Original Article

Changes of plasma angiogenic factors during Chronic resistance exercise in Type I Diabetic Rats

Shekarchizadeh Esfahani P[1], Gharakhanlou R[2], Karimian J[3], Safarzade A[4], Khazaei M[5]

ABSTRACT

Objective: Exercise has several beneficial effects on cardiovascular system. However, the exact mechanism is unclear. The purpose of this study was to evaluate the effects of chronic resistance exercise on some plasma angiogenic factors in type 1 diabetic rats.

Methodology: Thirty male Wistar rats were divided into three groups of control, diabetic and diabetic trained (n = 10 each). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg). The rats in the trained group undertook one training session per day, 3 days/week, for 4 weeks. Blood samples were taken and the concentrations of plasma glucose, lipid profile, nitric oxide (NO), vascular endothelial growth factor (VEGF) and soluble form of VEGF receptor-1 (sFlt-1) were determined.

Results: We found a significant reduction in plasma NO concentrations in diabetic rats compared to the controls ($p < 0.05$). After four weeks of resistance training, plasma NO concentrations increased ($p < 0.05$). Plasma VEGF concentrations were not significantly different between diabetic and control groups ($p < 0.05$). However, plasma sFlt-1 concentrations in diabetic rats were significantly higher than the controls ($p > 0.05$). There were no significant differences in plasma VEGF and sFlt-1 concentrations between diabetic sedentary and trained groups ($p > 0.05$). Moreover, VEGF/sFlt-1 ratios in diabetic animals were lower than the control group and resistance exercise could not increase this ratio in diabetic animals ($p > 0.05$)

Conclusion: Resistance exercise could not change plasma VEGF, sFlt-1 and VEGF/sFlt-1 ratio. However, it increased plasma NO concentrations in diabetic animals. More studies are needed to determine the effects of this type of exercise on the angiogenesis process.

KEY WORDS: Nitric Oxide, Vascular Endothelial Growth Factor, Exercise, Diabetes.

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1. Shekarchizadeh Esfahani P,
Dept. of Physical Education and Sports, Tarbiat Modarres University, Tehran, School Management and Medical Informatics, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Gharakhanlou R,
Department of Physical Education and Sports, Tarbiat Modarres University, Tehran, Iran.
3. Karimian J,
School Management and Medical Informatics, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Safarzade A,
Dept. of Exercise Physiology, University of Mazandaran, Babolsar, Iran.
5. Khazaei M,
Dept. of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence:
Majid Khazaei,
Dept. of Physiology,Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: khazaei@med.mui.ac.ir

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INTRODUCTION

Diabetes is a chronic metabolic disorder accompanied by acute and chronic complications including retinopathy, nephropathy and impaired coronary collateral formation.[1] Thus, diabetes is a major risk factor for cardiovascular diseases.

The sprouting of new vessels from pre-existing vessels in response to angiogenic molecules and hypoxia is called angiogenesis. This is vital in the development of diabetic complications. Vascular endothelial growth factor (VEGF) is a main angiogenic factor.[2,3] It is a 45-kDa glycoprotein which stimulates the proliferation and migration of endothelial

cells and inhibits apoptosis resulting in formation of collateral vessels.[4] VEGF has two tyrosine kinase receptors named VEGFR-1 and VEGFR-2. The soluble form of VEGFR-1 (sFlt-1) is found in circulation and can prevent VEGF actions by direct isolation.[5,6] Nitric oxide (NO) is a soluble gas with a half-life of 6-30 seconds. It is continuously synthesized from the amino acid l-arginine in endothelial cells by the constitutive calcium-calmodulin dependent enzyme nitric oxide synthase.[7] NO signaling regulates vascular tone, prevents portions of the atherogenic process, and influences myocardial energy expenditure.[8,9]

Various studies have been performed to evaluate the beneficial effects of different types of physical exercise on cardiovascular diseases especially in diabetic subjects. Based on whether oxygen is used or not, exercise can be divided to aerobic (endurance exercise) or anaerobic (resistance training). In this study, we evaluated the effects of resistance training on plasma angiogenic factors (NO, VEGF and sFlt-1) in type-1 diabetic rats.

METHODOLOGY

Animals and experimental groups: Thirty male Wistar rats (288 ± 22 g) were used in this study. The animals were obtained from Pasteur Institute (Tehran, Iran) and maintained in the Central Animal House, School of Medical Sciences, Tarbiat Modarres University, Tehran. The animals were housed in the cages under controlled light/dark cycles (12/12h) at 22 ± 2°C. They were provided with food and water ad libitum. The animals were randomly divided into three groups of control, diabetic, and diabetic trained (n = 10 each). The trained group undertook four weeks of resistance training program. All methods used in the study were approved by the Ethics Committee of the School of Medical Sciences, Tarbiat Modarres University.

