The Effects of Chronic Resveratrol Treatment on Vascular Responsiveness of Streptozotocin-Induced Diabetic Rats

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Deficiency in the vasorelaxant capacity is a result of an oxidative stress in diabetic animals and seems to be an etiological factor of vascular complications of diabetes. The present study was designed to examine whether resveratrol (RSV), a polyphenolic compound which is naturally present in grape and red wine, has a protective effect on diabetic aorta. Resveratrol (5 mg/kg/d, i.p.) was administered for 42 d to streptozotocin (STZ) (60 mg/kg) induced diabetic rats. Loss of weight, hyperglycemia, and elevated levels of plasma malondialdehyde (MDA) were observed in diabetic rats. Resveratrol treatment was significantly effective for these metabolic and biochemical abnormalities. The contractile responses of the aorta were recorded. Compared with control subjects, the aorta showed significantly enhanced contractile responses to noradrenaline (NA), but not to potassium chloride (KCl), in diabetic rats. Treatment of diabetic rats with resveratrol significantly reversed the increases in responsiveness and sensitivity of aorta to noradrenaline. In diabetic aorta, the relaxation response to acetylcholine (Ach) was found to be significantly decreased compared with control subjects, and resveratrol treatment reversed this; no such change was observed in the relaxation response to sodium nitroprusside (SNP). These results indicated that resveratrol significantly improved not only glucose metabolism and oxidative injury but also impaired vascular responses in streptozotocin induced diabetic rats.

Key words diabetes; oxidative stress; resveratrol; vascular reactivity

Resveratrol (3,4′,5-trihydroxystilbene) (RSV) is found in dietary plants, and it is a phytoalexin substantially present in grapes and wines, especially in red wines. Resveratrol has been reported to possess a wide range of pharmacological effects, including anti-inflammatory, anticarcinogenic, antioxidant, estrogenic, antiplatelet, and antimicrobial properties.[1—7] Resveratrol in red wine has been suggested to be responsible for the "French paradox", decreased risk for coronary artery disease in France.[8] The beneficial effects of these compounds seem to be due to their antioxidant/antiradical activities protecting the vascular walls from oxidation, inflammation, platelet aggregation, and thrombus formation.[9]

Several reports point to protective acute effects of resveratrol on vascular bed, most indicating its vasodilator effects through direct and/or endothelium dependent mechanisms.[10—12] The key regulators of the vasomotor function are vasodilatator nitride oxide (NO) and the vasoconstrictor endothelin-1 (ET-1). Alnaeb *et al.* demonstrated the number of ET-1 receptors was significantly higher whereas, the expression of endothelial NO synthase (eNOS) was significantly lower when comparing aortas from normal rabbits with these from diabetics ones.[13] eNOS expression in aorta was significantly lower in insulin-resistant and diabetes mellitus (DM) rats compared with normal control rats and the decrease was more pronounced in DM rats.[14] Resveratrol significantly increased the expression of the gene encoding eNOS, which synthesis the vasodilator molecule NO, and decreased expression of the potent vasoconstrictor, endothelin-1 (ET-1).[15] Resveratrol reduces the generation of H_2O_2 and normalizes the levels of oxidized glutathione reductase and myeloperoxidase (MPO) activities. By normalization of the reductive oxygen species (ROS) levels, resveratrol limits the oxidative stress, which inhibits NO synthesis by eNOS necessary for vasorelaxation.[9] Resveratrol inhibits ET-1 promoter activity, ET-1 mRNA level and strain induced ET-1 secretion. Furthermore, resveratrol inhibits ET-1 surproduction and cytosolic phospholipase A_2 activity stimulated by oxidative stress.[9]

Diabetes mellitus is an important health problem and mortality from cardiovascular complications is almost three fold higher in the diabetic population than in the general population.[16,17] Oxidative stress is a major factor in the genesis of both macroangiopathy and microangiopathy in diabetes.[18] Unlike single angiogenic factor antagonists, resveratrol has therapeutic value (even at very low concentrations of 1—2.5 μM), as it blocks multiple angiogenic pathways including protein kinase C, vascular endothelial growth factor and cyclooxygenase.[9] Resveratrol might have a protective effect on diabetic vascular degeneration with its vasorelaxant, antiangiogenic and hypoglycemic features.

The purpose of this study was to investigate the chronic effects of resveratrol on diabetes and vascular response in streptozotocin (STZ) induced diabetic rats. Our study is the first to involve a long term RSV treatment of diabetic rats.

