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NON CHOLESTEROL LOWERING DOSE OF ATORVASTATIN AMELIORATES DIABETIC VENTRICULAR MYOCYTE DYSFUNCTION IN MICE

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ABSTRACT

The aim of this study was to investigate whether atorvastatin ameliorates diabetes-induced cardiomyocyte dysfunction, independently of cholesterol-lowering effect. Streptozotocin-induced diabetic mice were treated with atorvastatin (10 mg/kg, daily, orally) for two weeks. Ventricular cardiomyocytes were isolated and contractile properties including peak shortening (PS), time to PS (TPS), time to 90% relengthening (TR_{90}) and maximal velocity of shortening/relengthening (+/- dL/dt) were analysed using video-based edge detection. Diabetes caused mechanical dysfunction with dampened stress tolerance of myocytes at high stress frequencies, all of which were significantly alleviated by atorvastatin without affecting hyperglycemia and dyslipidemia. In addition, changes in oxidative stress parameters (CAT activity, GSH and MDA levels) were also normalized by atorvastatin. These data indicate that atorvastatin, independently of its lipid-lowering capacity, reduces myocardial oxidative stress resulting in improved myocyte mechanical function in an experimental model of diabetes. Our results supports the concept that restriction of myocardial oxidative stress is a fundamental goal in the treatment of diabetic cardiomyopathy.

Keywords: Atorvastatin, diabetes mellitus, mouse, oxidative stress, ventricular myocyte

Abbrevations: AGES, advanced glycation endproducts; ATV, atorvastatin; CAT, catalase; DM, diabetes mellitus; DCM, diabetic cardiomyopathy; GSH, glutathione; HF, heart failure; MDA, malondialdehyte; LV, left ventricular; NADPH, nicotinamide adenine dinucleotid phosphate; OS, oxidative stress; RAS, renin angiotensin system; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is an independent risk factor for left ventricular (LV) dysfunction and associated with a specific diabetic cardiomyopathy (DCM) contributing to cardiac morbidity and mortality. It is charactherised by impaired ventricular contraction, relaxation, and wall compliance. The etiology of DCM is complex and oxidative stress (OS), glucose and lipid toxicity have been speculated to play an essential role, and effective therapeutic strategy against this specific myopathy remains elusive [1-8]. Statins (HMG-CoA reductase inhibitors) are the most widely prescribed lipid-lowering drugs worldwide and they are attracting much attention because of their beneficial cardiovascular pleiotropic effects such as antioxidant, anti-inflammatory, antithrombotic, fibrinolytic and angiogenic effects [9-

[18]. Some of these beneficial effects have also been observed in human cardiovascular tissues and could be the result of a direct action on the vessels and heart [17,19,20]. In diabetes, many of these effects have been ascribed to restriction of the reactive oxygen species (ROS) production induced by hypergylcemia. Excessive ROS production has been implicated in all stages of the development of heart failure (HF), from cardiac hypertrophy to fibrosis, contractile dysfunction, and failure in diabetes [2,7].

In general, pharmacological intervention using different antioxidants may have significant implications in the prevention of the pro-oxidant feature of DM and protect the redox status of the cardiovascular cells [6,21,22]. Growing evidence has suggested that antioxidant effects of statins have an important roles on cardiovascular disease independent of cholesterol lowering effects [9, 11,14,18,19,23]. Many of these pleiotropic effects are mediated by antagonism of isoprenoid mediated activation of small GTP-binding proteins, such as Rac 1. Nicotinamide adenine dinucleotid phosphate (NADPH) oxidase which is regulated by the small GTP-binding protein Rac 1 is a main source of ROS in myocardium. Statins inhibit cardiac hypertrophy through an antioxidant mechanism involving inhibition Rac1 geranylgeranylation [16,19,24,25]. Further, atorvastatin (ATV) has free-radical scavenging ability via its metabolites [26]. Among various statins, ATV is the most widely used statin for the treatment of hypercholesterolemia. This study was designed to investigate whether ATV has beneficial effect on diabetes-induced cardiomyocyte dysfunction independently of cholesterol-lowering effect. Although a large number of groups have investigated the cardioprotective effects of ATV, independent of its hypolipidemic effect [13,19,27-30], it's effect on diabetes-induced cardiac myocyte dysfunction has not been investigated at a single cell level after *in vivo* treatment.

