Vascular reactivity in the simvastatin treated experimental diabetes with endothelial disfunction

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Citation

S Yalc?n, C Köksoy, E Ergül, E Demirel, B Yalc?n. *Vascular reactivity in the simvastatin treated experimental diabetes with endothelial disfunction*. The Internet Journal of Internal Medicine. 2008 Volume 8 Number 1.

Abstract

Background: Vascular diseases are the principal causes of death and disability in people with diabetes. The bioavailability of nitric oxide (NO) represents a key marker in vascular health. Statins have anti-oxidant, anti-thrombotic and angiogenetic effects. They also increase NO by up-regulating nitric oxide synthase. It is aimed to asses the effect of simvastatin treatment on the general characteristics of diabetes and vascular reactivity in 14 week-old streptozotosin-diabetic rats. Methods: Twenty-four Sprague-Dawley male rats are divided into four groups as control, control-statin, diabetes and diabetes-statin. Four rings were taken from every rat's thoracic aorta. After taking a submaximal contraction with phenilephrine at the ring with endothelium, an acetylcholine relaxation was taken. While Na-nitroprusside relaxation reaction was gaining at one of the rings without endothelium, a cumulative contraction with phenilephrine and a cumulative contraction with KCL were obtained.
Results: We observed that hyperglycemia and weight-loss seen in diabetic rats are treated with simvastatin partially, but still different from the control group. Simvastatin treatment has lowered the plasma triglyceride and cholesterol levels. In isolated aortic rings of diabetic rats, after pre-contraction is performed with single dose Phenilephrine, it is observed that decreasing response in endothelial releasing responses with acetylcholine is treated partially with simvastatin treatment. After thapsigargin, endothel-related acetylcholine releasing responses are decreased; the releasing response in diabetes-statin group differs from the diabetic group. Conclusion: Simvastatin treatment in diabetic rats, in addition to treatment of diabetic dislipidemia, has also treated endothel-related releasing response in diabetes partially. We observed that thapsigargin reduces the response of the aortic rings to the current substance.

INTRODUCTION

Diabetes mellitus affects approximately 100 million people worldwide [1] . Five to ten percent have type 1 (formerly known as insulin-dependent) and 90% to 95% have type 2 (non–insulin-dependent) diabetes mellitus. It is likely that the incidence of type 2 diabetes will rise as a consequence of lifestyle patterns contributing to obesity [2] . Cardiovascular physicians are encountering many of these patients because vascular diseases are the principal causes of death and disability in people with diabetes. The macrovascular manifestations include atherosclerosis and medial calcification. The microvascular consequences, retinopathy and nephropathy, are major causes of blindness and end-stage renal failure. Physicians must be cognizant of the salient features of diabetic vascular disease in order to treat these patients most effectively.

Statin therapy is a widely used treatment for hypercholesterolemia, reduces the risk of stroke and improves the cardiovascular functions. The statin family of drugs is competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, the rate limiting enzyme in the synthesis of cholesterol which converts HMG-CoA to mevalonate. Statins have anti-oxidant, anti-thrombotic and angiogenetic effects [3] . They also increase nitric oxide (NO) by up-regulating nitric oxide synthase (NOS) enzyme [3] .

Thapsigargin is a sarco/endoplasmic reticulum Ca^{+2} - ATPase (SERCA) inhibitor. Thus, it empties the Ca^{+2} - storage at the sarco/endoplasmic reticulum (SER) by inhibiting the active transportation of Ca^{+2} to the SER.

In this study, it is aimed to asses the effect of simvastatin treatment on the general characteristics of diabetes and vascular reactivity in 14 week-old streptozotocin (STZ) -

diabetic rats.

MATERIAL AND METHODS

The Ethics Committee of Ankara University School of medicine approved the experimental procedures in this study. All of the guiding principles in the care and use of laboratory animals were strictly adhered throughout the entire study.

Twenty-four Sprague Dawley male rats, weighting between 200 and 250 g, were housed in climate controlled (relative humidity of 30-70% and temperature of 22 ^{o} C) animal-care facility, with a 12-hour light/dark cycle. The animals were provided with standard rodent chow and water as ad libitum.

