

## Effect of Resistance Training on Plasma Nitric Oxide and Asymmetric Dimethylarginine Concentrations in Type I Diabetic Rats

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### ABSTRACT

**Background:** Asymmetric dimethylarginine (ADMA) has a predominant role in progression of some cardiovascular diseases, including diabetes. It interferes with L-arginine in production of nitric oxide (NO) by inhibition of NO synthase. The purpose of this study was to evaluate the effect of resistance training on plasma NO and ADMA concentrations in type 1 diabetic male rats.

**Methods:** Thirty-six male wistar rats were randomly divided into four groups: (1) control; (2) diabetic; (3) diabetic trained, and (4) control trained ( $n = 9$  each). In the trained groups, the animals undertook one training session per day, 3 days/week, for 4 weeks. At the end of experiment, blood samples were taken and the concentrations of plasma glucose, insulin, lipid profile, NO and ADMA concentrations were determined.

**Results:** plasma ADMA concentration showed a significant increase in diabetic rats compare to control group ( $0.73 \pm 0.07$  vs.  $0.62 \pm 0.04 \mu\text{mol/l}$ ;  $P < 0.05$ ). The plasma ADMA level in the trained diabetic and control were lower than the sedentary groups, although it was not statistically significant. Plasma NO concentration in diabetic group was lower than control ( $P < 0.05$ ). Resistance training significantly increased plasma NO concentration in diabetic animals ( $P < 0.05$ ).

**Conclusion:** Elevated ADMA level in diabetic animals can normalize during resistance exercise. Reduced ADMA level and increased NO level following resistance training might improve cardiovascular risk in diabetic subjects.

**Keywords:** Asymmetric dimethylarginine, diabetes, nitric oxide, resistance training

### INTRODUCTION

Asymmetric dimethylarginine (ADMA) is a naturally occurring chemical found in blood plasma. ADMA is derived from the methylation of arginine residues during the normal protein turnover in many tissues, including vascular endothelial cells.<sup>[1]</sup> A major endothelial-derived vasoactive mediator that synthesized from the amino acid precursor L-arginine is nitric

oxide (NO) which is involved in the maintenance of vascular homeostasis.<sup>[2-4]</sup> ADMA interferes with L-arginine in the production of NO by inhibition of NO synthase.<sup>[5]</sup>

It is believed that ADMA has a predominant role in progression of some cardiovascular diseases, especially atherosclerosis,<sup>[1]</sup> hypercholesterolemia,<sup>[6]</sup> hypertension,<sup>[7]</sup> insulin resistance and diabetes.<sup>[8]</sup> Tobacco use, aging, or congestive heart failure have also been reported to increase plasma ADMA levels.<sup>[9]</sup> Besides, evidences demonstrated that endothelial dysfunction are associated with high circulating ADMA levels.<sup>[8,10]</sup> In patients with elevated ADMA levels, NO synthase is blocked by ADMA, and NO-dependent vasodilation and the manifold inhibitory effects of NO on cell-cell interactions, cell proliferation, and free radical reactions in the blood vessel are impaired.

Type 1 diabetes is associated with higher incidence of microvascular and macrovascular complications and elevated cardiovascular risk compare to non-diabetic individuals.<sup>[11]</sup> Also, endothelial dysfunction in the course of type 1 diabetes is the earliest feature for the vascular complications<sup>[12]</sup> that along with changes in NO synthase pathway.<sup>[11]</sup> Elevated ADMA in diabetic subjects in part can assist to the impaired nitric oxide synthase (NOS) pathway.<sup>[11]</sup> Exercise is considered for management of diabetic subjects and the beneficial effects of exercise on cardiovascular system in diabetic subjects have been documented in several studies.<sup>[13-15]</sup> Thus, we hypothesized that resistance training can normalize elevated ADMA concentrations in diabetic animals and therefore contributes to cardiovascular risk reduction in these subjects.

## METHODS

### Animals and experimental groups

In this experiment, we used thirty-six male wistar rats ( $288 \pm 22$  g). The animals were purchased from Pasteur's Institute (Tehran, Iran) and kept in the central animal house of the university. The animals were housed three per cage in an air-conditioned animal room with 12 h light/dark cycle, at a room temperature between  $22 \pm 2^\circ\text{C}$ , and provided with food and water *ad libitum*. The animals were divided into four groups: (1) control, (2) diabetic, (3) diabetic trained, and (4) control trained ( $n = 9$  each).

The ethical committee of the Tarbiat Modarres University approved all methods used in the study.