MATHERIALS AND METHODS

Chemicals: ATV was supplied from Eczacıbaşı-Zentiva Ltd, Turkey, glutathione reagant assay kit from Cayman Chemical, MI, USA, glucose, cholesterol, triglyceride strips from Roche Diagnostics, Germany, collagenase D from Boehringer Mannheim Biochemicals, Germany. All the other chemicals were purchased from Sigma-Aldrich Co, St Louis, USA.

Induction of diabetes and the treatment protocols: All animal procedures described here were approved by Animal Use and Care Committee at Ankara University (Turkey). The principles of laboratory animal care (NIH publication No.85-23, revised 1996) were observed. Male adult albino mice (n:40) were injected freshly prepared streptozotocin (STZ: 200 mg/kg, i.p., in 0.01 M citrate buffer with a pH of 4.3). Weight-matched control mice received citrate buffer only. After one week, blood samples were obtained by tail prick, and diabetes was confirmed by fasting blood glucose value of 300 mg/dL higher using glucose meter (Accu-Chek go®, Roche diagnostics, Germany). The experimental groups comprised control and diabetic untreated or treated with ATV (10 mg, oral gavage). The dosage of ATV was choosen as non lipid lowering dose according to the previous experimental studies [12,13,31]. Mice were treated with ATV for a period of two weeks, beginning one week after STZ injection. They were maintained on a 12:12-h light-dark illumination cycle with access to food and water *ad libitum*. At the end of the treatment period, blood was collected for the measurement of plasma triglycerides and cholesterol concentrations using Reflotron® analyser (Roche diagnostics, Germany), and the hearts were rapidly removed from anesthetized mice.

Isolation of ventricular myocytes: Cardiomyocytes were enzymatically isolated as described by Ren and Bode [32]. Hearts were removed and perfused (37 ^{0}C) with Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 HEPES, and 11.1 glucose, with 5% CO_2 and 95% O_2, in Langendorff perfusion system. Hearts were subsequently perfused with a Ca^{2+}-free KHB-buffer that containing collagenase D (0.9 mg/ml) for 10 minutes. After perfusion, left ventricules were removed and minced to disperse the individual cardiomyocytes in Ca^{2+}-free KHB-buffer. Extracellular Ca^{2+} was added incrementally back to 1.25 mM. Isolated myocytes were used for functional studies between 1 and 8 h after isolation and only rod-shaped myocytes with clear edges were selected for recording of mechanical properties.

Cell shortening / relengthening: Mechanical properties of cardiomyocytes were assessed using a SoftEdge MyoCam system (IonOptix Corp, Milton, MA, USA). Cells were placed in a chamber mounted on the stage of an inverted microscope (Nikon, TT-SM, USA) and superfused at 25^0 C with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES, at pH 7.4. Cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz, 3-msec duration, using a pair of platinum wires placed on opposite sides of the

chamber connected to a FHC stimulator (IonOptix). The myocyte being studied was displayed on a computer monitor using an IonOptix MyoCam camera, which rapidly scans the images. A SoftEdge software (IonOptix) was used to capture changes in cell lenght during shortening and relengthening. Cell shortening and relengthening were assessed using the following indexes: Peak shortening (PS): peak ventricular contractility, time- to- PS (TPS): contraction duration, time- to- 90 % relengthening (TR_{90}): cardiomyocyte relaxation duration, maximal velocity of shortening (+ dL/dt) and relengthening (- dL/dt): maximal velocities of ventricular rise/fall. In the case of altering stimulus frequency from 0.1 to 5.0 Hz (stress tolerance experiments), the steady-state contraction of myocyte was achieved (usually after the first six beats) before PS amplitude was recorded at 0.1, 0.5, 1.0, 3.0 and 5.0 Hz.

Measurements of oxidative stress parameters: The following parameters were measured as markers for OS in heart homogenates:

Glutathione (GSH) levels were measured by a commercially available assay kit (Cayman, Ann Arbor, MI,USA). This kit utilises an enzymatic recycling method based on the reaction between GSH and 5,5-dithiobis-2-nitrobenzoic acid that produces a yellow colored compound 5-thio-2-nitrobenzoic acid that produces a yellow colored compound 5-thio-2-nitrobenzoic acid production is directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of 5-thio-2-nitrobenzoic acid at 412 nm provides an accurate estimation of GSH in the sample. Due to the presence of GSH reductase, which reduces the disulphide dimer of GSH to GSH, in the reaction buffer, both GSH and disulphide dimer of GSH are measured and the assay reflects total GSH present in the sample.