The rats are divided into four groups as control (C), control-statin (CT), diabetes (D) and diabetes-statin (DT). Diabetes is performed by 45mg/kg single dose STZ injection via tail-vein and the rats, whose blood glucose levels are 250 mg and above, are accepted as diabetic at the third day after injection. Their plasma glucose levels and weights were measured everyday. 8 weeks after the diabetes is performed, 1mg/kg simvastatin treatment was given via intraperitoneal route to both of the control-statin and diabetes-statin groups. During the 14 week-long experiment, without food or water restriction, all rats are taken care under standard conditions. Plasma glucose, total cholesterol, triglyceride and lipoprotein (VLDL, LDL, HDL) levels were measured fourteen weeks after the STZ injection.

Rats were anesthetized with intraperitoneal injection of ketamine hydrochlorur (50mg/kg) (Ketalar, Parke-Davis) and xylasine (10 mg/kg) (Rompun, Bayer) at the end of 14 weeks. The surgical field was shaved and prepared with 1% antiseptic povidine-iodine solution and an incision was made along the thoracal midline. The thoracic aorta was quickly removed, and the isolated aorta cut into rings of 3 mm in length. The rings were suspended between two triangular-shaped stainless steel stirrups in a jacketed organ chamber filled with 20 ml modified Krebs–Henseleit solution. The modified Krebs–Henseleit solution was comprised of (in mM); 118 NaCl, 4.6 KCl, 1.2 MgSO4, 1.2 KH2PO4, 11.1 glucose, 27.2 NaHCO3, 0.03 Na_2-ethylene-diamine-tetra-acetic acid (EGTA) and 1.8 CaCl2. The chamber solution was kept at 36.5 C and oxygenated with 95% O2 and 5% CO2. Organ bath was changed at every ten minutes. The lower stirrup was anchored and the upper stirrup was attached to a force-displacement transducer (Nihon Kohden TB-652T, Tokyo, Japan) to record the isometric force. All rings were stretched to generate a resting tension of 2 g.

After 40 minutes of incubation an acetyl-choline induced relaxation was tested to asses the viability of endothelium.

Four rings were taken from every rat aorta (One with endothelium, three without endothelium). After taking a submaximal contraction with phenilephrine (10^{-7} M) at the ring with endothelium, an acetylcholine (10^{-9} -10^{-5} M) relaxation was gained. After a submaximal contraction with phenilephrine at the rings without endothelium a Na-nitroprusside (10^{-11} -10^{-6} M) relaxation reaction was gained. A cumulative contraction with phenilephrine and a cumulative contraction with KCL were obtained at the other two rings separately. All the experiments were repeated after addition of 10^{-6} M thapsigargin to the organ bath.

Data are expressed as mean ± SEM. Statistical evaluations of vascular-response were obtained by Wilcoxon Signed Ranks for paired groups, and Kruskal Wallis Variance Analyze to compare four groups together. When a difference found a poly-variance analyze was performed. Data analyzed with SPSS 11.5 for Windows.

RESULTS

Hyperglycemia and weight-loss is observed in simvastatin treated diabetic rats partially, but still different from the control group. Simvastatin treatment has lowered the plasma triglyceride, very low density lipoprotein (VLDL) and cholesterol levels, which are the signs of dislipidemia, in diabetic rats to the control levels (Figure.1). There was no statistically significant difference between all groups for high density lipoprotein (HDL) levels.

Figure 1

Figure.1. Triglycerid, VLDL, HDL, plasma cholesterol, plasma glucose levels and weights of the groups at the end of the experiment. Group1(c) - Control, Group 2(d) - diabetic, Group 3 (ct) - Control + Simvastatin, Group 4 (dt) – Diabetic + Simva

