Microvascular Research 80 (2010) 422–426

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Regular Article

# Does detraining restore influence of exercise training on microvascular responses in streptozotocin-induced diabetic rats?

Ali Heidarianpour *

*Department of Physical Education and Sport Science, Bu-Ali Sina university, Hamedan, Iran*

ARTICLE INFO

*Article history:*
Accepted 20 September 2010
Available online 27 September 2010

*Keywords:*
Exercise training
Detraining
Diabetes
Vascular dysfunction
L-ARG/ Nitric oxide pathway

ABSTRACT

Several findings demonstrated that exercise training has therapeutic and protective effects in type 1 diabetes and could correct micro and macro vascular dysfunction. Regarding all benefits of exercise training on vascular function, however, the mechanisms and persistence of these benefits are only partially understood. Method: Male Wistar rats ($220 \pm 10$ g, $N = 85$) were made diabetic by streptozotocin (60 mg/kg, subcutaneously). One week after diabetes induction, animals were submitted to exercise training for 10 weeks on a treadmill and detrained for 5 weeks. To characterize cutaneous microvascular responses by Laser Doppler flowmetery, animals were deeply anaesthetized by intra peritoneal injection of pentobarbital sodium and placed on a heating pad, a rectal thermometer was inserted, and body temperature was maintained at $37 \pm 0.5$ °C. A tracheotomy was performed to minimize respiratory difficulties.
Results in diabetic rats after training: (1) L-arginine (L-ARG) and Acetylcholine (Ach)-induced cutaneous perfusion were increased. However, these effects were reversed by detraining and Nw-nitro-L-arginine (L-NNA); (2) sodium nitroprusside (SNP)-induced cutaneous perfusion did not significantly change; and (3) cutaneous microvascular responses to SNP were reduced after detraining.
The results suggest that in diabetic rats, beneficial effect of regular exercise on cutaneous microvascular endothelium-dependent dilatation and L-ARG/Nitric oxide pathway activity was approximately reversed by 5 weeks of detraining. Training did not affect endothelium-independent dilatation; however, detraining reduced it significantly.



## Introduction

Vascular endothelial cells have a vital and complex role in regulating blood flow by producing important chemicals such as endothelium-derived relaxing factor/nitric oxide (EDRF/NO), prostacylin, and endothelin that regulate both hemostasis and vascular tone (Khan, 2000; Daniel, 2004). Abnormalities in the endothelium/NO pathway have been reported in human and animal models of type 1 diabetes, in both micro and macro vessels (Khan, 2000; Fuchsjäger-Mayrl et al., 2002). Despite the severity of the vascular complications of diabetes, there is a relative paucity of data specifically on the pathogenesis, prevention, and treatment of the vascular complications of diabetes.

A considerable body of evidence now suggests that in type 1 diabetes, the observed impairment of endothelial function may involve inactivation of NO by oxygen-derived free radicals. There is considerable support for the view that exercise training has therapeutic and protective effects in type 1 diabetes. It decreases oxidative stress and improves anti oxidative capacity of the vascular wall (Kingwell, 2000; Kojda and Hambrecht, 2005; Linke et al., 2005). During exercise, as core temperature rises, skin blood flow increases to facilitate the convective transfer of heat from core to skin (Johnson, 1998). Adjusting the control of cutaneous microcirculation with physical training can increase the capacity of the circulation to transport and to eliminate heat (Johnson, 1998). Furthermore, mechanical alteration or deformation of the endothelium during exercise as a result of increased pulsatile flow can plausibly contribute to EDRF release and NO synthase up regulation (Wang, 2005; Daniel, 2004). Therefore, increased cutaneous blood flow during exercise training may alter endothelial function and increase the sensitivity of stimulated EDRF/NO release in skin vasculature. This is consistent with our previous study in which Acetylcholine-induced cutaneous perfusion was improved by exercise training in streptozotocin-induced diabetic rats and adaptive changes were induced by physical conditioning in the cutaneous vasculature in diabetic rats, mainly by NO production (Heidarianpour et al., 2007).

It has been reported that both plasma and vascular tissue arginine (ARG) concentrations are decreased in diabetes, and that this decrease is associated with both diminished Acetylcholine-mediated relaxation and cGMP production, a measure of NO production. Acute addition of L-ARG but not D-ARG restored relaxation and cGMP production similar to that observed in untreated and ARG-treated control blood vessels (Newsholme et al., 2009).