MATERIALS AND METHODS

Animals Male Wistar albino rats (8 weeks old, weighing 180—230 g) were divided randomly into four experimental groups; Group I: Nondiabetic rats were used as a control group ($n=10$); Group II: RSV control group ($n=8$); Group III: STZ-induced diabetic group ($n=8$); Group IV: STZ induced and RSV treated diabetic group ($n=8$). 20% ethanol as vehicle was injected to Group I and III. No inflammation was observed at the intraperitoneally (i.p.) injected site of rats. The rats were kept under standard animal room conditions (temperature 21±1 °C; humidity 55—60%) with food and water continuously available for one week before the experiment. The experimental protocols were approved by the Medical School, Animal Ethical Committee of Düzce University, Düzce.

Resveratrol (RSV) Treatment The rats in group II (RSV-treated control group) and group IV (RSV-treated

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STZ-diabetic group) were i.p. injected with RSV (5 mg/kg/d) for 42 d.

Induction of Experimental Diabetes Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg). Two days later, diabetes was verified by estimating hyperglycemia in tail vein blood samples (Glucometer; Elite 2000; Bayer, Leverkusen, Germany). Rats who had 170 mg/dl or greater blood glucose levels were considered diabetic. In all experimental groups, nonfasting blood glucose levels in blood obtained from the tail vein was measured on day 0 (basal), 3, 30 and 42. All rats in 4 groups including STZ diabetic rat survived until the end of study before sacrificing.

Preparation of Rat Thoracic Artery Samples and Recording of Contraction–Relaxation Six weeks after the induction of diabetes, food was withdrawn for 18 h before killing the rats. Rats were anesthetized with 60 mg/kg thiopental sodium (i.p.). Then blood samples (3—5 ml) were collected in heparinized injectors by the intracardiac route. Descending thoracic aortas were carefully excised and placed in cold Krebs–Henseleit solution (KHS) containing in mM: NaCl 118.5, KCl 4.74, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.18, $NaHCO_3$ 24.9, and glucose 10.0. The aortas were cleaned of excess connective tissue and fat and cut into rings of approximately 3—5 mm length. In all experiments, great care was taken to avoid damaging the luminal surface of the endothelium. Aortic rings were suspended in 10 ml jacketed organ baths filled with 10 ml of KHS continuously aerated with a mixture of 5% CO_2, 95% O_2, pH 7.4 at 37 °C. Contraction was measured by suspending the rings between two stainless-steel hooks, one of which was attached to the end of a bathing tube and the other to an isometric force transducer (MAY FDT10, Commat Ltd., Ankara, Türkiye) connected to a multichannel acquisition and analysis system (MAY MP30, Commat Ltd., Ankara, Türkiye).

Experimental Protocol The rings were equilibrated for 90 min under a resting tension of 2 g before the experiment. During the equilibration period, the rings were washed every 20 min. At the end of the equilibration, dose–response curves were obtained first with noradrenaline (NA; 10^{-9}—10^{-4} mol/l) and then with potassium chloride (KCl; 10, 20, 40, 80 mmol/l) NA, or KCl was added in a cumulative manner until a maximal response was achieved. After the addition of each dose, a plateau response was obtained, which was followed by the addition of a subsequent dose.

After rinsing with KHS to base-line tension, rings were equilibrated for 30 min by observing acetylcholine (Ach) induced responses. Rings were then contracted with a submaximal concentration of NA, which produced 70—80% of maximal response. After reaching a plateau of contraction, cumulative concentration–response curves to Ach (10^{-8}—10^{-4} mol/l) and sodium nitroprusside (SNP; 10^{-10}—10^{-6} mol/l) were obtained for relaxations. At the end of each experiment, the tissue was blotted dry, measured and weighed.

Biochemical Measurements Aortic blood samples were centrifuged immediately upon sampling, at 1000 ***g*** for 10 min. Serum was separated from blood as soon as possible and stored at −20 °C. Serum samples were used to determine malondialdehyde (MDA) levels and total antioxidant status (TAS). Lipid peroxidation was used as an indirect measure of oxidative damage induced by ROS. Lipid peroxidation in serum was determined by the thiobarbiturate reaction measuring the formation of MDA.[19] Briefly, 0.5 ml of 0.5% butylated hydroxytoluene was added to 2 ml of serum to prevent lipid auto-oxidation. To precipitate the proteins, 2 ml of 20% trichloroacetic acid was added to 2 ml of serum. After mixing and centrifuging, 1 ml of 0.67% thiobarbiturate–water solution was added to the supernatant, and the mixture was boiled for 60 min. After it was cooled, the optical density at 530 nm was assayed 1,1,3,3-tetraethoxypropane was used as standard MDA levels are expressed as nmol/ml.