### Resistance training protocol

The rats in the trained group undertook one training session per day, 3 days/week, for 4 weeks, i.e., 12 sessions plus an initial familiarization session in total as previously described.<sup>[16]</sup> Training was done with the use of a 1 m high ladder inclined at  $80^\circ$ . There were 26 rungs evenly spaced on the ladder. The rats in the trained group were acquainted with the exercise by practicing climbing from the ladder, before inducing diabetes. The rats were placed at the bottom of the climbing apparatus and motivated to climb the ladder by touching and grooming to their tail. We used of electrical stimulation, forced air, food restriction/reward, and cold water to encourage the rats to perform the exercise training and in order to minimize the stress. The rats rested when they reached the top of the ladder. After 7 days injection of streptozotocin (STZ) rats started the training protocol, using the climbing ladder and weights which were attached to the base of the tail with 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: The preliminary phase of 2 weeks duration followed by the flat load resistance exercise training phase of 2 weeks duration. Prior to the commencement of the preliminary phase, those rats allocated to one of the two training groups were familiarized with the ladder climbing exercise. In the preliminary phase, the rats were adjusted to climbing the ladder with progressive loading on each consecutive training day. The training group of rats was undertaken six repetitions ascending the ladder interspersed with 1 min rest intervals. After 3 min rest, a second set of six repetitions was performed with 1 min rest intervals. On the 1<sup>st</sup> day, rats trained with the equivalent of 30% body mass (BM) as load appended to their tail (6 reps/2 sets). On the 2<sup>nd</sup> day the training load was elevated to 50% BM (6 reps/2 sets), and on the 3<sup>rd</sup> day an additional set of repetitions was performed with 50% BM (6 reps/3 sets). Thereafter, when the training load reached to 100% BM, the training

load was progressively increased until the 7<sup>th</sup> day (familiarization day and six progressive resistance training days). In the flat load resistance training phase, the rats continued to train with 100% BM, 6 repetitions per set, 3 sets per day, and 3 days per week until the end of 4<sup>th</sup> week. Warming-up and cooling down consisted of 2 repetitions climbing the ladder without weights appended to the tail, immediately pre and post each training session. Non-trained (sedentary) rats were controlled on the same days and times as the trained groups in order to minimize any stress imputable to handling.

### Induction of diabetes

A single intraperitoneal injection of STZ at a dose of 55 mg/kg (Sigma-Aldrich, St. Louis, MO) was used for induction of diabetes. STZ was dissolved (20 mg/ml) in a cold 0.1 M citrate buffer (pH 4.5). Non-diabetic rats received similar volume of citrate buffer only. Blood glucose concentrations were measured using tail vein following overnight fasting, 5 days after the STZ injection. Blood glucose level higher than 16 mmol/l was considered as indicative of diabetes.<sup>[17]</sup>

### Sacrificing and sampling

After 4 weeks, the rats were anaesthetized intraperitoneally with a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg). The animals were sacrificed between 9.00 and 12.00 am. The abdominal cavity was opened following the median line of the abdomen and approximately 6 ml of blood was obtained from the abdominal vena cava. The bloods were centrifuged (3000 rpm; 15 min) and the plasma samples were maintained at -70°C for further analyses.

### Plasma glucose, insulin, and lipid profile

Plasma glucose level was measured by an enzymatic colorimetric method (glucose oxidase phenol4-aminoantipyrineperoxidase, Pars Azmoun, Tehran, Iran). Enzyme-linked immunosorbent assay (ELISA) kits specific for the rats were used to determine plasma insulin level (Mercodia AB, Uppsala, Sweden). Plasma high-density lipoprotein cholesterol (HDL-C) was assigned by direct colorimetric method (Randox, Antrim, UK). Enzymatic colorimetric methods (Pars Azmoun,

Tehran, Iran) assessed total triglyceride (TG) and total cholesterol (TC). Serum free fatty acid concentrations were assessed by a colorimetric method (Randox, Antrim, UK) following the manufacturer's instructions. To determine low-density lipoprotein cholesterol (LDL-C), the Friedewald equation was used.

### Plasma NO measurement

The plasma NO concentrations were determined by evaluation of its stable oxidation product (nitrite) using the Griess reaction method (Promega Corp., Madison, USA) as previously described.<sup>[18]</sup> Briefly, samples were added to 96-well enzymatic assay plate. Then, sulfanilamide and N-1-naphtylethylenediamine dihydrochloride solutions were added to the samples, respectively. Absorbance was measured by a microplate reader at the wavelength of 520 nm. The plasma NO concentrations were determined in comparison to nitrite standard reference curve. The limit detection was 2.5 µm nitrite.

