

## The vasorelaxant effect of simvastatin in isolated aorta from diabetic rats

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### Short Communication

#### Abstract

**BACKGROUND:** The increasing incidence of diabetes mellitus (DM) is of great clinical significance. In this study, we aimed to investigate whether exposure of endothelium-intact aortic rings to simvastatin could have a vasorelaxant effect in diabetic rats.

**METHODS:** For induction of diabetes, streptozotocin (STZ) (60 mg/kg, i.p., single dose) was used. After 1 month, the cumulative reaction of isolated endothelium-intact aortic rings was determined to KCl and phenylephrine (PE) in the absence and presence of nitric oxide (NO) synthase inhibitor, i.e., nitro-L-arginine-methyl ester (L-NAME), and prostaglandin synthesis inhibitor, i.e., indomethacin. Meanwhile, the role of extracellular calcium was assessed in this respect.

**RESULTS:** At the end of the study, the addition of simvastatin (at a concentration  $\geq 10^{-5}$  M) caused a significant concentration-dependent relaxation response of PE-precontracted aortic rings for both control and diabetic groups (at a significant difference of  $P < 0.050$ ), and this difference did not exist for KCl-precontracted aortic rings. Furthermore, both L-NAME (100  $\mu$ M) and indomethacin (10  $\mu$ M) significantly diminished the vasorelaxant response following simvastatin addition. Meanwhile, there was no statistically significant difference between control and diabetic groups in the absence of extracellular calcium.

**CONCLUSION:** The results of this study suggest that simvastatin is able to relax PE-precontracted aortic rings isolated from STZ-diabetic rats via modulation of NO- and prostaglandin-dependent signaling and its effect is not via modulation of calcium mobilization from intracellular stores.

**Keywords:** Simvastatin; Aorta; Diabetes Mellitus

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#### Introduction

The increasing epidemic of diabetes mellitus (DM) is of significant concern worldwide.<sup>1,2</sup> Vascular complications in DM, as known as vasculopathy, include macro- and micro-vascular dysfunction and abnormality that is the principal reason of morbidity and mortality in patients with DM.<sup>1,2</sup> Endothelial dysfunction in DM plays a key role in the development and progression of vasculopathy due to DM.<sup>1</sup> Urgent medical interventions are necessary to lower DM vascular complications which require new preventive and treatment strategies.<sup>3</sup> Earlier strategies for controlling and managing the cardiovascular complications of DM mainly target a group of well-defined risk factors such as hyperglycemia, lipid abnormalities, and hypertension.<sup>4</sup> Considering the great developments in human knowledge about DM diversity and knowing that it is one of the major health, social and economic problems worldwide, a need for

finding effective treatments with fewer side effects, to treat DM and its complications has been arisen.<sup>5</sup>

Simvastatin is a drug that is generally used in clinics for the treatment of hypercholesterolemia.<sup>6</sup> Simvastatin acts via blocking the key enzyme in the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and has found to be useful for lowering plasma low-density lipoprotein cholesterol (LDL-C).<sup>7</sup> Clinical trials have also proven that such enzyme inhibitors are capable to lower cardiovascular disorders and associated morbidity and mortality in patients with coronary artery disease (CAD).<sup>8</sup> In addition, research evidence have indicated that such inhibitors could restore endothelium-dependent responses in the vascular system.<sup>9</sup> Furthermore, it has found out that simvastatin could produce a vasodilation in conductance and small arteries that are an endothelium-dependent response that engages both nitric oxide (NO) and eicosanoid vasodilators.<sup>6</sup>

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Simvastatin is also effective and well-tolerated in the management of lipid disorders in DM.<sup>10</sup> Since no researches have been done on the beneficial effect of simvastatin on the vascular response in diabetic state, therefore, we wanted to explore whether an exposure of endothelium-intact aortic rings to simvastatin could have a vasodilatory effect in streptozotocin (STZ)-diabetic rats and to explore the engagement of NO and prostaglandin and intracellular Ca<sup>2+</sup> pathways.