Malondialdehyte (MDA), an end product of unsaturated fatty acid peroxidation, can react with thiobarbituric acid to form a colored complex called thiobarbituric acid reactive substances, thus thiobarbituric acid reactivity was assayed by the method of Uchiyama and Mihara [33]. Butanol phase was measured with microplate reader at 532 nm. The results were expressed with tetramethoxypropane standart curve within the range from 0-20 nmol.

Catalase (CAT) and superoxide dismutase (SOD) activities in homogenates were measured spectrophotometrically following the procedures of Aebi and Sun et al. respectively [34,35]. The molar extinction coefficient of 43.6 M/cm was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/min per mg protein. SOD activity was assayed using nitro blue tetrazolium. The samples were subjected to ethanol-chloroform (5:3) extraction before the enzyme activity assay. The nitro blue tetrazolium was reduced to blue formazan by O_2, which has a strong absorbance at 560 nm. The calculated SOD activity was expressed as IU/mg of protein in the tissue.

Total protein concentration in the homogenates were determined by Bradford method using bovine serum albumin as a standart [36].

Statistical analyses: For each experimental series, data are presented as means ± SE. Statistical significance ($P<0.05$) comparisons were performed by ANOVA followed by the Newman-Keul's post hoc test. The number of replicates for each experiment is expressed in the figure legends.

RESULTS

General characteristics of animals*:* The data, at the end of the study, are shown in Table 1. Body weight, hyperglycemia and dyslipidemia in diabetic (d) group remained unchanged after ATV treatment (dt group) ($p>0.05$, n: 8-10 each group). The heart weight or heart-to-body weight ratio was significantly increased in d group and it was normalized after ATV treatment.

Cell shortening/relengthening properties of myocytes*:* In myocytes, diabetic state did not significantly alter resting cell length, peak shortening amplitude (PS) and maximal velocity of shortening (+dL/dt), but significantly prolonged time to PS (TPS), time to 90% relengthening (TR_{90}), and decreased maximum velocity of relengthening (- dL/dt) ($p<0.05$, n: 120-150 cells from 5 mice per group). ATV treatment countered these alterations and exerted little effect on myocyte mechanics in control group (Figure 1).

To evaluate the impact of diabetes on cardiac contractile function under physiological, but high stress frequencies we increased the stimulating frequency up to 5.0 Hz (300 beats/min) and recorded steady-state PS. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady state before concerning the frequency response from 0.1 to 5.0 Hz. PS obtained at all recording frequencies was normalised to that at 0.1 Hz of the same myocyte. In these stress tolerance experiments, myocytes from diabetic group displayed a significantly enhanced depression in PS at all frequencies studied and ATV treatment countered the alterations (Figure 2).

Oxidative stress parameters: The results are summarised in Table 2. GSH content was decreased (21% lower vs. control group), and MDA content was increased (48 % higher vs. control group) in heart of diabetic animals ($p<0.05$, n:5 each group). CAT activity was significantly higher (51 %) than that in the control group ($p<0.05$, n:5 each group). ATV treatment alleviated these alterations ($p<0.05$, n:5 each group). SOD activity was similar in the four groups studied ($p>0.05$, n:5 each group), however there was a slight decrease in diabetic group but it did not reach to statistically significance.

DISCUSSION

The present study investigated for the first time beneficial effect of ATV on diabetes-induced cardiac myocyte dysfunction independent of cholesterol lowering. This effect is associated with reduction in OS.