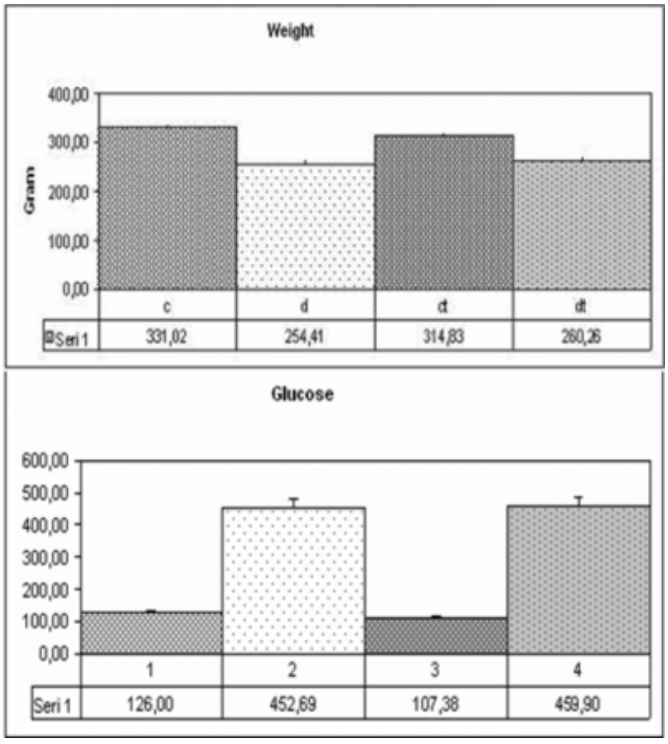


Figure 2

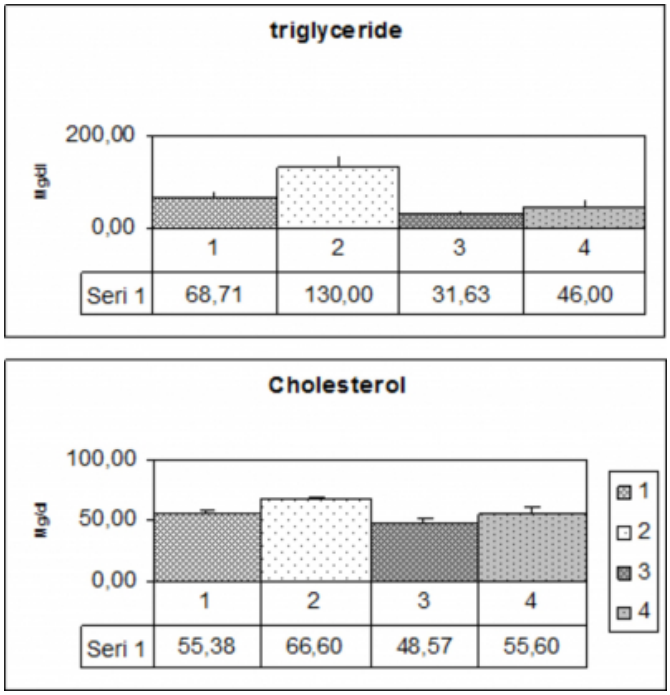
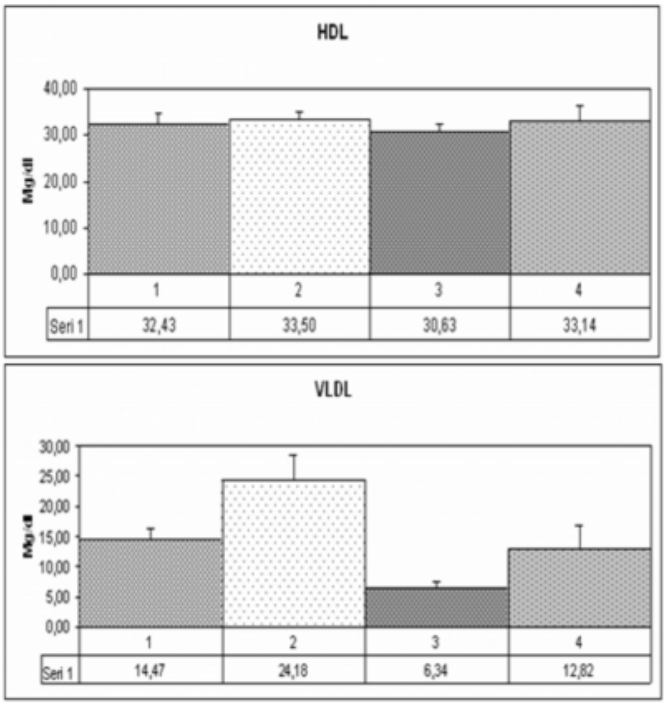