Induction of diabetes: Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg (Sigma-Aldrich, St. Louis, MO). STZ was dissolved (20 mg/ml) in a cold 0.1 M citrate buffer (pH: 4.5). Control rats were injected with a similar volume of citrate buffer only. Five days after the STZ injection, blood glucose concentrations were measured using tail vein blood samples obtained from rats following overnight fasting. A blood glucose level higher than 16 mmol/L was considered indicative of diabetes.

Resistance training protocol: The rats in the trained group undertook one training session per day, 3 days/week, for 4 weeks (a total of 12 sessions) plus an initial familiarization session as previously described.[10] Training was accomplished using a 1-meter ladder inclined at 80°. There were 26 rungs evenly spaced on the ladder. Before inducing diabetes, the rats in the trained group were familiarized with the exercise by practicing climbing up the ladder. The rats were positioned at the bottom of the climbing apparatus and motivated to climb the ladder by touching and tapping their tails. In order to minimize the stress to the rats, we utilized electrical stimulation, forced air, food restriction/reward, and cold water to encourage the rats to perform the exercise training. When the rats reached the top of the ladder, they were allowed to rest.

Resistance training was initiated 7 days after injection of STZ by climbing the ladder while weights were attached to the base of the tail with an adhesive tape and a clip. All animals were weighed once every 4 days to monitor weight gains and, for the resistance trained animals, to determine the amount of weight to append to their tails for the remainder of the week. The study was divided into two parts, i.e. the 2-week preliminary phase was followed by the 2-week flat load resistance exercise training phase. Prior to the commencement of the preliminary phase, the rats allocated to one of the two training groups were familiarized with the ladder climbing exercise. In the preliminary phase, the rats were adapted to climbing the ladder with progressive loading on each successive training day.

The training group of rats performed 6 repetitions of ascending the ladder interspersed with 1-minute rest intervals. A second set of 6 repetitions with 1-minute rest intervals was performed after a 3-minute rest. On the first day, rats exercised with a load equivalent to 30% of body mass (BM) appended to their tail (6 repetitions/2 sets). On the second day, the training load was increased to 50% of BM (6 repetitions/2 sets) while on the third day, an additional set of repetitions was undertaken with 50% of BM (6 reps /3 sets). Thereafter, the training load was gradually increased until the seventh day (i.e. the familiarization day plus six progressive resistance training days) when the training load reached 100% of BM.

In the flat load resistance exercise training phase, the rats continued to exercise with 100% of BM, 6 repetitions per set, 3 sets per day, and 3 days per week until the end of the 4th week. Warm-up and cool–down stages consisted of 2 repetitions of climbing the ladder without weights appended to the tail, immediately pre and post each training session. Non-trained (sedentary) rats were handled on

Table-I: Plasma glucose, insulin and lipid profile at the end of the experiment in all groups.

Factor/group	*Diabetic trained*	*Diabetic*	*Control*
Glucose (mmol/l)	352.33 ± 7.68	353.22 ± 12.09	126.12 ± 2.05*
Insulin (IU/ml)	0.158 ± 0.018	0.176 ± 0.023	0.540 ± 0.096*
LDL-C (mg/dl)	37.33 ± 2.09	33.311 ± 2.77	34.50 ± 3.19
HDL-C (mg/dl)	28.82 ± 1.45	29.78 ± 1.26	27.95 ± 0.757
Triglyceride (mg/dl)	71.44 ± 2.52	70.89 ± 4.87	64.62 ± 3.87
Total cholesterol (mg/dl)	80.44 ± 2.46	76.66 ± 2.01	75.37 ± 3.42

HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol;
*: P < 0.05 compared to other groups. Data is expressed as mean ± SE.

the same days and times as the trained groups in order to minimize any stress attributable to handling.

Sacrifying and sampling: To minimize any residual effects of the last training bout, 48 hours after the last training session, the rats were anaesthetized intraperitoneally with a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg). The rats were sacrificed between 9.00 and 12.00 am after an overnight fasting. The abdominal cavity was opened following the median line of the abdomen and approximately 6 ml of blood was collected from the abdominal vena cava. Blood was centrifuged (3000 rpm; 15 minutes) and the plasma was kept for further analyses.