## Materials and Methods

In this experimental study, male Wistar rats (obtained from Razi Institute, Tehran, Iran), weighing 200-240 g were kept in a housing room on a light/dark cycle (22 ± 2 °C and a humidity of 40-50%) and supplied with standard diet and tap water with no limitation. The used procedures for animals and their care were conducted in accordance to NIH guidelines for the care and use of research animals.

The animals (n = 18) were randomly divided into two groups: control (n = 8) and diabetic (n = 10). For induction of diabetes, STZ (60 mg/kg, i.p., single dose) was used. STZ was freshly dissolved in cold normal saline immediately before its use. For verification of diabetes, serum glucose level > 250 mg/dl was considered using glucose oxidation method (Glucose Oxidase Kit, Zistchimie, Tehran, Iran). The timing of study design has been shown in figure 1.

### Aortic rings preparation

At the end of the study, (after 4 weeks), the rats were deeply anesthetized under diethyl ether, euthanized, and after exposing the abdomen and thorax, descending thoracic aorta was excised with extreme care and immediately transferred to cold saline solution [physiological salt solution (PSS)] containing (in mM): NaCl-118, KCl-4.6, MgSO<sub>4</sub>-1.2, KH<sub>2</sub>PO<sub>4</sub>-1.2, glucose-11.1, NaHCO<sub>3</sub>-27.2, and CaCl<sub>2</sub>-1.8. Thereafter, the aorta was cleaned from extra connective tissue and fat and cut into separate rings of about 4 mm in length. Aortic rings were suspended between two triangular-shaped stainless-steel wires. One wire was attached to a tissue holder in a 50 ml isolated tissue bath containing PSS (pH = 7.4) kept at 37 °C and continuously gassed

with a combination of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The other end of each wire attached (via a cotton thread) to an isometric force transducer (Ugo Basile, Comerio, Italy) coupled to a signal amplifier and connected to a computer via an A/D board. Recording and data analysis was conducted using specially designed software.

In all experimental procedures, special care was paid to prevent damage of the luminal surface of aortic rings. The rings were allowed to equilibrate for 90 minutes under a resting tension of 1.5 g before further experiments were started. This resting tension was found to be optimal in our pilot experiments for all groups under study. During equilibration period, the rings were washed every 30 minutes. For assessing the endothelial integrity, phenylephrine (PE, 1 μM)-precontracted rings were exposed acetylcholine (ACh-10 μM).

For exploring the engaged mechanisms for vasodilatory effect of the simvastatin, isolated aortic rings were pretreated with nitro-L-arginine-methyl ester [L-NAME, nitric oxide synthase (NOS) blocker, 100 μM] and indomethacin (10 μM, prostanoid synthesis inhibitor) separately or in combination 30 minutes before the application of the vasoconstrictors and the simvastatin.

For determination of the engagement of intracellular Ca<sup>2+</sup> mobilization in the vasodilatory effect of the simvastatin, a Ca<sup>2+</sup>-free PSS was made by replacing CaCl<sub>2</sub> with MgCl<sub>2</sub> and the addition of ethylene-glycol-tetraacetic acid (EGTA) (0.5 μM) to chelate any free Ca<sup>2+</sup> in the solution. After a 15-minutes preincubation period with 3-4 washings, PE (1 μM) was applied to stimulate the release of intracellular Ca<sup>2+</sup> and the contraction recorded for 3 minutes. A similar procedure was applied with Ca<sup>2+</sup>-free PSS containing the simvastatin (10<sup>-5</sup> M).

### Drugs and chemicals

Simvastatin, PE, ACh-HBr, indomethacin, L-NAME, and STZ were supplied from Sigma-Aldrich (MO, USA). All other chemicals and reagents were supplied from Merck Co. (Germany) and local market. Indomethacin was dissolved in 0.5% w/v sodium bicarbonate. Further dilutions of these drugs were made in PSS. In addition, STZ was freshly dissolved in 0.9% normal saline.