The antioxidant system has many components, and deficiency of any of the components can cause a reduction in the overall antioxidant status of an individual. The relative efficacy of the contribution of each antioxidant does not define the actual importance of the antioxidant. Therefore, TAS measurements provide a tool for establishing links between the antioxidant capacity and the risk of disease, as well as for monitoring antioxidant therapy.[20] Serum TAS was determined using a Shimadzu UV-1200 spectrophotometer and a commercially available TAS kit (Cat No. NX3223, Randox laboratories Ltd., Crumlin, U.K.) as described by Miller and Rice-Evans (1995).[21] Both water- and lipid-soluble antioxidants in the serum samples under investigation inhibit the production of the radical cation 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate). The production levels of radical cations were measured spectrophotometrically. TAS results are expressed as nmol/ml of trolox (6-hydroxy-2,5,7-tetramethylchroman-2-carboxylic acid, a water-soluble analogue of a-tocopherol) equivalent.

Drugs Streptozotocin, (−) noradrenaline bitartrate, acetylcholine chloride, sodium nitroprusside, KCl, thiopental sodium and resveratrol were purchased from Sigma (Chemical Co., St. Louis, MO, U.S.A.). All concentrations are expressed as the final molar concentration of the base in the organ bath.

Data and Statistical Analysis Contractile responses to NA and KCl were expressed as the increase in tension (in milligrams) in response to the agonist per milligram of aorta. Relaxations to acetylcholine and sodium nitroprusside were calculated as above. Agonist pD_2 value ($= -\log EC_{50} = -\log$ contractile $\text{concentration}_{50}$) was calculated from each agonist concentration–response curve by linear regression analysis of the linear portion of the curve and taken as a measure of sensitivity of the tissues to each agonist.[22] All values are expressed as "means ± S.E.M." Comparisons of concentration–response curves were analyzed by one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. The results were considered significantly different if $p < 0.05$.

RESULTS

General Characteristics At the end of 30 and 42 d, the body weight of the diabetic rats (Groups III and IV) was found to be significantly decreased compared with control rats Untreated DM (Group III) was found significantly lower than DM plus RSV group (Group IV) at 42nd day measurements (Fig. 1).

Blood glucose levels of control, RSV treated, diabetic, and RSV treated diabetic groups are represented in Fig. 2. Measurements on 30th and 42nd days revealed that RSV signifi-

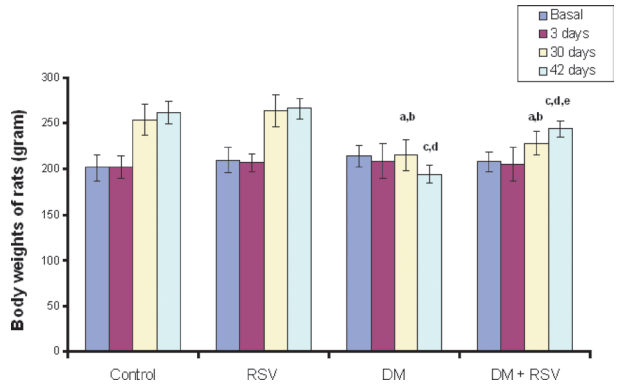

Fig. 1. Body Weights of Rats (Mean (g)±S.E.M.)

Control group (n=10), resveratrol group (RSV, n=8), diabetic group (DM, n=8) and resveratrol treated diabetic group (RSV+DM, n=8). [a] Significant from $Control_{30}$ $p<0.001$; [b] significant from RSV_{30} $p<0.001$; [c] significant from $Control_{42}$ $p<0.001$; [d] significant from RSV_{42} $p<0.05$; [e] significant from DM_{42} $p<0.001$.

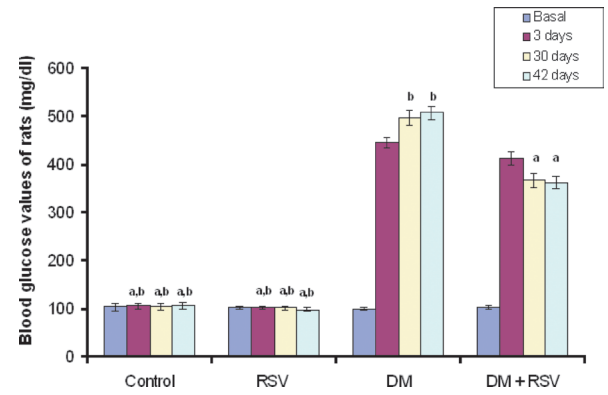

Fig. 2. Nonfasting-Blood Glucose Values of Rats (Mean (mg/dl)±S.E.M.)