Somewhat similar to earlier observations [32,37-40] , STZ-induced diabetes prolonged TPS and TR90 with normal PS and maximum velocity of shortening in ventricular myocytes even after only two weeks of diabetes induction. One of the major complications in diabetes including STZ-induced experimental diabetes is DCM, which develops independent of any macro/micro-vascular disease and is characterized by both systolic and diastolic dysfunction. Even short-term STZ-diabetes has been shown to induce ventricular myocyte dysfunction [37,39], and these mechanical defects are beleived to be mediated through a number of cellular events including impaired intracellular Ca^{2+} handling, altered excitation-contraction coupling and dysregulated cellular enzyme activity or gene expression in cardiac myocytes. There is a robust literature documenting that delayed intracellular Ca^{2+} clearence, and dampened intracellular Ca^{2+} release in cardiac myocytes are considered hallmarks of DCM [1-7,41]. Although we did not measure intracellular Ca^{2+} movements in the present study, the diminished myocyte contractile response (PS) to increased stimulating frequency during our stress tolerance experiments (PS-frequency relationship) indicates a much slower replenishing process of intracellular Ca^{2+} pool or significantly smaller pool size under diabetic condition. Previously, Fura-2 fluorescence experiments revealed that myocytes from STZ-diabetic mice overloaded resting intracellular Ca^{2+} and reduced intracellular Ca^{2+} clearing rates. This is consistent with prolongation in TR90, similar to its effect on cell shortening [39]. While studies have unraveled numerous mechanisms for the abnormal Ca^{2+} handling, investigations have indicated that much of the contractile dysfunction and adverse remodeling that occurs in DCM and HF involves OS. Increased production of free radicals is a well-known phenomenon in hyperglycaemic conditions, and STZ-diabetic model is a prototype of T1DM with an intense OS due to hyperglycemia [6,8,42,43]. The auto-oxidation of glucose, the formation of advanced glycation end-products (AGEs) and the activation of NAPDH oxidase have been suggested as possible sources of the augmented OS in diabetes. Free radicals disrupt intracellular Ca^{2+} homeostasis through inhibition of L-type Ca^{2+} currents, sarcoplasmic Ca^{2+} load, Na/Ca^{2+} exchange, and sarcoplasmic Ca^{2+} uptake. The prolonged duration of relaxation (TPS) may be a result of impaired sarco (endo) plasmic reticulum ATPase (SERCA) and/or other Ca^{2+} regulating proteins such as Na^{+}/Ca^{2+} exchange which has been reported in diabetes. Numerous studies suggest that these defects stem in part from perturbation in intracellular Ca^{2+} cycling [1,2,6,21,41,44]. Further, ryanodine receptors (RyR2) are redox-sensitive ion channels in heart and become leaky during diabetes and this defect may also be responsible to the reduced sarcoplasmic Ca^{2+} load in myocytes [45,46].

The heart posesses a relatively low antioxidant capacity contributed by enzymatic and non-enzymatic free radical scavengers or antioxidants, thus making it a prime target for oxidative insults. In the present study, ATV treatment had an overall positive effect on the OS parameters measured in the heart. ATV dosage used in the study did not significantly affect the cholesterol levels, as expected, allowing us to investigate *in vivo* direct antioxidant effect of the drug. Thus, our results indicate that the lipid lowering effect did not contribute to the antioxidant effect of ATV in the heart. Statins produce antioxidant effects by two general mechanisms: 1) by preventing oxidant production (*e.g.*, by inhibiting NADPH oxidase assembly and function), and 2) by subsequently blocking the harmful effects of ROS (*e.g.*, by scavenging ROS and increasing the activity of cellular antioxidants) [11,22]. In diabetes, both the activity of NADPH oxidase system and the levels of NADPH subunits were significantly increased and NADPH oxidase inhibitors were demonstrated to significantly inhibit the increased ROS formation [47]. Statins block the isoprenylation and activation of members of the Rho family, such as RhoA and Rac1. Rac1 regulates NADPH oxidase, which is a major source of ROS in cardiovascular cells [9,16,23,25,48]. Vecchione et al. have reported that ATV treatment (1 mg / kg, day) inhibits Rac-1 activation and consequently exert antioxidant effect on the STZ-

diabetic mice vessel [20]. Riad et al. have also observed that low dose (50 mg/kg/day) 48-day ATV treatment of STZ- diabetic rats reduces NADPH oxidase activity associated with reduced inflammatory markers [12]. Similar positive effects were also observed in the clinical trials [19,49]. Further, the drug protected the heart of mice against doxorubicin- induced OS [30].

The increased ROS generation because of up-regulated angiotensin signalling pathway via diabetes is another important event in the pathogenesis of diabetic cardiac dysfunction. Hyperglycemia locally activates myocardial RAS (renin angiotensin system) thus angiotensin II leads to excessive production of ROS via activation of NADPH oxidase and to hypertrophy, proliferation, thus RAS inhibitors exert antioxidant effects [7,8,50,51]. Statins has been shown to prevent cardiac hypertrophy, induced by angiotensin II infusion in rat in a cholesterol-independent manner. The mechanism is due partly to inhibition of Rho geranylgeranylation and reduction of Rac1-induced superoxide anion production in cardiomyocytes [25,52] . Further, ATV down-regulates AT1-receptor and expression of NADPH oxidase subunit independent of cholesterol synthesis in aorta from normocholesterolemic hypertensive rat [53]. In a new study, ATV, exerted anti-remodeling effects partially by inhibition of NADPH oxidase-mediated cardiac OS in hypertensive rats [54]. Because of the critical role of NADPH oxidase in the regulation of both myocardial and vascular redox signaling, its inhibition by statins, irrespective of cholesterol, (e.g., by suppressing Rac activation) could modify OS in the entire cardiovascular system.