* PO Box: 65174, I.R. Iran. Fax: +98 8118273236.
*E-mail address:* heidarian317@gmail.com.

0026-2862/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2010.09.006

A number of questions, however, remain: (1) How long are these beneficial effects retained in the cutaneous vasculature in diabetic rats? (2) Does chronic exercise potentiate L-ARG vascular function? (3) What is effect of detraining on endothelium-dependent and -independent dilation?

In the present study, we used laser Doppler to evaluate changes induced by 5 weeks of detraining after 10 weeks of training, on endothelium-dependent and -independent dilation, with an emphasis on the L-ARG/NO pathway. Analysis of cutaneous microcirculation was performed after transdermal iontophoresis of acetylcholine (Ach) and sodium nitroprusside (SNP) as specific endothelium-dependent and -independent vasodilators, respectively, in skin vasculature of STZ-induced diabetic and control rats.

## Materials and methods

### *Animals and experimental design*

Experiments were performed on 85 male Wister rats (8 weeks old and body weight 220 ± 10 g). They were taken from Razi Institute, Tehran, Iran, and received standard laboratory feed and water ad libitum. The animals were housed in standard cages in a temperature-controlled room (22–25 °C) with a 12 h dark–light cycle. All procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals (http://www.nap.edu/readingroom/books/labrats) and approved by the Institutional Animal Care and Use Committee. The rats were randomly assigned to one of six groups: sedentary control (SC), sedentary diabetic (SD), trained diabetic (TD), trained control (TC), Detrained control (DTC) and Detrained diabetic (DTD). After weeks of familiarization period, animals were made diabetic by a single dose injection of STZ (60 mg/kg, S.C; Sigma Chemical Co.) dissolved in citrate buffer, pH 4.5. The rats were fasted overnight before STZ injection. Age matched control rats received sham injection of equal volume of vehicle. 72 h after the injection was performed; the induction of diabetes was confirmed by glucose testing of the blood using glucometer (ACCU-CHEK Active, Ireland). Animals with at least glucose concentration 300 mg/dl were considered diabetic and used in these experiments.

### *Exercise-training protocol*

Animals were submitted to low-intensity exercise training 1 week after diabetes induction. They ran on a motor-driven treadmill (Arian Instruments, Iran) set at an 8% incline, according to a program adapted from Plourde et al. (1991). The rats were trained twice a day, 6 h apart, 5 days a week, for 10 weeks; they initially ran for10 min at 22 m/min for 3 weeks, then 40 min at 28 m/min for 3 weeks, finally 60 min at 31 m/min for 4 weeks, and then the DTD and DTC groups detrained for 5 weeks. However the trained group continues exercise with 60 min at 31 m/min in this time.

### *Measurement of plasma glucose, body weights, heart rate and mean arterial pressure*

Plasma glucose, body weights, mean arterial blood pressure and heart rate were measured by glucometer (ACCU- CHEK Active, Ireland), scale (Sartorius universal, Germany) and tail-cuff (Narco Bio-system, PE- 300) respectively in each five weeks after the administration of STZ (Tables 1, 2).

### *Assessment of cutaneous blood flow*

To avoid the short-term effects of exercise, 24 h after the last training session, animals were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg) and placed on a heating pad. A rectal thermometer was inserted, and body temperature was maintained at 37 ± 0.5 °C. A tracheotomy was performed to minimize respiratory difficulties. The arterial oxygen saturation percentage was monitored continuously with pulse oximeter (Radiometer, Copenhagen, Denmark). Systemic arterial blood pressure and heart rate were measured by using a tail-cuff during assessment of cutaneous blood flow (Table 2). Laser Doppler flow meter (MBF3, Moor Instruments, Axminster, UK) was used to measure the relative changes in skin blood flow as described in previous studies (Badavi et al., 2000; Hajizadeh et al., 2005; Khorasani et al., 2006; Heidarianpour et al., 2007). Laser Doppler perfusion measurements also were used to determine the responses of the skin vasculature on plantar paw surface of the rat by transdermal iontophoresis of polar drugs. The technique of iontophoresis allows polar drugs to cross the skin using a small, direct current. It has been shown to be possible to assess reactivity of the microvascular system when blood perfusion is being measured simultaneously in the same area (Andreassen et al., 1998). A combined probe holder, for iontophoresis and perfusion measurement, was fixed with double-sided adhesive tape on the plantar paw surface of the rat after the skin had been cleaned with isopropyl alcohol and left to dry in the air. The Perspex probe holder had a small chamber for deposition of the test solutions which was then in direct proximity to the laser Doppler probe. A powered constant current stimulator battery (MIC 1, Moor Instruments, England) was used to provide a direct current for the drug iontophoresis. The active electrode was made of platinum, and charged according to the active ions of the drug. To avoid current-induced stimulation of local sensory nerves, currents higher than 0.2 mA or total charges greater than 8mC were limited (Andreassen et al., 1998). A commercially available monitor was used for LDF (MBF 3D, Moor Instruments, Axminster, UK). A sampling frequency of 40 Hz and a time constant of 0.1 s were