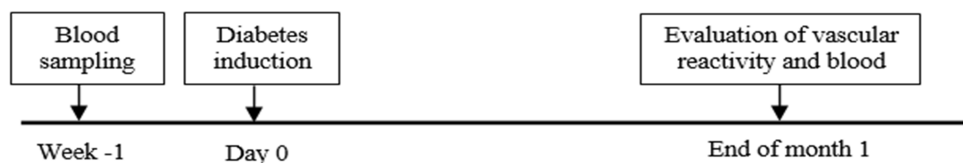


Figure 1. Study timing design

All values were reported as a mean  $\pm$  standard error (SE). Data were analyzed by statistical tests, i.e., Student's t-test for paired samples, independent sample t-test, and one-way analysis of variance followed by Tukey post-hoc test at a significant level of  $P < 0.050$ .

### Results

During the study, 2 rats were excluded from the diabetic group due to a morbid and fatal condition. After 1 month, the body weight of diabetic animals significantly decreased from an average of 227.5 to 181.6 g ( $P = 0.008$ ). Regarding serum glucose level, it significantly increased to  $415.3 \pm 21.8$  mg/dl from the initial level of  $127.2 \pm 8.3$  mg/dl ( $P < 0.001$ ) (Table 1).

Addition of high  $K^+$  (80 mM)-containing PSS to the tissue bath induced a maximal tension of  $258.2 \pm 15.3$  and  $175.6 \pm 17.4$  mg in control and diabetic groups, respectively. However, the found difference was not statistically significant ( $P = 0.080$ ). The application of simvastatin produced a concentration-dependent relaxation in both control and diabetic groups (Figure 2-A). In this respect, simvastatin-induced vasorelaxation of rings from diabetic group was not significantly lower as compared to control rats ( $P = 0.130$ ).

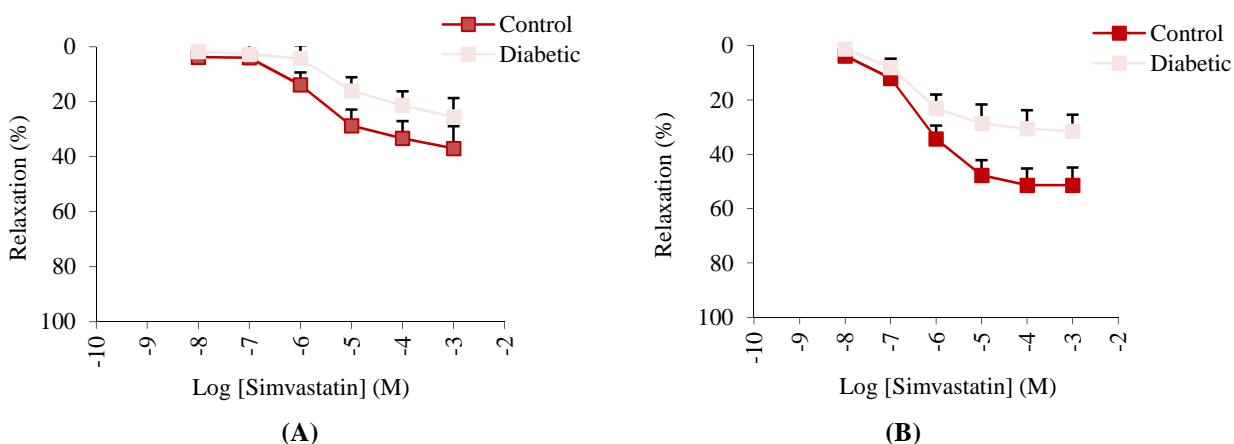
With respect to contractile response of aortic rings, PE (1  $\mu$ M) induced a sustained contraction of the rat aorta with a peak tension of  $487.3 \pm 24.9$  and  $619.5 \pm 26.8$  mg in control and diabetic groups, respectively. This difference was statistically significant ( $P = 0.007$ ). Meanwhile, addition of simvastatin produced a concentration-dependent relaxation in both control and diabetic groups (Figure 2-B). In this regard, simvastatin-induced vasodilation of aortic rings isolated from diabetic rats was significantly lower as compared to control rats at concentrations  $> 10^{-5}$  M ( $P = 0.020$  and  $P = 0.040$ ).

