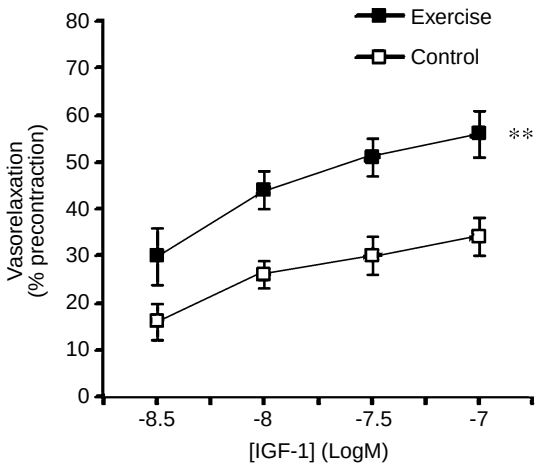

Fig. 2. Concentration-response curves for IGF-1 (3×10^{-9}-10^{-7} M)-induced vasorelaxation in rat aortas after the single exercise session. $^{**}P < 0.01$, control *vs*. exercise (n = 12).

the vasorelaxant responses to various concentrations of SNP (3×10^{-11}-3×10^{-8} M, Merck, Darmstadt, Germany), a direct vasodilator of vascular smooth muscle, were also examined to observe whether the SNP-mediated vasorelaxation was affected by the single exercise session.

Statistical Analysis

All data presented in the figures were means ± SEM. Sample sizes were indicated by "n". The responses of vasorelaxation were analyzed by the analysis of variance using the general linear model (GLM) in a one between (control and exercise) and one within (different drugs) design. Differences in the responses of vasorelaxation among different drugs were subsequently tested as single group repeated measures with contrast transformation. In all cases, a difference at $P < 0.05$ was considered statistically significant.

Results

At the end of the experiments, there was no significant difference in the body weight between control and exercise groups (380.8 ±18.4 g *vs*. 382.6 ± 18.7 g for control *vs*. exercise groups, respectively; n = 12).

Effects of the Single Exercise Session on Insulin-Induced and IGF-1-Induced Vasorelaxation

The vessel rings were precontracted with phenylephrine (10^{-7} M), and then exposed to various concentrations of insulin (3×10^{-7}-10^{-5} M) or IGF-1 (3×10^{-9}-10^{-7} M). After the phenylephrine-induced contraction of the isolated aortic rings had reached a plateau level, insulin or IGF-1 was added cumulatively. The administration of insulin (3×10^{-7}-10^{-5} M) caused a concentration-dependent vasorelaxation in both control and exercise groups (Fig. 1). After the single exercise session, the insulin-induced vasorelaxation was significantly ($P < 0.01$) improved in aortas of the exercise group compared with that in the control group (Fig. 1). Similar results were found in the IGF-1-induced vasorelaxation. The administration of IGF-1 (3×10^{-9}-10^{-7} M) also evoked a concentration-dependent vasorelaxation of aortas in both control and exercise groups (Fig. 2). Similar to that found in the insulin-induced vasorelaxation, the IGF-1-induced vasorelaxation was also significantly ($P < 0.01$) improved by the single exercise session (Fig. 2).

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

The insulin-induced vasorelaxation in the aortas of control and exercise groups was greatly diminished by the pre-incubation with wortmannin (3×10^{-7} M; an inhibitor of PI3K) or L-NAME (3×10^{-7} M; a NOS inhibitor). Prior to the administration of wortmannin or L-NAME, the vascular responses to 3×10^{-6} M of insulin were significantly higher in the exercise group than that in the control group ($P < 0.01$; Fig. 3). After the administration of wortmannin, the insulin-evoked vasorelaxation was significantly reduced in either control or exercise group, and the group difference between these two groups disappeared. Similar results were observed when the vessels were pretreated with L-NAME (Fig. 3). The pretreatment of L-NAME also

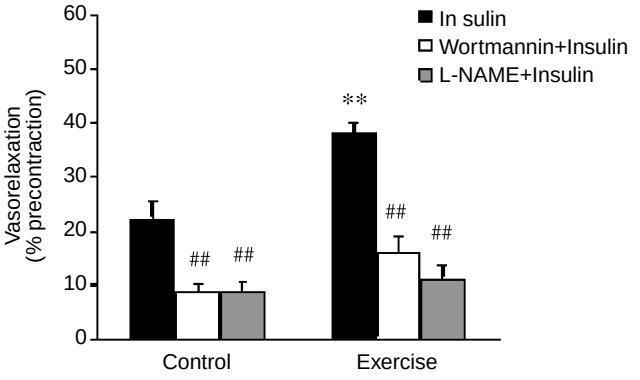

Fig. 3. Vascular responses to insulin (3×10^{-6} M) without or with the pretreatment of wortmannin (3×10^{-7} M) or L-NAME (3×10^{-7} M). $^{**}P < 0.01$, control *vs*. exercise; $^{\#\#}P < 0.01$, with the inhibitor (wortmannin or L-NAME) *vs*. without the inhibitor (n = 10).