### Plasma asymmetric dimethylarginine measurement

The plasma level of ADMA was determined using a commercially available ELISA kit ((DLD) diagnostica GmbH, Germany) based on manufacturer's guidelines. The ADMA assay is a competitive ELISA involving polyclonal capture and secondary antibodies specific for ADMA. The minimum level of detection is 0.05 µmol/l. The interassay coefficient of variation is 4.5%.<sup>[19]</sup>

### Statistical analysis

Results are reported as mean ± standard error. Data was analyzed using One-Way ANOVA with tukey's *post hoc* test. SPSS 16 was used for statistical analysis. *P* less than 0.05 was considered statistically significant.

## RESULTS

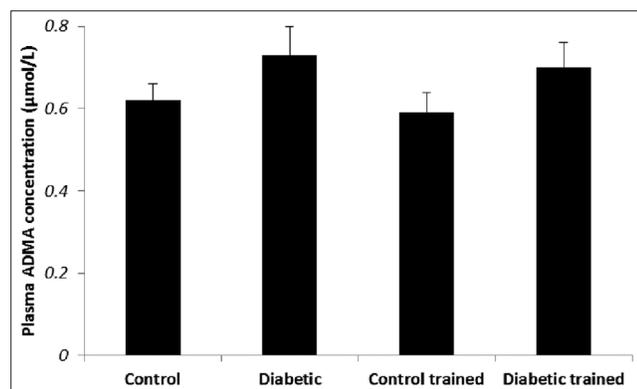
Table 1 illustrates the results of plasma glucose, insulin, and free fatty acid (FFA) and lipid profile at the end of study in experimental groups. Plasma glucose level was higher and insulin level was lower in diabetic animals compare to control (*P* < 0.05). Resistance training could not change plasma glucose and insulin concentrations in control and diabetic

animals ( $P < 0.05$ ). Evaluation of lipid profile showed that there were no significant differences between experimental groups ( $P < 0.05$ ).

Before exercise, the diabetic animals had significantly higher ADMA concentration than control ( $0.73 \pm 0.07$  vs.  $0.62 \pm 0.04$   $\mu\text{mol/l}$ ;  $P < 0.05$ ). ADMA plasma level showed a decrease upon resistance training in the trained diabetic and control rats, although it was not statistically significant [Figure 1]. Plasma NO concentration in diabetic group was lower than control ( $P < 0.05$ ). Resistance training significantly increased plasma NO concentration in diabetic animals ( $P < 0.05$ ; Figure 2).

## DISCUSSION

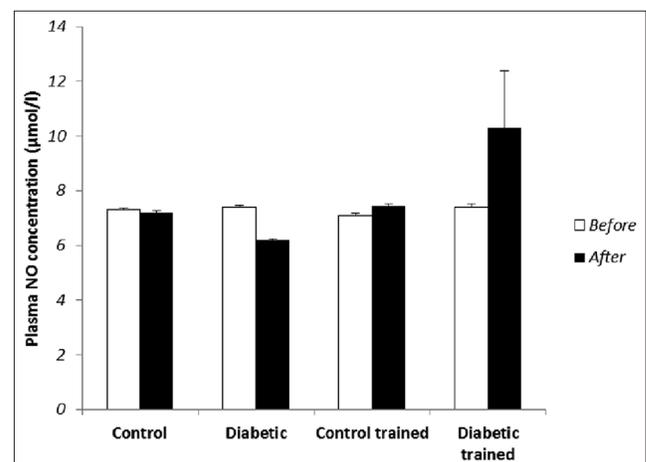
Cardiovascular disease is the main cause of morbidity and mortality in diabetic patients and endothelial dysfunction is a major risk factor for cardiovascular diseases. NO is one of the most important endothelium-derived relaxing factor which has several vascular protective effects.<sup>[20]</sup> The



**Figure 1:** Plasma ADMA concentrations in experimental groups ( $n = 9$  in each group)

aim of the present study was to determine the effect of 8 weeks and 4 weeks resistance training on plasma ADMA and NO concentrations in type I diabetic rats. In this study, we found a decreased plasma NO concentration in diabetic animals compared to sedentary group which support the results of previous studies.<sup>[21-23]</sup> Suppression of endothelial NO synthase expression and activity, increased superoxide generation and activation of protein kinase C are the suggested mechanisms responsible for lowered NO bioavailability in hyperglycemic status and diabetes.<sup>[23,24]</sup>

In this study, we found that diabetic animals had higher plasma ADMA concentration compared to control. Elevated plasma ADMA concentration has been demonstrated in several cardiovascular risk factors including hyperlipidemia, diabetes mellitus and peripheral artery disease.<sup>[25]</sup> It is documented that there is a positive correlation between high plasma ADMA level and cardiovascular mortality