Pretreatment of the tissues with L-NAME and not indomethacin markedly and significantly attenuated the inhibitory effect of simvastatin against PE (1  $\mu$ M)-induced contraction in both control ( $P = 0.020$ ) (Figure 3-A) and diabetic ( $P = 0.030$ ) groups (Figure 3-B). Meanwhile, in the absence of extracellular  $Ca^{+2}$ , PE produced a transient contraction in control and diabetic groups. This difference was not found out to be statistically significant ( $P = 0.070$ ). Furthermore, pretreatment of the aortic rings with simvastatin did not significantly reduce the contractions induced by PE for both control ( $P = 0.140$ ) and diabetic ( $P = 0.110$ ) groups in the absence of extracellular calcium (Figure 4).

**Table 1.** Body weight and serum glucose level in different groups and weeks

Group	Body weight (g)		Serum glucose (mg/dl)	
	Baseline (mean $\pm$ SD)	After 1 month (mean $\pm$ SD)	Baseline (mean $\pm$ SD)	After 1 month (mean $\pm$ SD)
Control	221.8 $\pm$ 9.6	241.9 $\pm$ 10.3	131.7 $\pm$ 10.4	125.7 $\pm$ 12.5
Diabetic	227.5 $\pm$ 10.2	181.6 $\pm$ 11.8*	127.2 $\pm$ 8.3	415.3 $\pm$ 21.8**

\* $P = 0.008$  (vs. baseline in the same group), \*\* $P < 0.001$  (vs. baseline in the same group). Student's t-test for paired samples SD: Standard deviation



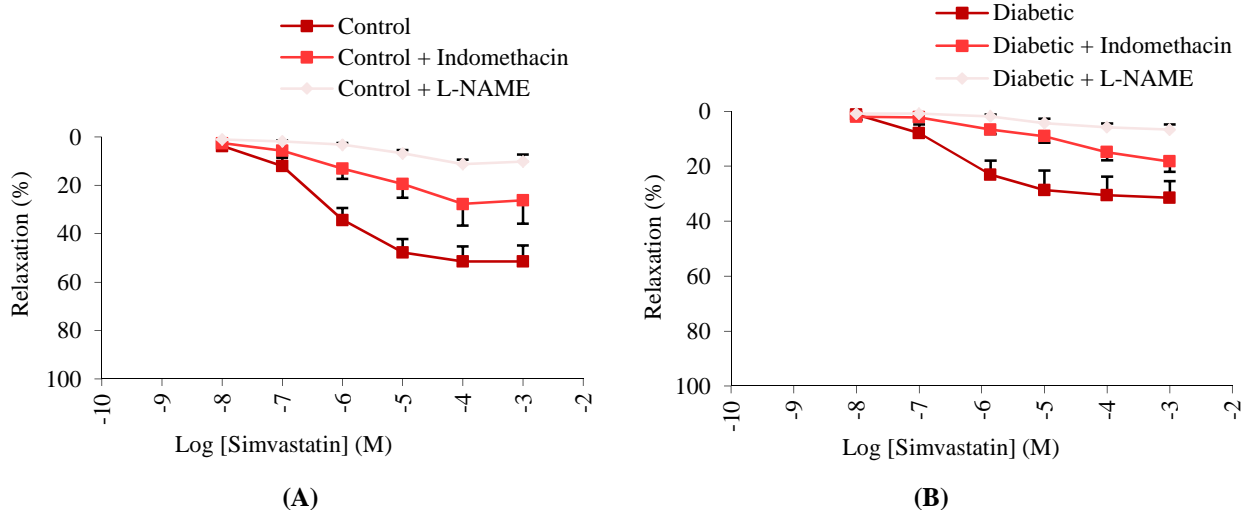
**Figure 2.** Vasorelaxant effect of simvastatin against KCl (A) and phenylephrine (B)-induced contractions in aortic rings from control (n = 6) and streptozotocin-diabetic (n = 7) rats. Unpaired Student's t-test was used for comparing control and diabetic groups at each concentration of simvastatin ( $P = 0.020$ ,  $P = 0.040$ ) (vs. diabetic).