Cardiac hypertrophy is one of the charachteristics of DCM and eventually leads to HF. Studies suggest that increased ROS production, in particular NADPH oxidase dependent ROS generation, plays an important role in the pathophysiology and progression of CM hypertrophy [8]. Statins inhibit cardiac hypertrophy by cholesterol-independent mechanisms [10]. In our study, ATV treatment prevented changes in the heart / body weight ratio in diabetic mice. Recently, it has been reported that ATV at low dose (5 mg/kg/day) slows the progression of cardiac remodeling in mice with pressure overload [55]. Thus, ATV could protect diabetic heart from HF.

Formation of advanced glycation endproducts (AGEs) also play a major role in enhancing OS in diabetes. AGEs exert OS and inflammation mainly through the receptor for AGEs (RAGE). RAGE generates OS via activation of NADPH oxidase, amplification mechanism in the mitochondria may further drive ROS production [5,7,56]. ATV, via its anti-oxidative property, decreases serum levels of AGEs in T2DM patients [57], renal accumulation and renal RAGE expression in STZ-diabetic rat [58]. Thus, AGE-lowering effect of ATV may also contribute to its cardioprotective properties.

Inducible NOS (iNOS) is activated in diabetes by inflammatory mediators, which makes iNOS uncoupling a predominant contributor for oxidative/nitrosative stress in diabetic myocardium. Studies suggest that there is a large nitroso-redox imbalance with HF and correcting this imbalance may be able to restore myocyte contraction and improve heart function [6,59]. $ONOO^-$ can modify tyrosine residues in various proteins to form nitrotyrosine, which can lead to damage that alters protein function and stability. Statins may restore physiological balance between NO and ROS production [43]. On the other hand, a considerable body of evidence suggests that increasing endothelial NO bioavailability via antioxidant therapy is an important intervention not only for endothelial dysfunction but also other cardiovascular abnormalities induced by DM. ATV have been shown to normalise the reduced eNOS production in heart caused by STZ-diabetes, in mice [13].

On the other hand, antioxidative effect of ATV is not only by inhibition of ROS generation, but also by the scavenging action of the radicals. As shown by Aviram et al. the ortho- and para-hydroxy metabolites which represent 70 % of active ATV demonstrate free-radical scavenging ability and inhibit lipoprotein oxidation *in vitro* [26]. The parent compound has no antioxidant effects on lipoprotein oxidation *in vitro* as compared to the two major hydroxy metabolites. The hydroxyl group, attached to the carboxamide portion of the molecule, enable these metabolites to act as electron donors, and hence as potent antioxidants.

Enzymatic antioxidants form the first line of the antioxidant defense mechanisms to protect cells from ROS- mediated oxidative damage. Increased antioxidant enzyme activities is a compansatory response to OS due to an elevation of hydrogen peroxide and lipid peroxidation, and their activities and/or levels were shown to be changed in diabetic hearts [43,44,60]. Increased production of MDA, which is an index of OS and lipid peroxidation reported in diabetic hearts [43,44,51,60]. Similarly, our data suggest that peroxidative injury may be involved in diabetic

myocyte dysfunction and ATV exerts antioxidant effects and protects the heart from lipid peroxidation. In the current study diabetes induced an increase in CAT activity. This may be a response to increased production of hydrogen peroxide as a compensatory mechanism to degrade it. As reported by several authors, under *in vivo* conditions, OS may result in compensatory elevation in the activity of antioxidant enzymes in diabetes [60,61]. Statins up-regulate antioxidant enzymes, but very few studies have directly addressed the question of statin-mediated antioxidant enzyme induction [9,11,14] . ATV treatment of hypertensive rats increases both CAT expression and activity in rat aorta, but with no changes seen in SOD or in GSH peroxidase [62]. Recently, we have found that fluvastatin treatment, at low dose, attenuated increased activity of CAT and MDA levels, but did not affect SOD activity in heart of STZ-diabetic rat [43]. In the present study, SOD activity also did not change between the groups. While these studies are few in number and not entirely consistent, it is clear that various statins do have the potential to change cellular and tissue levels of the antioxidant enzymes. On the other hand, non enzymatic antioxidant GSH also plays an excellent role in preventing the cells from oxidative damage. In our study its content was decreased in diabetic heart might be due to increased utilization for scavenging free radicals by GSH peroxidase. ATV treatment protected this depletion which could be due to low utilization of GSH. This effect of ATV on GSH level might be relevant if demonstrated in human, because in diabetic patients plasma GSH is lower and is in more oxidized state than that in healthy control subjects [63].