Control group (n=10), resveratrol group (RSV, n=8), diabetic group (DM, n=8) and resveratrol treated diabetic group (RSV+DM, n=8). [a] Significant from DM $p<0.001$, [b] significant from DM+RSV $p<0.001$.

cantly reduced nonfasting blood glucose levels of rats.

The Effects of Diabetes and Resveratrol on the Oxidative Stress TAS and MDA levels of RSV treated diabetic rats were normalized compared to those of the diabetic group. TAS levels of RSV treated diabetic rats were significantly different from those of diabetic and control groups. The MDA concentration in diabetic rats was significantly higher than the controls. Although treatment with RSV decreased MDA concentrations with approximately 35%, this result was not statistically significant and still was 43% higher than controls. The data were shown in Fig. 3.

Agonist-Induced Contractions Cumulative addition of NA (10^{-9}—10^{-4} mol/l) to the isolated organ bath resulted in concentration dependent contractions in aortae of all groups (Fig. 4). The contractile responses of aortic rings to NA were found significantly different from DM group (maximum effect (E_{max}): 351.74±15.89 mg tension/mg aorta), (pD_2: 6.388±0.054). RSV partially reversed the contraction abnormalities (263.36±12.70) in diabetic groups. On the other hand, the responses to KCl (E_{max}: 88.427±14.69) in diabetic rats could not be reversed by RSV treatment (E_{max}: 107.085±17.34). The data were shown in Figs. 5 and 6.

Agonist-Induced Relaxations Endothelium-dependent relaxation of aortic rings precontracted with NA to acetylcholine (pD_2 and inhibition %) was summarized in Figs. 7

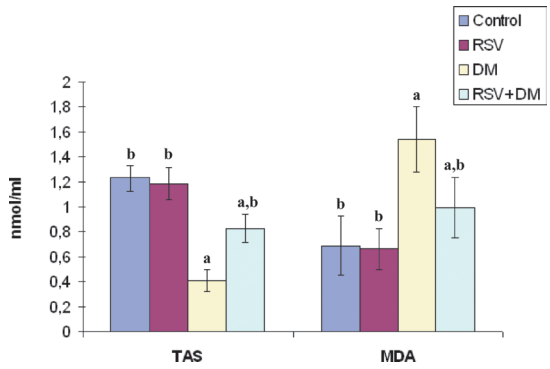

Fig. 3. Values of Total Antioxidant Status (TAS) (nmol/ml) and Malondialdehyde (MDA) (nmol/ml) in the Plasma (Mean±S.E.M.)

Control group (n=10), resveratrol group (RSV, n=8), diabetic group (DM, n=8) and resveratrol treated diabetic group (RSV+DM, n=8). Significantly different from control: [a] $p<0.05$, significantly different from DM: [b] $p<0.05$.

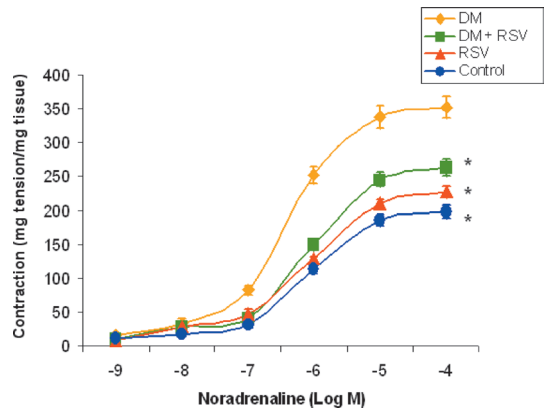

Fig. 4. The Concentration–response Curves to Noradrenaline in Aortas from RSV-Treated and Untreated Controls and Diabetic Rats (Mean±S.E.M.)

Control group (●, n=10), resveratrol group (▲, n=8), diabetic group (◆, n=8) and resveratrol treated diabetic group (■, n=8). Significantly different from DM: * $p<0.001$.