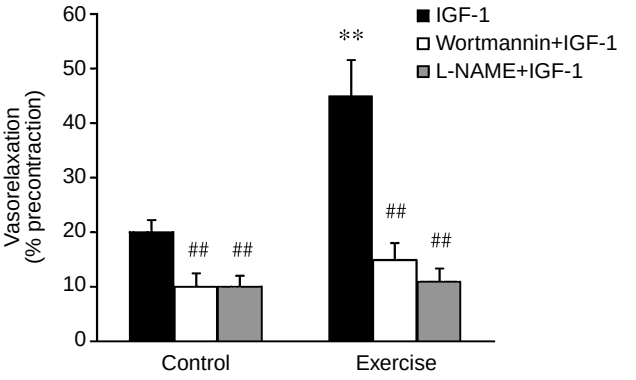

Fig. 4. Vascular responses to IGF-1 (3×10^{-8} M) without or with the pretreatment of wortmannin (3×10^{-7} M) or L-NAME (3×10^{-7} M). $^{**}P < 0.01$, control *vs*. exercise; $^{\#\#}P < 0.01$, with the inhibitor (wortmannin or L-NAME) *vs*. without the inhibitor (n = 10).

inhibited the insulin-evoked vasorelaxation in either control or exercise group and abolished the group difference between these two groups.

The IGF-1-induced vasorelaxation in control or exercise group was also greatly diminished by the pre-incubation with wortmannin (3×10^{-7} M) or L-NAME (3×10^{-7} M). Similarly, the vascular responses to 3×10^{-8} M of IGF-1 in the exercise group were significantly higher than that in the control group before the administration of wortmannin or L-NAME ($P < 0.01$; Fig. 4). Moreover, the pretreatment of wortmannin or L-NAME significantly inhibited the IGF-1-evoked vasorelaxation and abolished the group difference between control and exercise groups (Fig. 4).

Effects of the Single Exercise Session on SNP-Induced Vasorelaxation

The administration of SNP (3×10^{-11}-3×10^{-8} M), a direct vasodilator of vascular smooth muscle, caused a concentration-dependent vasorelaxation in both control and exercise groups (Fig. 5). However, the vasorelaxant responses to SNP between control and exercise groups were similar (Fig. 5). It indicated that the sensitivity of vascular smooth muscle to NO was not affected by the single exercise session.

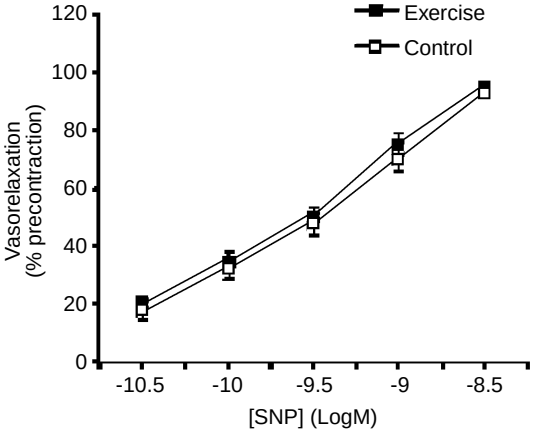

Fig. 5. Concentration-response curves for SNP (3×10^{-11}-3×10^{-8} M)-induced vasorelaxation in rat aortas after the single exercise session. There was no significant difference of SNP-induced vasorelaxation between control and exercise groups (n = 12).

Discussion

Our results indicated that the single exercise session significantly enhanced not only insulin-induced vasorelaxation but also IGF-1-induced vasorelaxation. After the pre-administration of wortmannin or L-NAME, the exercise-enhanced vasorelaxation was abolished. It suggested that the augmentation of NO bioavailability was mainly involved in the exercise-induced improvement of vascular function. However, the SNP-induced vasorelaxation was not affected in the exercise group compared with the control group.

Both the single exercise session and exercise training exert beneficial effects on the cardiovascular functions in animal and human studies (30, 33). Especially, the single bout of exercise induces favorable changes for the modification of cardiovascular risk factors. For example, it acutely reduces serum triglyceride, increases high-density lipoprotein cholesterol (HDL-C), and improves glucose control (6, 9, 18, 25). In addition, many studies have indicated that the single exercise session and exercise training both significantly improve endothelial function, NO