Biochemical measurements: Plasma glucose was determined by an enzymatic (glucose oxidase phenol 4-aminoantipyrine peroxidase (GOD-PAP)) colorimetric method (Pars Azmoun, Tehran, Iran). Enzyme-linked immunosorbent assay (ELISA) kits specific for the rats were used to determine plasma insulin (Mercodia AB, Uppsala, Sweden). Plasma high-density lipoprotein cholesterol (HDL-C) was determined by direct colorimetric method (Randox, Antrim, UK). In addition, total triglyceride (TG) and total cholesterol (TC) were assessed by enzymatic colorimetric methods (Pars Azmoun, Tehran, Iran) while serum free fatty acid concentrations were evaluated by a colorimetric method (Randox, Antrim, UK) following the manufacturer's instructions. The Friedewald equation was used to determine low-density lipoprotein cholesterol (LDL-C).

Plasma NO measurement: Plasma NO concentrations were determined by evaluation of NO metabolite (nitrite) using Griess reagent method (Promega Corp, Madison, USA) as described elsewhere.[11]

Plasma VEGF and sFlt-1 measurements: Plasma VEGF and sFlt-1 assays were performed using a sandwich enzyme immunoassay kit and reagents (R&D systems, USA) according to the manufacturer's instructions. The minimum sensitivity of VEGF assay is 3.9 pg/ml with intra- and inter-assay variation coefficients of < 10% and < 5%, respectively. The sFlt-1 assay has a lower limit of sensitivity of 3.8 pg/ml and intra- and inter-assay variation coefficients of less than 10% and 5%, respectively.

Statistical analysis: Data is reported as mean ± SE. One-way analysis of variance (ANOVA) was used for comparison of data between groups using Tukey's post-hoc test. P values less than 0.05 were considered as statistically significant.

RESULTS

Physiological parameters: The effects of resistance training on physiological parameters are shown in Table-I. Plasma glucose concentrations in trained and untrained diabetic rats were significantly higher than control rats ($p < 0.05$). Plasma glucose levels tended to be lower in trained diabetic rats than in untrained diabetics although the difference was not statistically significant ($p > 0.05$). Plasma insulin in control rats was significantly higher than the diabetic groups ($p < 0.05$). No significant differences in TC, LDL-C, HDL-C, and TG ($p > 0.05$) were found when control rats were compared with trained and untrained diabetic rats. Resistance training demonstrated no significant changes in these physiological parameters.

Plasma NO concentration: We found a significant difference in plasma NO concentrations between

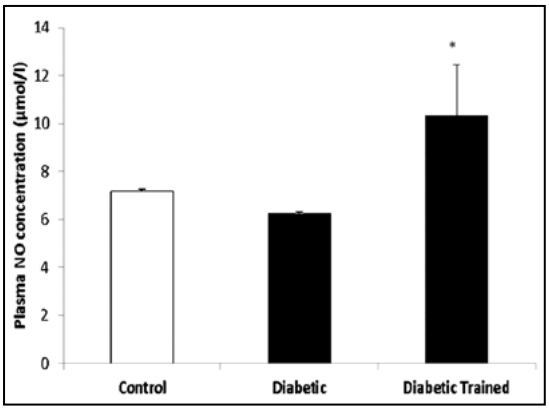

Fig.1: Plasma nitric oxide (NO) concentrations in the control, diabetic and diabetic trained groups (n = 10 in each group).

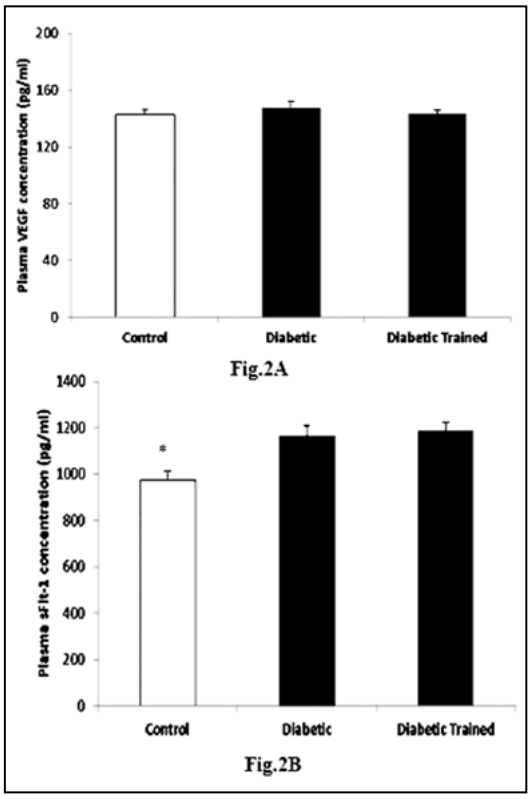

Fig.2: Plasma vascular endothelial growth factor (VEGF) (A) and VEGF receptor-1 (sFlt-1) concentrations in the experimental groups (n = 10 in each group); *: P < 0.05 compared to the control group.

the control and diabetic rats ($p < 0.05$). After 4 weeks of resistance training, plasma NO concentrations increased which led to significant differences between diabetic and diabetic trained groups ($p < 0.05$) (Fig.1).