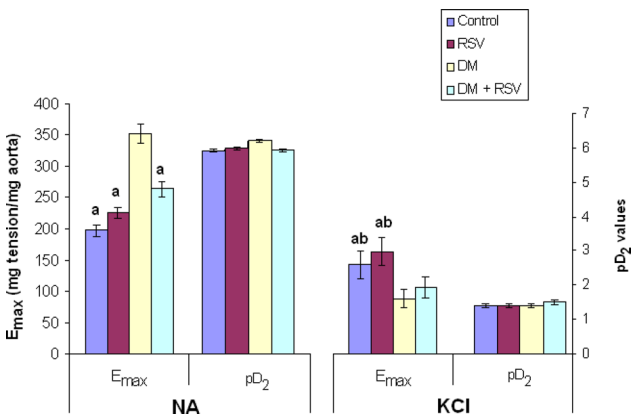

Fig. 5. E_{max} (mg Tension/mg Aorta) and pD_2 Values to NA and KCl in Aortas from RSV-Treated and Untreated Control and Diabetic Rats (Mean±S.E.M.)

Control group (n=10), resveratrol group (RSV, n=8), diabetic group (DM, n=8) and resveratrol treated diabetic group (RSV+DM, n=8). [a] Significant from DM $p<0.01$, [b] significant from DM+RSV $p<0.001$.

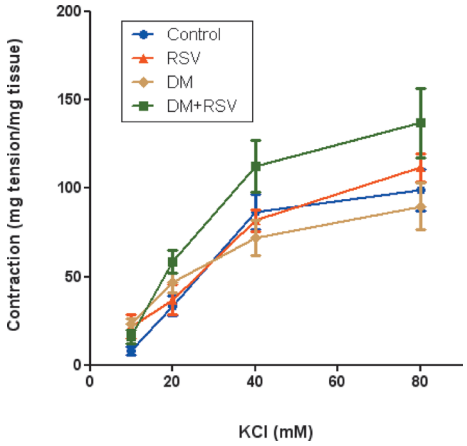

Fig. 6. Contraction Responses of Rat Aortic Strips to KCl Dose Dependently Were Presented

There were no statistically differences between the groups. Control group (●, $n=10$), resveratrol group (▲, $n=8$), diabetic group (◆, $n=8$) and resveratrol treated diabetic group (■, $n=8$).

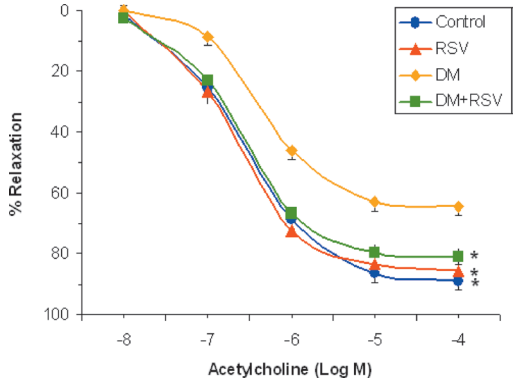

Fig. 7. Graph Showing Endothelium-Dependent Relaxation in Response to Acetylcholine (Ach) in Aorta Wistar Albino Rats Treated for 6 Weeks

Control group (●, $n=10$), resveratrol group (▲, $n=8$), diabetic control group (◆, $n=8$) and resveratrol treated diabetic group (■, $n=8$). Asterisks indicate significant differences from DM group (* $p<0.001$, mean±S.E.M.).

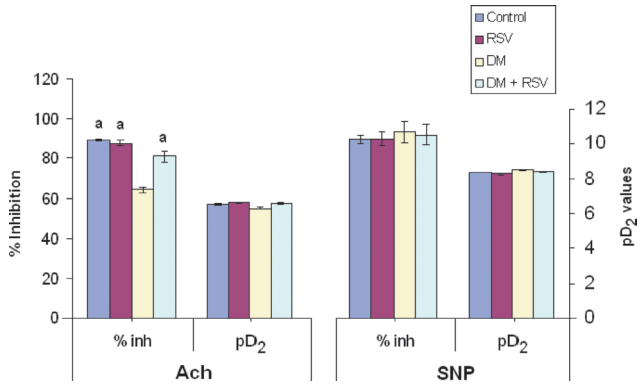

Fig. 8. Inhibition % and pD_2 Values to Ach and SNP in Aortas from RSV-Treated and Untreated Control and Diabetic Rats (Mean±S.E.M.)

Control group ($n=10$), resveratrol group (RSV, $n=8$), diabetic group (DM, $n=8$) and resveratrol treated diabetic group (RSV+DM, $n=8$). [a] Significant from DM $p<0.05$.