It has not been unequivocally demonstrated in humans that prolonged statin treatment exerts effects independent of cholesterol lowering because the use of statins always resulted in reduced LDL cholesterol levels, but in their elegant study, Landmesser et al. showed that despite the similar reduction in LDL cholesterol levels caused by simvastatin and ezetimibe, a cholesterol absorption inhibitor, only simvastatin resulted in beneficial effects on endothelial dysfunction [64]. Considering cardiovascular beneficial effects and since DCM usually subclinical for a longtime before the appearence of clinical symptoms statin therapy may be prescribed early, irrespective of the plasma cholesterol level.

On the other hand, the clinical dosage of ATV for human adults is usually 10-40 mg/day, which corresponds to a dosage per g body weigt of 0.17-0.66 mg/kg given to a patient with an avarage body weight of 60 kg. The dosage of ATV used in our study is not comparable (higher) to usual dosage used clinically. The peculiarities specific to lipid metabolism in rodents make these drugs to use high dosage, but, experiments on rodents is suitable for examining the extra-lipid effect of statins [13]. For e.g., ATV at the dose of 50 mg/kg/day did not reduce plasma lipids in STZ-diabetic rat but improved endothelial dysfunction through its antioxidant effects or reduced myocardial inflammation and fibrosis resulting in improved LV function [12,13]. 20 mg/kg/day ATV dosage reduced OS and normalized vascular endothelial dysfunction without changing plasma LDL levels in STZ-diabetic rat model [31].

As a conclusion, our results indicate that ATV has beneficial effects in diabetic cardiomyocyte dysfunction, independent of cholesterol-lowering effect. Our results further support the concept that restriction of myocardial OS is a fundamental goal in the treatment of DCM. Thus, antioxidant property of ATV may have important clinical implications for the treatment and prevention of DCM. Whether this beneficial effect is clinically relevant remains to be determined. In a subclinical diastolic dysfunction, the early stage of diabetic cardiomyopathy, statin therapy can protect myocyte dysfunction even in non hyperlipidemic state.

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CONFLICT OF INTEREST:
The authors report no conflicts of interest in this work.