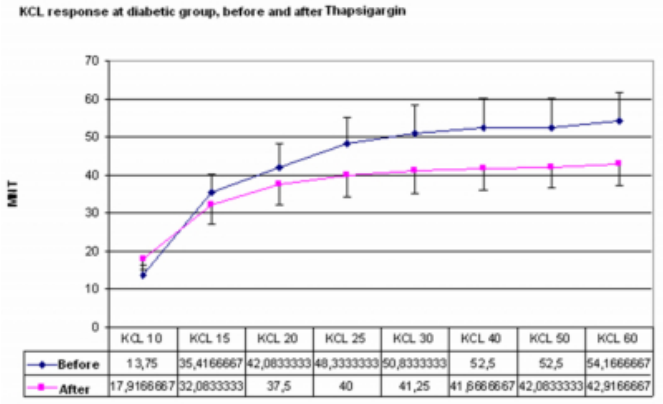
Figure 3



In isolated aortic rings without endothelium; phenilephrine has an increasing contraction response due to dose at all groups and there is no statistically significant difference at the same group before and after thapsigargin, and also between the groups (P>0.05). KCL has an increasing contraction response due to dose (10-60 µM) at all groups and there is no statistically significant difference at the same group before and after thapsigargin, except diabetic group (Figure.2). In diabetic group, a stronger contraction response is obtained before thapsigargin then the one after it. Na-Nitroprusside has an increasing relaxation response due to dose (10^{-11} -10^{-6} M) at all groups and there is no statistically significant difference at the same group before and after thapsigargin, except diabetic (Figure.3) and control-statin (Figure.4) groups.

Figure 4

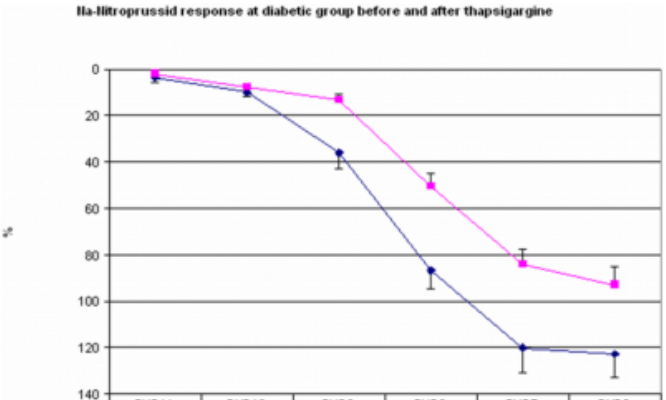
Figure.2 KCL response at the diabetic group before and after thapsigargin. KCL has an increasing contraction response due to dose (10-60 ÂµM) at all groups and there is no statistically significant difference at the same group before and after thapsigargin, except diabetic group. (p<0.05)



	KCL 10	KCL 15	KCL 20	KCL 25	KCL 30	KCL 40	KCL 50	KCL 60
Before	13,75	35,4166667	42,0833333	48,3333333	50,8333333	52,5	52,5	54,1666667
After	17,9166667	32,0833333	37,5	40	41,25	41,6666667	42,0833333	42,9166667

Figure 5

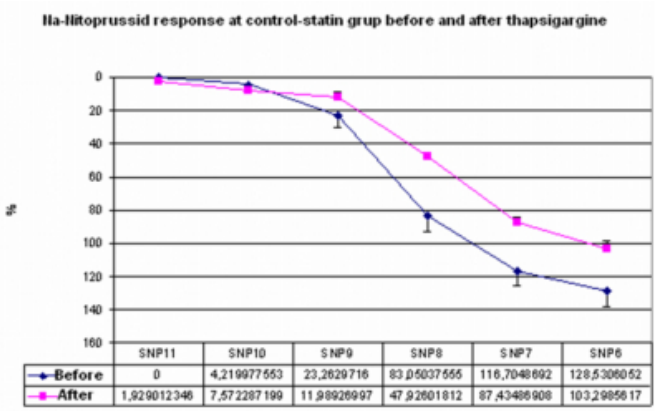
Figure.3 Na-Nitroprusside response at the diabetic group before and after thapsigargin. At diabetic group Na-Nitroprusside has an increasing relaxation response due to dose (10 -10 M). (p



	SNP11	SNP10	SNP9	SNP8	SNP7	SNP6
Before	3,52980353	9,980936937	36,04410083	86,88847384	120,0951705	122,7935832
After	2,104585976	7,696843423	13,21945153	50,33404783	84,09487555	93,05447299

Figure 6

Figure.4 Na-Nitroprusside response at the control-statin group before and after thapsigargin. At control-statin group Na-Nitroprusside has an increasing relaxation response due to dose (10 -10 M). (p