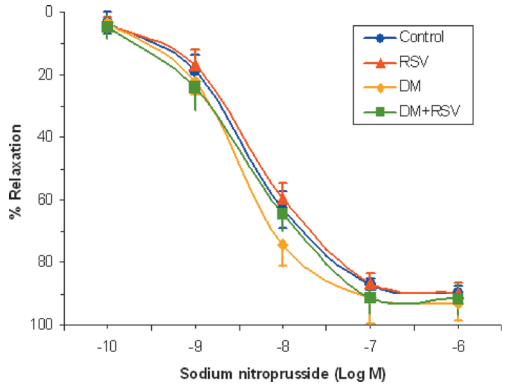

Fig. 9. Graph Showing Endothelium-Independent Relaxation in Response to Sodium Nitroprusside (SNP) in Aorta Wistar Albino Rats Treated for 6 Weeks

Control group (●, $n=10$), resveratrol group (▲, $n=8$), diabetic control group (◆, $n=8$) and resveratrol treated diabetic group (■, $n=8$) (mean±S.E.M.).

and 8. Acetylcholine-induced relaxations in untreated diabetic rats (pD_2: 6.318±0.0248 and inh. %: 64.25±1.15) were decreased compared with age-matched controls (pD_2: 6.54±0.03, $p<0.05$ and inh. %: 89±0.689, $p<0.001$). The impaired relaxation to acetylcholine in aortic rings after 6 weeks of diabetes reached near to untreated control levels within 6 weeks of RSV-treatment (DM+RSV) (pD_2: 6.591±0.0394, $p<0.001$ and inh.%: 81±2.8, $p<0.001$), (Fig. 7). RSV treatment significantly ameliorated Ach induced relaxations in diabetic rats.

Endothelium-independent relaxations to sodium nitroprusside (SNP) in aorta rings precontracted with NA (submaximally, approximately 90% effective concentration (EC_{90})), were not significant when compared to control groups (untreated and RSV-treated) and STZ-diabetic groups (untreated and RSV treated) animals regarding % inhibition and pD_2 values. Data were shown in Figs. 7 and 9.

DISCUSSION

The study demonstrated, in agreement with previous studies, that STZ induced DM caused reactive oxidative stress and impaired endothelium dependent relaxation response to agonists such as Ach in diabetic rat aorta. The present study is the first to show the protective effects of long term RSV treatment on vascular bed of STZ induced diabetic rats.

We have shown that long term (42 d) RSV treatment in STZ diabetic rats improved blood glucose levels, body weight gain and partially vascular contraction responses against vasoactive agents compared to diabetic control groups.

Although some researchers noticed that RSV decreased blood glucose levels insignificantly[23] the results in the present study were compatible with a great majority of the previous researchers.[24,25] The study reveals that RSV decreased blood glucose levels in STZ diabetic rats. The mechanism of hypoglycemic effect of RSV is still unknown and seems to be a different mechanism in diabetics and nondiabetics. RSV, like glibenclamide, inhibits K^+ channels in the cells of insulinoma cell-lines, leading to an increase in insulin secretion *in vitro*.[26] RSV increased insulin secretion associated with a decline in plasma glucose in normal rats, but not in STZ diabetic rats. Additionally, it was shown that RSV increased glucose uptake to tissues without increasing the insulin level in STZ diabetic rats.[25] The antioxidant effect of RSV decreased the oxidative-stress related damage in dia-

betic tissues and made cells function normally.[26)]

The average weight in the control group was expected to increase by the end of the 6 week-study period. No increase in the weight of diabetic group can be explained by less food and fluid intake or by impaired lipid and carbonhydrate metabolism, nor can even any decrease be explained this way. The resumption of moderate body growth in STZ-DM rats with RSV treatment strongly suggested that sugar and lipid metabolism in these animals was improved.

In the present study, total antioxidant status and MDA levels were significantly different in DM and controls even if RSV treatment did not completely improve the diabetic effects but ameliorated them.

Vascular complications account for almost 80% of deaths among diabetic patients. Three major mechanisms that play a role in the pathogenesis of vascular changes in diabetics are nonenzymatic glycosylation of proteins and lipids, oxidative stress and protein kinase C activation with subsequent alteration in growth factor expression.[27)] Deficiency in the vasorelaxant capacity is a result of oxidative stress in diabetic animals and seems to be an etiological factor for vascular complications of diabetes.[28,29)]

Contraction response to NA in aorta of diabetic rats followed for six weeks was increased, and RSV normalized that response. Increased contraction response to NA in STZ diabetic rats has been observed in most of studies.[30—33)] The mechanism is still unknown. However, impaired endothelial activity,[30)] increased response to Ca^{2+},[34)] increase in vasoconstrictor prostanoids due to increase superoxide anion (prostaglandin F2 alpha ($PGF_{2\alpha}$)) or prostaglandin H_2/thromboxane A_2)[35)] might be responsible for increased contraction responses in diabetic rats, which could be improved by RSV treatment.