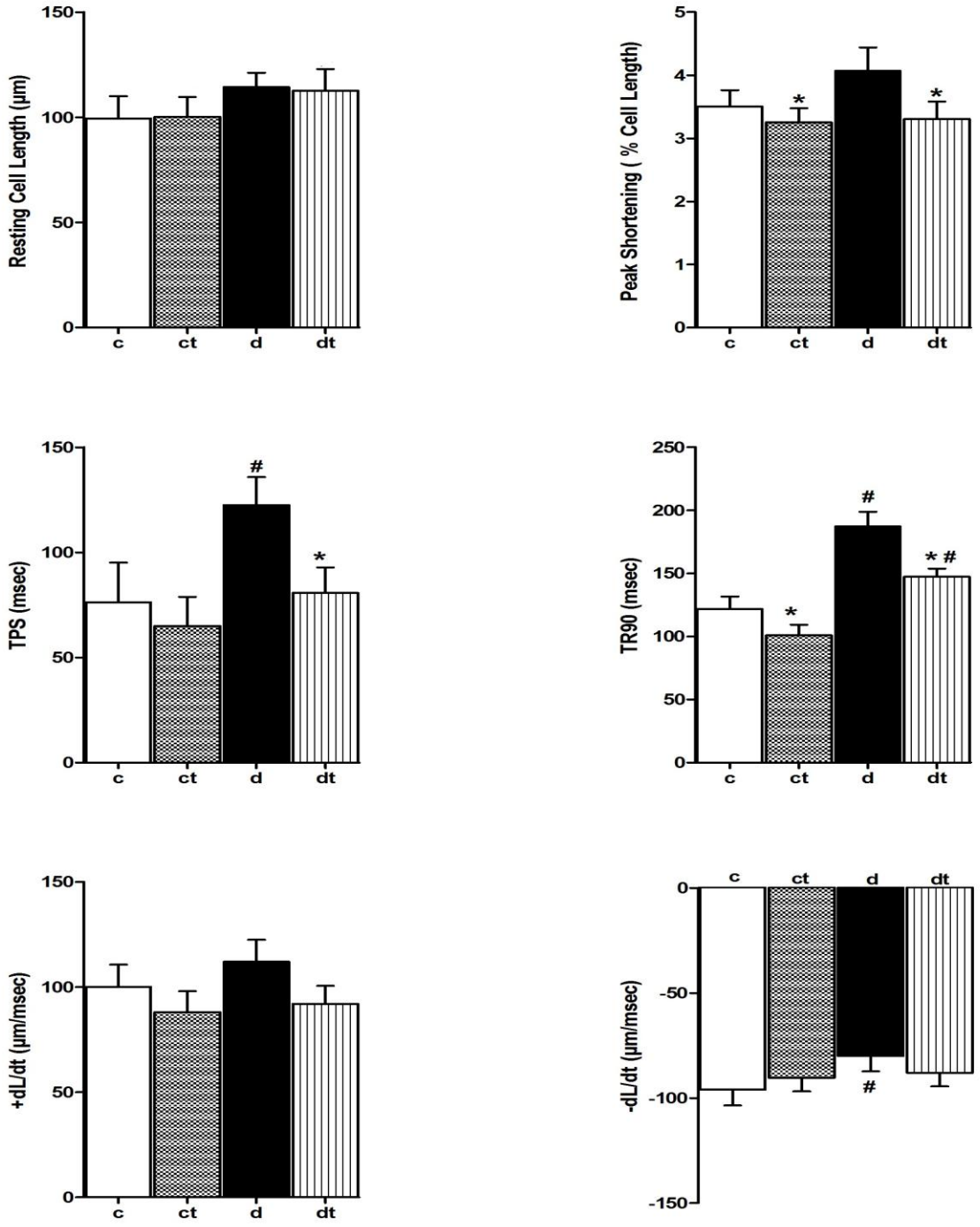

Figure 1: Contractile properties of cardiac ventriculer myocytes isolated from control (c), control + atorvastatin treated (ct), diabetic (d), diabetic + atorvastatin treated (dt) hearts (n: 5 animals each). Values are mean ± SE (*n:*120-150 cells from 5 mice per group). *P< 0.001 vs. d group, $^{\neq}$P < 0.001 vs. c group. PS: peak shortening, TPS: time-to-peak shortening, TR90: time- to- 90% relengthening, ± dL/dt: maximal velocity of shortening/relengthening.