**Table 1**
Body weight, Blood glucose in SC, TC, DTC, SD, TD and DTD at 5, 10 and 15 week after the STZ injection in rats.

| Group | Body weight(g) | | | Blood glucose(mg/dl) | | |
|---|---|---|---|---|---|---|
| | The fifth week | The tenth week | The fifteenth week | The fifth week | The tenth week | The fifteenth week k |
| SC | 265 ± 7 | 335 ± 6.3 | 437 ± 6.2 | 89 ± 9 | 101 ± 5 | 99 ± 6 |
| TC | 261 ± 3.6 | 320 ± 5.7 | 375 ± 4.8 | 82 ± 4 | 85 ± 3 | 83 ± 5 |
| DTC | 281 ± 4.2 | 312 ± 4.6 | 400 ± 8 | 86 ± 5 | 80 ± 5 | 93 ± 4 |
| SD | 187 ± 5.3* | 171 ± 7** | 147 ± 4** | 403 ± 11*** | 421 ± 9*** | 431 ± 11*** |
| TD | 234 ± 8 | 222 ± 6* | 197 ± 3.2* | 330 ± 12+ | 400 ± 10 | 415 ± 12 |
| DTD | 238 ± 4.5 | 217 ± 6.2* | 151 ± 5** | 324 ± 10+ | 405 ± 10 | 550 ± 15# |

SC, sedentary control; TC, trained control; DTC, Detrained control; SD, sedentary diabetic; TD, trained diabetic; DTD , detrained diabetic .Values are mean ± S.E.M. *$p$<0.05, **$p$<0.01 and ***$p$<0.001 vs. SC, +$p$<0.05 vs. SD and #$p$<0.05 vs. TD.

**Table 2**
Heart rates and Mean arterial pressure in SC, TC, SD, TD and DTD in 5, 10 and 15 week after the STZ injection in rats.

| Group | HR(bpm) | | | MAP(mm Hg) | | |
|---|---|---|---|---|---|---|
| | The fifth week | The tenth week | The fifteenth week | The fifth week | The tenth week | The fifteenth week |
| SC | 320 ± 12 | 324 ± 11 | 310 ± 9 | 111 ± 5 | 105 ± 4 | 119 ± 3 |
| TC | 311 ± 9 | 308 ± 12 | 289 ± 7 | 108 ± 3 | 106 ± 8 | 115 ± 5 |
| DTC | 305 ± 8 | 288 ± 10 | 296 ± 6.8 | 99 ± 4.3 | 110 ± 5 | 116 ± 6 |
| SD | 280 ± 6* | 267 ± 10* | 243 ± 6** | 93 ± 4* | 82 ± 5* | 79 ± 11** |
| TD | 291 ± 8 | 301 ± 7+ | 307 ± 10++ | 106 ± 3.2 | 100 ± 4 | 90 ± 11 |
| DTD | 287 ± 4 | 297 ± 7+ | 255 ± 11* | 102 ± 3.2 | 98 ± 4 | 78 ± 5 |

HR, heart rate; MAP, mean arterial pressure; SC, sedentary control; TC, trained control; DTC, Detrained control; SD, sedentary diabetic; TD, trained diabetic; DTD , detrained diabetic .Values are mean ± S.E.M. *$p$<0.05 and **$p$<0.01 vs. SC, +$p$<0.05 and ++$p$<0.01 vs. SD.