Our study, similar to some other studies,[22,36)] also showed that long term diabetes and RSV treatment did not affect rat aorta KCl responses although there are a number of contrary reports.[37)]

The results of this work revealed that the endothelium-dependent relaxant response was reduced in aorta of the streptozotocin-induced diabetic rats, and this reduction in relaxant responses could be recovered by RSV treatment. Although some researchers asserted that the relaxant response to acetylcholine was not influenced by diabetes,[36)] the results of this research, in accordance with those of many previous ones,[38,39)] revealed that diabetes decreased acetylcholine responses both in maximum relaxation and in sensitivity (pD_2), and RSV treatment reversed the decreased relaxation responses to normal level. The acetylcholine-induced relaxation response was endothelium-dependent and NO-mediated.[40,41)] The impairment in endothelium-dependent response in diabetic rats could be due to endothelial damage (injury) resulting from increased free oxygen radicals induced by diabetes mellitus. While NO synthesis is decreased from damaged endothelium, vasoconstrictors like endothelin-1 are increased.[42)] The free radical scavenging activity of RSV might have protected the endothelium in diabetic rats and further lead to normalization of acetylcholine-induced relaxation response. It has also been demonstrated that RSV increased acetylcholine-induced Ca^{2+} influx into epithelial cells.[34)] Ca^{2+} increase in epithelial cells increases eNOS levels, which, in turn, increases NO production and secretion. This is comparable to the results of studies indicating that RSV increased NO synthesis. The second possible mechanism of improvement in the acetylcholine-induced relaxation response might be due to increased NO secretion *via* acetylcholine-induced Ca^{2+} influx into epithelial cells in RSV treated rats.[34)]

SNP response is independent from endothelium. Thus, it was not influenced by DM and did not change with RSV treatment. Some of the previous studies claimed that the NO donor sodium nitroprusside decreased the endothelium-independent relaxation responses in diabetes.[43)] However, most of others showed that diabetes had no effect on SNP responses.[22,44)] The results of our study showed that the SNP responses changed neither with diabetes nor with RSV treatment. Our results were in agreement with those of most previous studies.[22,44)]

Our results indicated that resveratrol significantly improved both glucose metabolism and oxidative injury, and it also impaired vascular responses in streptozotocin induced diabetic rats. RSV may be a candidate molecule for preventing the chronic vascular complications of DM by its both antioxidant and vasodilator effects.

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REFERENCES

1) Das S., Das D. K., *Inflammation Allergy Drug Targets*, **6**, 168—173 (2007).
2) Meeran S. M., Katiyar S. K., *Front Biosci.*, **13**, 2191—2202 (2008).
3) Orallo F., *Curr. Med. Chem.*, **13**, 87—98 (2006).
4) Silan C., Uzun O., Comunoglu N. U., Gokçen S., Bedirhan S., Cengiz M., *Biol. Pharm. Bull.*, **30**, 79—83 (2007).
5) Ho S. M., *J. Cell Biochem.*, **91**, 491—503 (2004).
6) Vilar S., Quezada E., Santana L., Uriarte E., Yánez M., Fraiz N., Alcaide C., Cano E., Orallo F., *Bioorg. Med. Chem. Lett.*, **16**, 257—261 (2006).
7) Chan M. M., *Biochem. Pharmacol.*, **63**, 99—104 (2002).
8) Renaud S., de Lorgeril M., *Lancet*, **339**, 1523—1526 (1992).
9) Delmas D., Jannin B., Latruffe N., *Mol. Nutr. Food Res.*, **49**, 377—395 (2005).
10) Buluc M., Demirel-Yilmaz E., "Advances in Recent Cardiovascular Research," ed. by Varro A., Vegh A., Monduzzi Editore, Bologna, 2002, pp. 55—59.
11) El-Mowafy A. M., *Biochem. Biophys. Res. Commun.*, **291**, 1218—1224 (2002).
12) Naderali E. K., Doyle P. J., Williams G., *Clin. Sci.*, **98**, 537—543 (2000).
13) Alnaeb M. E., Thompson C. S., Seifalian A. M., Hamilton G., Mikhailidis D. P., *In Vivo*, **21**, 1069—1074 (2007).
14) Wu J., Lei M. X., Liu L., Xie X. Y., *Zhonghua Xin Xue Guan Bing Za Zhi*, **35**, 265—270 (2007).
15) Nicholson S. K., Tucker G. A., Brameld J. M., *Proc. Nutr. Soc.*, **67**, 42—47 (2008).
16) Jarrett R. J., *Diabetes Metab. Rev.*, **5**, 547—558 (1989).
17) Stamler J., Vaccaro O., Neaton J. D., Wentworth D., *Diabetes Care*, **16**, 434—444 (1993).
18) Karasu C., Ozansoy G., Bozkurt O., Erdoğan D., Omeroğlu S., *Metabolism*, **46**, 872—879 (1997).
19) Ohkawa H., Ohishi N., Yagi K., *Anal. Biochem.*, **95**, 351—358 (1979).
20) Miller N. J., Rice-Evans C., Davies M. J., *Biochem. Soc. Trans.*, **21**, 95 (1993).
21) Miller N. J., Rice-Evans C. A., *Clin. Chem.*, **41**, 1789 (1995).
22) Ozyazgan S., Senses V., Ince E., Sultuybek G., Utkan T., Akkan A. G., *Pharmacol. Res.*, **38**, 73—79 (1998).
23) Ates O., Cayli R. S., Yucel N., Altinoz E., Kocak A., Durak M. A.,