Plasma VEGF and sFlt-1 concentrations: Our results showed that plasma VEGF concentrations were not significantly different between the diabetic and control groups ($p > 0.05$). Resistance exercise did not significantly change plasma VEGF concentrations in diabetic trained rats (Fig.2A). Plasma sFlt-1 concentrations in diabetic rats were significantly higher than the controls ($p < 0.05$). There were no significant differences in plasma sFlt-1 concentrations between diabetic sedentary and trained groups ($p > 0.05$) (Fig.2B). VEGF/sFlt-1 ratio in diabetic animals was lower than the control group and resistance exercise could not increase this ratio in diabetic animals ($p > 0.05$) (Fig.3).

DISCUSSION

In this study, we could not find any differences in plasma glucose, insulin and lipid profiles between diabetic and resistance trained diabetic groups.

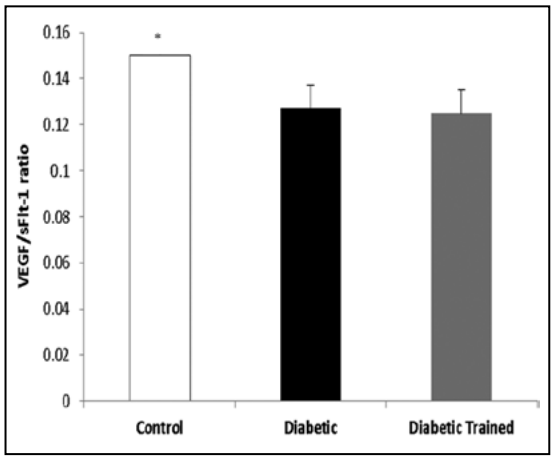

Fig.3: Vascular endothelial growth factor (VEGF) to VEGF receptor-1 (sFlt-1) ratio in the control, diabetic and diabetic trained groups (n = 10 in each group); *: P < 0.05 compared to the diabetic and diabetic trained groups.

Plasma sFlt-1 in diabetic animals was higher than the controls. Exercise significantly improved plasma NO concentration and reduced plasma sFlt-1 in diabetic animals. However, it did not alter plasma VEGF concentrations.

The beneficial effects of exercise on cardiovascular system in diabetic subjects have been documented in several studies.[12-14] Some studies suggested that these effects are related to reduced plasma glucose and/or improvement of plasma lipid profile.[15] However, others suggested that the benefits of different types of exercise on cardiovascular system did not relate to improvement of these parameters.[16-18] In the present study, we found no changes in the above mentioned parameters after resistance exercise in diabetic animals.

Angiogenesis is an important process involved in physiological and pathological conditions such as wound healing, coronary collateral formation or tumor growth. Several angiogenic and antiangiogenic factors, including NO, VEGF and VEGF receptors, have been candidate for physiological and pathological angiogenesis. Evidence has shown different types of exercise to be able to improve plasma NO levels,[19-21] even in diabetic subjects.[22] NO has several effects, such as angiogenesis, on cardiovascular system.[23,24] VEGF and VEGF receptors are the most important factors during the angiogenesis process.[25] SFlt-1 and VEGFR-2 have been found to have antiangiogenic effects and pro-angiogenic properties, respectively.[19,25] In the present study, although plasma VEGF was not different between control and diabetic animals, plasma sFlt-1 was higher in the diabetic animals than the controls. Our results are in agreement with previous studies.[12] SFlt-1 binds

with a high affinity to VEGF and inhibits VEGF activity and angiogenesis processes.[26] Thus, reduced plasma NO concentrations and increased sFlt-1 may be responsible for impaired angiogenesis in diabetic subjects.

Although plasma VEGF and VEGF receptors are indicators of angiogenesis processes, in a recent study by Chang et al., VEGF/sFlt-1 ratio was considered as a better index for angiogenesis process.[27] In the present study, we also found VEGF/sFlt-1 ratio to be lower in diabetic animals compared to the control group. Moreover, we found exercise to increase plasma NO levels but it, did not change VEGF, sFlt-1 and VEGF/sFlt-1 ratios in the diabetic group.

In conclusion, plasma angiogenic factors were reduced in type 1 diabetic animals. In addition, resistance exercise increased plasma NO concentrations without changes in VEGF, sFlt-1 and VEGF/sFlt-1 ratio. More studies are needed to determine the role of this type of exercise on angiogenesis.

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