production, and insulin sensitivity in normal or diseased models (2, 4, 8, 11, 24, 36). However, whether exercise could affect insulin-induced or IGF-1-induced cardiovascular function in normal or diseased states has not been totally understood. Recently, we have reported that long-term exercise training improves both insulin-induced and IGF-1-induced vasorelaxant responses in normal vessels (37). In the present study, we found that the single exercise session could elicit similar changes in these vasorelaxant responses, *i.e.*, enhanced insulin-induced and IGF-1-induced vasorelaxation. Our results clearly indicated that acute exercise also exerted positive effects on the vascular function mediated by insulin and IGF-1 in the normal animal model. Under normal physiological conditions, insulin and IGF-1 have specific vascular actions in human and experimental animals, such as an increase in the muscular blood flow, a decrease in the vascular resistance, and the regulation of vascular tone and blood pressure (10, 26, 31). In addition, some investigators have found that insulin-mediated vasodilatory actions are associated with muscle glucose uptake in healthy subjects (5). Therefore, the enhancement of insulin- and IGF-1-mediated vascular functions from exercise could be beneficial for the improvement of normal glucose metabolism and cardiovascular health. Whether exercise-enhanced insulin- and IGF-1-mediated vascular functions positively affect glucose metabolism and cardiovascular health needs further investigation. The derangement of insulin or IGF-1 actions is known to result in the development of cardiovascular diseases in the states of insulin resistance or diabetes mellitus (10, 31). Further studies should be conducted to elucidate exercise effects on insulin-induced and IGF-1-induced vascular function in the diseased models, such as the models of obesity, insulin resistance, and diabetes mellitus.

Insulin and IGF-1 mediate vascular relaxation mainly through the NO-dependent pathway involving the PI3K and NOS activities (15, 20, 40). In the current study, after the administration of wortmannin (an inhibitor of PI3K) or L-NAME (a NOS inhibitor), no significant difference of the insulin-induced or IGF-1-induced vasorelaxation was found between control and exercise groups. These findings suggested that the insulin-induced and IGF-1-induced vasorelaxation enhanced by the single exercise session was mainly due to the elevated activities of vascular PI3K and NOS in the NO-dependent vasorelaxant pathway. However, the underlying mechanisms of the upregulation in vascular PI3K and NOS remain unknown. Previous study has reported that insulin treatment ameliorates IGF-1-induced vasorelaxation by inducing the overexpression of endothelial NOS (eNOS) and IGF-1 receptor level in thoracic aortas of streptozotocin (STZ)-induced diabetic rats (19). It indicated that this change of vasorelaxant response was associated with the alteration of IGF-1 receptor level in diabetic rats. However, whether increases in insulin-induced and IGF-1-induced vasorelaxation after the single exercise session result from the alteration of insulin or IGF-1 receptor levels and their downstream signaling molecules must be further explored.

In addition to the enhancement of the ACh-induced vasorelaxation (4), our study indicated the increase of insulin- and IGF-1-induced vasorelaxation from acute exercise. One previous study demonstrated that acute exercise enhanced ACh-induced vasorelaxation through upregulating muscarinic receptor (subtype M_3) in normal rats (4). However, the involvement of insulin and IGF-1 receptor levels in exercise-enhanced insulin- and IGF-1-induced vasorelaxation remains unknown. According to different mechanisms of ACh-, insulin-, and IGF-1-induced vascular functions, the effects of acute exercise on their responses in normal and pathological conditions are speculated to be different. Thus, the physiological and pathological roles of ACh-, insulin-, IGF-1-induced vascular functions influenced by exercise need to be further examined.

In our study, immediately after the single exercise session, we compared the relaxation responses to SNP between control and exercise groups to elucidate the possibility that vascular smooth muscle becomes more sensitive to nitrovasodilators. Our results showed that the single exercise session did not affect the SNP-induced vasorelaxation in rat thoracic aortas. It indicated that the endothelium-independent NO pathway in vascular smooth muscle was not affected by the single exercise session. Similar results were found in previous studies. In normal, hypertensive, or atherosclerotic animals, exercise did not alter the sensitivity of smooth muscle cells to SNP and the SNP-induced vasorelaxant response, but enhanced ACh-induced vasorelaxation (2, 35-37). Therefore, the exercise effects on the improvement of vascular function may not be due to an increased sensitivity of vascular smooth muscle to NO.

Until now, exactly how exercise improves vascular function is still unclear. *In vitro* study showed that elevated blood flow increased the ACh-induced endothelium-dependent vasorelaxation (22). Also, aortic eNOS mRNA and protein expression was found to be increased during high shear stress (23). As the promoter region of eNOS gene contains a shear stress-responsive element (21), exercise may upregulate eNOS gene expression by an increase in blood flow or shear stress, and then facilitates NO release. In addition, our previous findings indicated that the increased ACh-induced vasorelaxation only occurred in the thoracic aorta where blood flow increases several folds during

exercise, but not in the common carotid artery where blood flow remains relatively constant during exercise (3, 35, 36). Therefore, the exercise-induced changes are likely due to local increases in blood flow or shear stress. The possible mechanisms need to be further examined.

In conclusion, this study demonstrated that the single exercise session acutely enhanced both insulin-induced and IGF-1-induced vasorelaxant responses, which were mediated through the PI3K-NOS-dependent pathway. The health benefit coming from single exercise, supported by our current study and previous studies, should strongly encourage individuals to engage in exercise, even any single exercise (7, 14, 17). Furthermore, our findings may provide one of the possible mechanisms for explaining acute effects of the exercise program on improving vascular functions. Further studies should elucidate exercise effects on insulin-induced and IGF-1-induced vascular function in the diseased status, such as the status of insulin resistance and diabetes mellitus.

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