Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 79 (2006) 2017–2021
Life Sciences
www.elsevier.com/locate/lifescie

Exercise training improves insulin-induced and insulin-like growth factor-1-induced vasorelaxation in rat aortas

Ai-Lun Yang [a], Chia-Ting Su [b], Ko-Long Lin [c], Jung-I Chao [a], Huei-Ping You [d], Shin-Da Lee [e,*]

[a] Department of Physical Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[b] Department of Occupational Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[c] Departments of Physical Medicine and Rehabilitation, Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan
[d] School of Physical Therapy, Chung Shan Medical University, Taichung, Taiwan
[e] School of Physical Therapy, China Medical University, Taichung, Taiwan

Received 19 February 2006; accepted 25 June 2006

Abstract

Improved vasorelaxant response is one of the beneficial effects of exercise training on vascular function. The mechanism for this response is, however, poorly understood. The aim of this study was to investigate the effects of exercise training on insulin-induced and insulin-like growth factor-1 (IGF-1)-induced vasorelaxation. Fourteen 6-week-old male Wistar rats were randomly divided into sedentary control and exercise groups. For 12 weeks, the exercise group ran on a treadmill 60 min/day, 5 days/week. After exercise training, insulin-induced and IGF-1-induced vasorelaxant responses were evaluated by measuring the isometric tension of aortic rings. The vasorelaxant role of phosphatidylinositol 3-kinase (PI3K) and nitric oxide synthase (NOS) was examined by applying inhibitors, such as wortmannin (an inhibitor of PI3K) and N^{ω}-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor). In addition, we examined the vascular response to the NO donor, sodium nitroprusside (SNP). We found that: (1) exercise training significantly enhanced both insulin-induced and IGF-1-induced vasorelaxation in rat aortas; (2) this vasorelaxant effect disappeared after the use of wortmannin or L-NAME; (3) there was no significant difference in SNP-induced vasorelaxation between control and exercise groups. Our findings indicate that exercise training enhances insulin-induced and IGF-1-induced vasorelaxant responses which are mediated through the PI3K-NOS-dependent pathway.

Keywords: Treadmill; Thoracic aortas; Phosphatidylinositol 3-kinase; Nitric oxide synthase

Introduction

Insulin and insulin-like growth factor-1 (IGF-1) both contribute greatly to cardiovascular health (Goke and Fehmann, 1996; Ren et al., 1999). Clinically, they are used to treat diabetes mellitus and/or cardiovascular disorders (Bondy et al., 1994; Sasali and Leahy, 2003; Sowers, 1997). Recent studies have indicated that insulin and IGF-1 have vasorelaxant effects dependent on the production of endothelium-derived nitric oxide (NO) (Schini-Kerth, 1999; Steinberg et al., 1994). Using a specific inhibitor of NO synthase (NOS), *N*-monomethyl-L-arginine (L-NMMA), insulin-induced and IGF-1-induced vasorelaxations have been shown to be highly dependent on vascular NO production. Signaling pathways of vascular NO formation involve phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase Akt activities (Isenovic et al., 2002; Zeng and Quon, 1996). Both insulin and IGF-1 mediate vascular relaxation mainly through activating PI3K and NOS, which further modulate vascular tone (Isenovic et al., 2001; Kuboki et al., 2000; Zeng and Quon, 1996).

It is well known that the physiological function, especially that of the cardiovascular system, benefits greatly from regular physical activity (Hawley, 2004; Shephard and Balady, 1999). Exercise training has been found to significantly improve endothelial function and enhance insulin action in both animal and human studies (Chen et al., 1996; Delp et al., 1993; Hamdy et al., 2003; Yen et al., 1995). In addition, regular exercise effectively reduces the risk of developing insulin resistance and cardiovascular disease

* Corresponding author. School of Physical Therapy, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 4 22053366x3101; fax: +886 4 22065051.
E-mail address: shinda@csmu.edu.tw (S.-D. Lee).

0024-3205/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2006.06.037

(Helmrich et al., 1991; Shephard and Balady, 1999). 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 (Chen et al., 1996; Chen and Li, 1993; Delp et al., 1993; Yang and Chen, 2003; Yang et al., 2003). Moreover, the training effect on endothelium-dependent vasoactive response has been connected with NOS gene expression and NO production (Chen and Li, 1993; Koller-Strametz et al., 1998; Sessa et al., 1994; Yang et al., 2002). Little has been known about the effects of exercise training on insulin-induced or IGF-1-induced vascular response. Therefore, in this study we investigate the effects of a 12-week exercise training program on insulin-induced and IGF-1-induced vasorelaxation in isolated thoracic aortas of normal rats. The vasorelaxant role of PI3K and nitric oxide synthase (NOS) was examined by applying selective inhibitors, such as wortmannin (an inhibitor of PI3K) and N^{ω}-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor). In addition, we evaluated the vascular response to sodium nitroprusside (SNP), a direct vasodilator of vascular smooth muscle.