Turkoz Y., Yologlu S., *J. Clin. Neurosci.*, **14**, 256—260 (2007).
24) Thirunavukkarasu M., Penumathsa S. V., Koneru S., Juhasz B., Zhan L., Otani H., Bagchi D., Das D. K., Maulik N., *Free Radical Biol. Med.*, **43**, 720—729 (2007).
25) Su H. C., Hung L. M., Chen J. K., *Am. J. Physiol. Endocrinol. Metab.*, **290**, E1339—E1346 (2006).
26) Chen W. P., Chi T. C., Chuang L. M., Su M. J., *Eur. J. Pharmacol.*, **30**, 269—277 (2007).
27) Aronson D., Rayfield E. J., *Cardiovasc. Diabetol.*, **1**, 1—10 (2002).
28) Oyama Y., Kawasaki H., Hattori Y., Kanno M., *Eur. J. Pharmacol.*, **132**, 75—78 (1986).
29) Kamata K., Miyata N., Kasuya Y., *Br. J. Pharmacol.*, **97**, 614—618 (1989).
30) MacLeod K. M., *Diabetes*, **34**, 1160—1167 (1985).
31) Harris K. H., MacLeod K. M., *Eur. J. Pharmacol.*, **153**, 55—64 (1988).
32) Abebe W., Harris K. H., MacLeod K. M., *J. Cardiovasc. Pharmacol.*, **16**, 239—248 (1990).
33) Ozçelikay A. T., Tay A., Dinçer D., Meral S., Yildizoğlu-Ari N., Altan V. M., *Gen. Pharmacol.*, **33**, 299—306 (1999).
34) Buluc M., Demirel-Yilmaz E., *Vascul. Pharmacol.*, **44**, 231—237 (2006).
35) Kanie N., Kamata K., *Gen. Pharmacol.*, **35**, 311—318 (2000).
36) Agrawal D. K., Bhimji S., McNeil J. H., *J. Cardovasc. Pharmacol.*, **9**, 584—593 (1987).
37) Ramanadham S., Lyness W. H., Tenner T. E., *Can. J. Physiol. Pharmacol.*, **62**, 418—423 (1984).
38) King G. L., Shiba T., Oliver J., Inoguchi T., Bursell S. E., *Ann. Rev. Med.*, **45**, 179—188 (1994).
39) Senses V., Ozyazgan S., Ince E., Tuncdemir M., Kaya F., Ozturk M., Sultuybek G., Akkan A. G., *J. Basic Clin. Physiol. Pharmacol.*, **12**, 227—248 (2001).
40) Furchgott R. F., Zawadzki J. V., *Nature* (London), **288**, 373—376 (1980).
41) Palmer R. M. J., Ferrige A. G., Moncada S., *Nature* (London), **327**, 524—526 (1987).
42) Gryglewski R. J., Botting R. M., Vane J. R., *Hypertension*, **12**, 530—548 (1988).
43) Kiff R. J., Gardiner S. M., Comptom A. M., Bennett T., *Br. J. Pharmacol.*, **103**, 1357—1362 (1991).
44) Rembold C. M., *Hypertension*, **20**, 129—137 (1992).