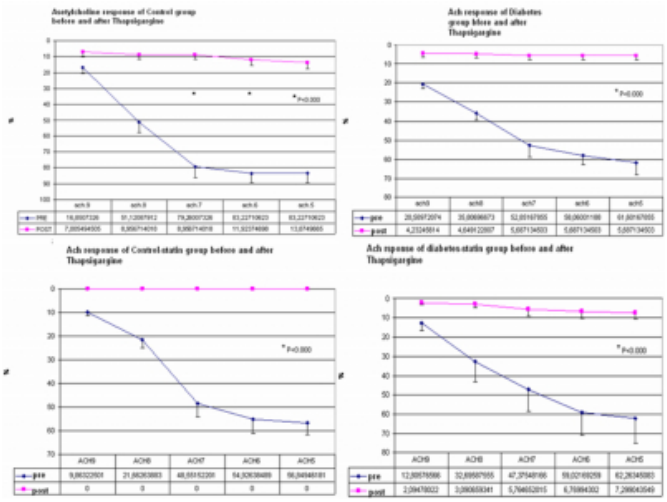


	SNP11	SNP10	SNP9	SNP8	SNP7	SNP6
Before	0	4,219977553	23,2629716	83,05037555	116,7048692	128,5306052
After	1,929012346	7,572287199	11,98926997	47,92601812	87,43486908	103,2985617

In isolated aortic ring of diabetic rats, after pre-contraction is performed with single dose Phenilephrine (10^{-7} molar) (before thapsigargin), it is observed that decreasing response in endothelial releasing responses with acetylcholine is treated partially with simvastatin treatment. After thapsigargin application at all groups, endothel-related acetylcholine releasing responses, are decreased (statistically significant) (Figure.5), the releasing response in treated group (diabetes-statin) differs from the diabetic group partially but more.

Figure 7

Figure.5 Acetyl-choline responses of the groups with endothelium.



DISCUSSION

Abnormalities in endothelial and vascular smooth muscle cell function, as well as a propensity to thrombosis, contribute to atherosclerosis and its complications.

Endothelial cells, because of their strategic anatomic position between the circulating blood and the vessel wall, regulate vascular function and structure. In normal endothelial cells, biologically active substances are synthesized and released to maintain vascular homeostasis, ensuring adequate blood flow and nutrient delivery while preventing thrombosis and leukocyte diapedesis. Among the important molecules synthesized by the endothelial cell is NO, which is constitutively produced by endothelial NO synthase (eNOS). The bioavailability of NO represents a key marker in vascular health. NO causes vasodilation by activating guanylyl cyclase on subjacent vascular smooth muscle cells. In addition, NO protects the blood vessel from endogenous injury by mediating molecular signals that prevent platelet and leukocyte interaction with the vascular wall and inhibit vascular smooth muscle cell proliferation and migration [4] . Endothelial dysfunction, as represented by impaired endothelium-dependent, NO-mediated relaxation, occurs in cellular and experimental models of diabetes. Similarly, many, but not all, clinical studies have found that endothelium-dependent vasodilation is abnormal in patients with type 1 or type 2 diabetes. Thus, decreased levels of NO in diabetes may underlie its atherogenic predisposition. Many of the metabolic derangements known to occur in diabetes, including hyperglycemia, excess free fatty acid liberation, and insulin resistance, mediate abnormalities in endothelial cell function by affecting the synthesis or degradation of NO. Thus, we performed the same procedures not only in aortic rings with endothelium but also without endothelium.

The intracellular glucose concentration of endothelial cells mirrors the extracellular environment. Experimental evidence supports the notion that hyperglycemia decreases endothelium-derived NO. When normal aortic rings are incubated in a hyperglycemic milieu, endothelium-dependent relaxation is impaired. Similarly, endothelium-dependent vasodilation is reduced in healthy subjects during hyperglycemic clamping. Hyperglycemia induces a series of cellular events that increase the production of reactive oxygen species (such as superoxide anion) that inactivate NO to form peroxynitrite [2] . Hyperglycemia may initiate this process by increasing superoxide anion production via the mitochondrial electron transport chain. Superoxide anion then promotes a cascade of endothelial processes that engage increasing numbers of cellular elements to produce oxygen-derived free radicals. Activation of protein kinase-C (PKC) by glucose has been implicated in the regulation and activation of membrane-associated NAD-(P)H-dependent oxidases and subsequent production of superoxide anion [5] .