**Figure 2:** Plasma nitric oxide (NO) concentrations before and after experiment in study groups ( $n = 9$  in each group). \*:  $P < 0.05$  compare to other groups

**Table 1:** Plasma biochemical parameters at the end of experimental study

Variable	Control sedentary	Diabetic sedentary	Control exercised	Diabetic exercised
Total cholesterol (mg/dl)	$75.4 \pm 3.4$	$76.7 \pm 2.0$	$79.6 \pm 3.6$	$80.4 \pm 2.5$
LDL (mg/dl)	$34.5 \pm 3.2$	$33.3 \pm 2.8$	$36.82 \pm 3.6$	$37.3 \pm 2.1$
HDL (mg/dl)	$28.0 \pm 0.8$	$29.8 \pm 1.3$	$27.6 \pm 1.2$	$28.9 \pm 1.5$
Triglyceride (mg/dl)	$64.6 \pm 3.5$	$70.9 \pm 4.9$	$59.6 \pm 6.4$	$71.4 \pm 2.5$
FFA (mmol)	$0.75 \pm 0.16$	$0.77 \pm 0.13$	$0.83 \pm 0.17$	$0.80 \pm 0.13$
FBS (mmol/l)	$7.0 \pm 0.1$	$19.6 \pm 0.7^*$	$7.2 \pm 0.1$	$19.6 \pm 0.6^*$
Insulin (IU/ml)	$0.53 \pm 0.10$	$0.18 \pm 0.03^*$	$0.16 \pm 0.02$	$0.54 \pm 0.11^*$

LDL=Low-density lipoprotein, HDL=High-density lipoprotein, FFA=Free fatty acid, FBS=Fasting blood sugar, IU=International unit, \*= $P < 0.05$  compare to control groups

and morbidity.<sup>[26,27]</sup> In agree to our results, a study in patients with type I diabetes mellitus showed that diabetic patients had higher ADMA level than the control before exercise.<sup>[28]</sup> Diabetic patients have endothelial dysfunction and have higher ADMA level than healthy person.<sup>[29]</sup> NO has several antiatherosclerotic properties such as inhibition of platelet aggregation, vasodilation, smooth muscle cell proliferation, leukocyte adhesion<sup>[20]</sup> and inhibition of NO production by elevated ADMA level in diabetic subjects can lead to endothelial dysfunction and contribute to increased cardiovascular risk and microvascular and macrovascular complications.<sup>[30]</sup>

In the present study, resistance training increased plasma NO concentration and reduced plasma ADMA concentration in diabetic animals, although it was not statistically significant. ADMA is a NO synthase inhibitor and inhibits NO production<sup>[31]</sup> and exercise-induced increases in NO bioavailability may be due to reduces in ADMA concentration.<sup>[32]</sup> In contrast to our results, a study reported that exercise is ineffective in lowering plasma ADMA concentration in patients with chronic heart failure.<sup>[33]</sup> However, it should be considered that these patients had normal ADMA concentration and exercise could not further reduce it. In another study, the 10 km runners had an increased plasma ADMA levels after exercise, while in marathon runners, plasma ADMA level decreased.<sup>[34]</sup> However, in agreement with the present study, exercise-induced decreases in plasma ADMA level have been previously reported.<sup>[28]</sup> Recently, Serre *et al.* showed that 12 weeks moderate-intensity exercise training lowered plasma ADMA level in patients with type 2 diabetes.<sup>[35]</sup> In the present study, we found that training reduced plasma ADMA level in control and diabetic animals, although it was not statistically significant. It should be considered that small changes of plasma ADMA concentration have a large effect on intracellular ADMA level and it is sufficient to improve NO production.<sup>[36]</sup>

High plasma ADMA in diabetes may be related to reduced renal clearance and dimethylarginine dimethylaminohydrolase (DDAH) activity in renal cortex.<sup>[37]</sup> Regular exercise may decrease ADMA concentration by up regulation of DDAH-1 and enhanced DDAH-1 messenger RNA [mRNA] expression, an enzyme that metabolize ADMA.<sup>[38]</sup> In this study, we did not measure renal function in the animals and this is the limitation of the present study.

In the present study, plasma glucose, insulin and lipids did not change after exercise which support the previous studies<sup>[39]</sup> and showed that changes of plasma ADMA and NO concentrations are independent of changes in plasma glucose and lipids concentrations.

## CONCLUSION

In summary, reduced plasma ADMA and increased NO concentration after resistance exercise might reflect an improved endothelial function in diabetic animals and by this mechanism, exercise may contribute to cardiovascular risk reduction in diabetic subjects.

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