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. Fourteen 5-week-old male Wistar rats purchased from the National Cheng Kung University Animal Center (Tainan, Taiwan) were housed in an environmentally controlled room (25±1 °C; 12 h light/12 h dark cycle), and fed standard rat chow and water ad libitum. Exercise protocol followed our previous study (Chen et al., 1996). After one week of familiarization, all rats were randomly divided into sedentary control group and exercise group. Rats in the exercise group were trained on a motor-driven treadmill (Model T510E, Diagnostic and Research Instruments Co., Taoyuan, Taiwan) at the speed of 12 m/min for 20 min on the first day. Running time was extended by 10 min/day, to 60 min/day. The running speed was increased 3 m/min every 2 weeks until the speed reached 27 m/min at the end of the training protocol. These rats were trained 5 days/week for 12 weeks. In contrast, animals in the sedentary control group were placed on the treadmill without running for 10 min each day.

Nineteen-week-old rats were sacrificed under ether-induced general anesthesia. To avoid acute effects of exercise, they were sacrificed 48 h after training. Thoracic aortas were immediately isolated in preparation for the experiments below.

Assay of citrate synthase activity

The exercise training effect is commonly confirmed by an increase in citrate synthase activity. Here, citrate synthase activity was measured using homogenized soleus muscle samples (in five volumes of 0.1 M of Tris buffer containing 0.1% Triton X-100) as described by Srere (1969). The enzyme activity was determined by spectrophotometric readings at 412 nm (UV-240, Shimadzu Co., Tokyo, Japan). All samples were run in duplicate. Enzyme activity was expressed as micromoles of substrate utilized per minute per gram of tissue (μmol/min/g).

Evaluation of vasorelaxant responses

The vasorelaxant response (vasorelaxation) is defined as a reduction in the tension of the walls of blood vessels, and expressed as the percentage of the contractile force. Isometric tension of thoracic aortic rings was used to measure vasorelaxant response following a previously described methodology (Chen and Li, 1993; Yang and Chen, 2003; Yang et al., 2003). Isolated vessel rings (3 mm long) were mounted on force transducers (Grass Instrument, Rhode Island, USA) and submerged in high oxygen bubble-aeration (95% O_2, 5% CO_2) organ chambers containing 37 °C Krebs–Ringer solution (composition in mM: 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). Aortic rings were stretched to the optimal passive tension (i.e., 2 g) where the phenylephrine contraction was strongest. 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.

SNP is a direct vasodilator of vascular smooth muscle. In some phenylephrine-precontracted vessels, the vasorelaxant responses to 10^{-9} M SNP (Merck, Darmstadt, Germany), were examined to determine the effect of exercise training on SNP-induced vasorelaxation.

Examination of PI3K and NOS in vasorelaxant responses

The possible roles of PI3K and NOS in the insulin-induced or IGF-1-induced vasorelaxant responses were examined without an inhibitor present or with preadministration 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;

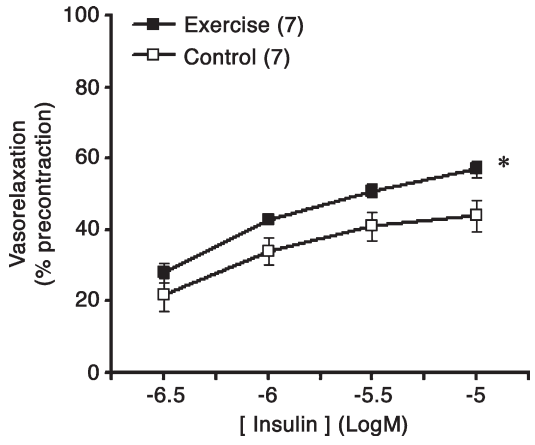

Fig. 1. Concentration–response curves for insulin (3×10^{-7}–10^{-5} M)-induced vasorelaxation in rat thoracic aortas after 12-week exercise training. *$P<0.05$, control vs. exercise. Numbers in parentheses indicate the numbers of animals used in each group.