The concept that hyperglycemia-induced oxidative stress medi-supported by the observations that intra-arterial infusion of ascorbic acid, a water-soluble antioxidant capable of scavenging superoxide anion, restores endothelium-dependent vasodilation in healthy subjects exposed to a hyperglycemic clamp and in patients with type 1 or type 2 diabetes [2] . Hyperglycemia also increases the production of the lipid second messenger diacylglycerol, which causes the membrane translocation and activation of protein kinase-C. Activation of PKC inhibits the activity of the phosphatidylinositol-3 kinase pathway which results in less NO production.

We observed that hyperglycemia and weight-loss seen in diabetic rats are treated with simvastatin partially, but still different from the control group. Simvastatin treatment has lowered the plasma triglyceride, VLDL and cholesterol levels in diabetic rats. Circulating levels of free fatty acids are elevated in diabetes because of their excess liberation from adipose tissue and diminished uptake by skeletal muscle. Free fatty acids may impair endothelial function through several mechanisms, including increased production of oxygen-derived free radicals, activation of protein kinase-C, and exacerbation of dyslipidemia. Infusion of free fatty acids reduces endothelium-dependent vasodilation in animal models and in humans in vivo [6] . Elevation of free fatty acid concentrations activate PKC and decrease insulin receptor substrate-1–associated phosphatidylinosital-3 kinase activity [7] . These effects on signal transduction may decrease NOS activity as discussed above. The liver responds to free fatty acid flux by increasing very-low-density lipoprotein production and cholesteryl ester synthesis [8] . This increased production of triglyceride-rich proteins and the diminished clearance by lipoprotein lipase results in hypertriglyceridemia, which is typically observed in diabetes. Elevated triglyceride concentrations lower HDL by promoting cholesterol transport from HDL to very-low density lipoprotein [8] . These abnormalities change LDL morphology, increasing the amount of the more atherogenic, small, dense LDL. Both hypertriglyceridemia and low HDL have been associated with endothelial dysfunction [9] .

In diabetes, endothelial cell dysfunction is characterized not only by decreased NO but also by increased synthesis of vasoconstrictor prostanoids and endothelin [10] . Endothelin may be particularly relevant to the pathophysiology of vascular disease in diabetes because endothelin promotes

inflammation and causes vascular smooth muscle cell contraction and growth [11] . Insulin increases endothelin-1 immunoreactivity in endothelial cells. Also, plasma endothelin-1 concentration increases after administration of insulin to healthy subjects and patients with type 2 diabetes mellitus [2] .

Previous studies have suggested that the diabetic dyslipidemia induced impairment in vascular reactivity is largely due to alterations in coronary smooth muscle intracellular Ca^{+2} (Ca^{+2} i) regulation. These studies have shown that diabetic dyslipidemia significantly impairs Ca^{+2} i effluxes from the cell [11] , increases basal Ca^{+2} i levels [11] , and increases sarcoplasmic reticulum Ca^{+2} i buffering [12] . Since it is currently thought that the increase in sarcoplasmic reticulum Ca^{+2} i buffering is a compensatory alteration due to the impairment in Ca^{+2} efflux and the rise in Ca^{+2} i levels, collectively these results suggest that pro-atherogenic factors present in the diabetic dyslipidemic state negatively impact the functional capacity of plasma membrane Ca^{+2} transporters [12] .

Thapsigargin is a SERCA inhibitor. Thus, it empties the Ca^{+2} -storage at the SER by inhibiting the active transportation of Ca^{+2} to the SER. In the present study we observed that thapsigargin reduces the response of the aortic rings to the current substance. We also figured out that it increases the stage of ischemia.

We did not come cross any study like ours. We evaluated the vascular (with and without endothelium) response to KCL, Na-Nitroprusside, and acetylcholine in control, control-statin, diabetic and diabetes-statin groups before and after thapsigargin administration.

In isolated aortic ring of diabetic rats, after pre-contraction is performed with single dose Phenilephrine (10^{-7} molar) (before thapsigargin), we observed that decreasing response in endothelial releasing responses with acetylcholine is treated partially with simvastatin treatment, as Pfaffman et al found [13] . Different responses of diabetic aorta to the same substances have been found by different authors [13-15] . Piper at al suggested that this variability is due to the duration of diabetes [15] .

Our results have shown that simvastatin treatment in diabetic rats, in addition to treatment of diabetic dislipidemia, has also treated endothel-related releasing response in diabetes partially. Thapsigargin has a negative effect on vascular response not only group with endothelium but also without it.

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