## Discussion

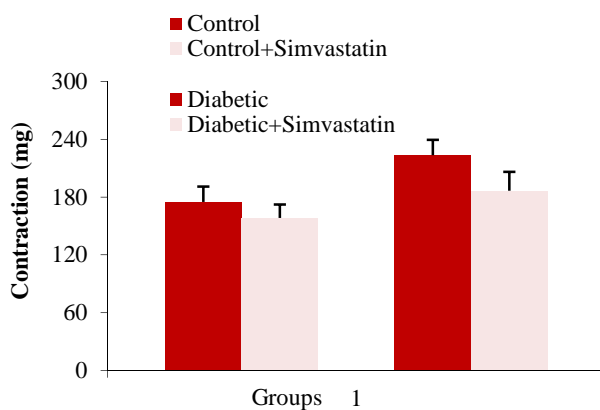
The present work was performed to investigate the involved mechanisms responsible for simvastatin-induced vasorelaxation in PE-precontracted aortae of STZ-diabetic rats. It was found out that simvastatin-induced and NO- and prostaglandin-dependent vasorelaxation in aortic rings from diabetic rats.

There are two mechanisms for the vasodilation response in the vascular system: the secretion of relaxant factor from vascular endothelium and inhibition of vasoconstriction. The former is mediated by bradykinin, prostacyclin, and NO.<sup>11</sup> The relaxant action of simvastatin was affected by both L-NAME and indomethacin, suggesting that its effect is mediated through endothelium-derived

NO and vasodilator eicosanoids. NO which is produced by endothelial NOS is a potent vasodilator by stimulating soluble guanylate cyclase and increasing cyclic guanosine monophosphate levels in smooth muscle cells.<sup>6</sup> Our results showed that pretreatment of aortic specimens with an NOS inhibitor, L-NAME significantly reduced the vasorelaxant effects of simvastatin. Therefore, our findings may suggest that simvastatin could relax the isolated rat aorta through endothelium-dependent NO pathway. In addition, indomethacin application could have lowered the vasodilatory response of simvastatin in isolated aortic rings from control and diabetic rats, indicating the important role of vasodilator prostaglandins in this regard.



**Figure 3.** Vasorelaxant effect of simvastatin against phenylephrine-induced contractions in the presence of nitro-L-arginine-methyl ester (L-NAME) or indomethacin in control (n = 6) (A) and diabetic (n = 7) (B) groups. One-way analysis of variance was used for statistical analysis of differences amongst the groups.



**Figure 4.** Effect of simvastatin on phenylephrine-induced transient contractions in normal and  $Ca^{2+}$ -free physiological salt solution (PSS) in control (n = 5) and diabetic (n = 6) groups. One-way analysis of variance was used for statistical analysis of differences amongst the groups.

The results of a previous study on relaxation response of simvastatin in normal rats showed that simvastatin is able to induce the synthesis and release of vasodilator products by a mechanism that is sensitive to superoxide scavengers like superoxide dismutase and is partly mediated through tyrosine kinase pathway.<sup>6</sup> Such mechanisms may have occurred in aortic rings from diabetic rats following an in vitro exposure to simvastatin in our study. Furthermore, it has been shown that simvastatin at appropriate concentration, which has occurred in our study, could promote endothelial-dependent relaxation through improving vasomotion at the level of smooth muscle.<sup>12</sup>

Some studies have also claimed that vasorelaxation response to simvastatin is endothelium-independent.<sup>6</sup> There are also some evidence that simvastatin is able to produce

relaxation of both aorta and small arteries in the absence of functional endothelium that might take place via inhibition of agonist-induced increase in cytosolic calcium involved in vascular smooth muscle contraction.<sup>13</sup> These mechanisms may include a decrease in the release of Ca<sup>2+</sup> from thapsigargin-sensitive pool, inhibition of inositol triphosphate-dependent Ca<sup>2+</sup> mobilization, and/or blockade of L-type Ca<sup>2+</sup> channels.<sup>13</sup>

### Conclusion

Taken together, our results show that simvastatin could relax the PE-preconstructed rings of aorta in STZ-diabetic rats through NO- and prostaglandin-related pathways and it could not affect the release and mobilization of calcium from the intracellular stores.

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### Conflict of Interests

Authors have no conflict of interests.

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