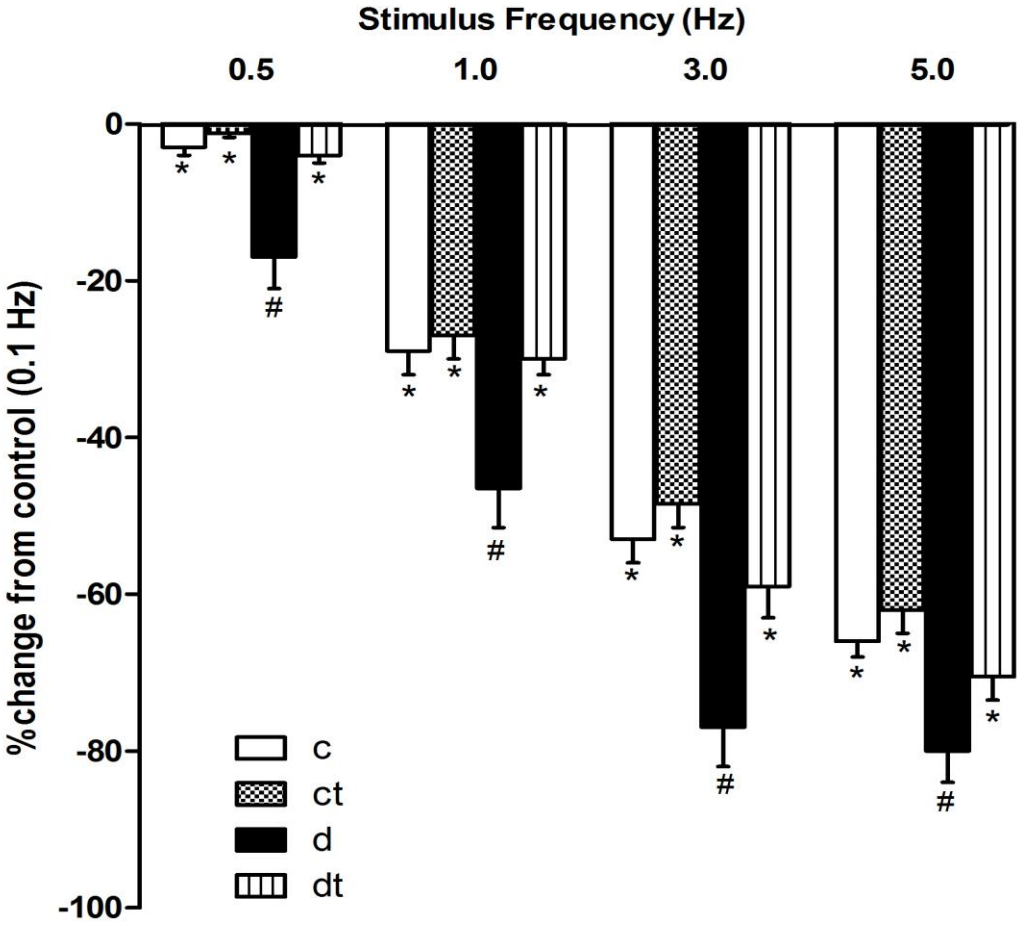

Figure 2: Effects of increased stimulus frequency (0.1 - 5.0 Hz) on cardiac ventriculer myocyte peak shortening (PS) amplitude in myocytes from control (c), control+atorvastatin treated (ct), diabetic (d), diabetic + atorvastatin treated (dt) hearts (n: 5 animals per group). PS was presented as percent change from respective PS obtained at 0.1 Hz of the same cell. Values are mean ± SE (*n:* 80-85 cells from per group). *P<0.001 vs. d group, $^{\neq}$P<0.001 vs. c group.

Table 1: General characteristics of experimental groups

GROUPS	c	ct	d	dt
Body weight, g	27.6 ± 0.3*	28.4 ± 0.9*	24.0 ± 0.3$^{\neq}$	27.2 ± 1.2*
Heart weight, mg	122 ± 16*	120 ± 22*	146 ± 11$^{\neq}$	133 ± 13*
Heart / body weight, mg/g	4.41 ± 0.53*	4.40 ± 0.24*	5.92 ± 0.37$^{\neq}$	4.81 ± 1.00*
Glucose, mg/dl	98 ± 4*	98 ± 5*	392 ± 7$^{\neq}$	382 ± 6$^{\neq}$
Total cholesterol, mg/dl	68 ± 2*	62± 2*	98 ± 3*	99 ± 4*
Triglyceride, mg/dl	75 ± 4*	70 ± 3*	124 ± 7*	128 ± 6$^{\neq}$

Values are mean ± SE of 8-10 animals. c: control group, ct: control + atorvastatin group, d: diabetic group, dt: diabetic + atorvastatin group. $^{\neq}$P<0.001 vs. c group, *P<0.001 vs. d group.

Table 2: Oxidative stress parameters in heart from the experimental groups

Groups	c	ct	d	dt
CAT (IU / mg prot.)	19.31 ± 2.92	15.65 ± 1.04	29.13 ± 1.05$^{\neq}$	23.2 ± 1.49*
SOD (IU / mg prot.)	0.47 ± 0.07	0.34 ± 0.05	0.34 ± 0.04	0.46 ± 0.03
GSH (nmol / mg prot.)	15.91 ± 1.03	13.62 ± 0.79	12.51 ± 0.36$^{\neq}$	14.86±0.35*
MDA (nmol / mg prot.)	1.62 ± 0.21	1.24 ± 0.13	2.39 ± 0.30$^{\neq}$	1.28 ± 0.14*

Values are mean ± SE of 5 animals. c: control group, ct: control + atorvastatin group, d: diabetic group, dt: diabetic + atorvastatin group. $^{\neq}P < 0.05$ vs. c group, *P < 0.05 vs. d group. CAT: Catalase, SOD: Superoxide dismutase, GSH: Glutathion, MDA: Malondialdehyde.

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