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Antioxidant treatment protects diabetic rats from cardiac dysfunction by preserving contractile protein targets of oxidative stress

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Abstract

Backgound: Animal studies suggest that reactive oxygen species (ROS) play an important role in the development of diabetic cardiomyopathy.
Hypothesis: Matrix metalloproteinase-2 (MMP-2) is activated by ROS and contributes to the acute loss of myocardial contractile function by targeting and cleaving susceptible proteins including troponin I (TnI) and α-actinin.
Methods: Using the streptozotocin-induced diabetic rat model, we evaluated the effect of daily in vivo administration of sodium selenate (0.3 mg/kg; DMS group), or a pure omega-3 fish oil with antioxidant vitamin E (omega-3E; 50 mg/kg; DMFA group), which has antioxidant-like effects, for 4 weeks on heart function and on several biochemical parameters related to oxidant stress and MMP-2.
Results: Although both treatments prevented the diabetes-induced depression in left ventricular developed pressure (LVDP) as well as the rates of changes in developed pressure (±dP/dt) ($P<.001$), the improvement in LVDP of the DMS group was greater compared to that of the DMFA group ($P<.001$). Moreover, these treatments reduced the diabetes-induced increase in myocardial oxidized protein sulfhydryl and nitrite concentrations ($P<.001$). Gelatin zymography and Western blot data indicated that the diabetes-induced changes in myocardial levels of MMP-2 and tissue inhibitor of matrix metalloproteinase-4 (TIMP-4) and the reduction in TnI and α-actinin protein levels were improved in both the DMS and DMFA groups ($P<.001$).
Conclusions: These results suggest that diabetes-induced alterations in MMP-2 and TIMP-4 contribute to myocardial contractile dysfunction by targeting TnI and α-actinin and that sodium selenate or omega-3E could have therapeutic benefits in diabetic cardiomyopathy.

1. Introduction

Cardiac dysfunction is an important component of diabetes. Chronic, severe diabetes in animal models produces a series of stable alterations in heart function [1]. Abnormalities in contractile function and prolongation of the action potential duration have been reported, in addition to impaired insulin signalling that occurs during electrical remodelling of the heart in diabetes [1,2]. It is a well known fact that hyperglycemia in diabetes causes changes in membrane function within days and changes in contractile function within weeks due to increased production of reactive oxygen species (ROS) and an altered cellular redox state [3–5].

Significant increases in oxidants trigger a cascade of pathological events, including activation of matrix metalloproteinases (MMPs) [6–9]. MMPs play an important role by cleaving many proteins comprising the extracellular matrix, thus leading to remodelling under pathological conditions [10,11]. Recent studies have demonstrated that MMP-2 is colocalized with sarcomeric and cytoskeletal proteins in cardiomyocytes and its activation in ischemia-reperfusion or peroxynitrite-induced myocardial injury preceded the loss in mechanical function, which was prevented by inhibition of MMP-2 activity [6–8,12]. In addition, recent studies show that MMP-9 may also contribute to the pathogenesis of chronic heart failure and tachycardia-induced cardiomyopathy [13]. In a recent article, McCawley and Matrisian [14] discussed the concept of the role of MMP just for not the matrix. Although the physiological consequences of MMP activation/degradation in mitochondria are well understood, it has been shown the presence of MMPs in the cardiac mitochondria and it is well recognized that ROS generated by mitochondria can drive both activation and expression [15,16].

To date, few investigations have examined the role of MMPs in diabetes-induced cell damage and organ dysfunction [17]. In animal myocardium [10,18] there is a predominant expression of MMP-2, which is also found directly in cardiomyocytes. It was also demonstrated that inhibiting MMP activity improves the cardiovascular dysfunction in response to oxidative stress injury [6–8,18,19]. Recently, we showed the beneficial role of the MMP inhibitor doxycycline in the streptozotocin (STZ) diabetic rat in preventing the development of diabetic cardiomyopathy and the accompanying degradation of TnI caused by increased MMP-2 activity [20].

Although results from a Cochrane review suggest no beneficial effects of antioxidant treatment for prevention of mortality in healthy participants and patients with various diseases [21], several studies

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have reported beneficial effects of antioxidant therapy against the cardiovascular consequences of diabetes [22–25]. It has been shown that trace elements such as selenium have beneficial effects on parameters of glucose metabolism [24,25] as well as mechanical and electrical dysfunctions of the diabetic rat heart [4].

Supplementation with long-chain (n-3) polyunsaturated fatty acids has also been of potential interest as a therapy for cardiovascular diseases. Studies showed that (n-3) polyunsaturated fatty acid supplementation results in a reduction in relative risk of 10–20% in fatal and nonfatal cardiovascular events [26]. Our previous data demonstrated that treatment of diabetic rats with a dietary supplement consisting of (n-3) polyunsaturated fatty acid enriched with vitamin E [omega-3 fish oil with antioxidant vitamin E (omega-3E)] induced marked protective effects including improved left ventricular contractile function and preservation of the diabetes-induced altered activities of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase and thioredoxin reductase [27].

Since MMP-2 contributes to the development of cardiac dysfunction in several cardiac injury models where there is an increased production of ROS, including diabetic cardiomyopathy [20], we aimed to investigate alterations in MMP-2 and its target contractile proteins and the possible protective actions of an antioxidant such as sodium selenate, or pure omega-3E, which has antioxidant-like effects.

2. Methods

2.1. Animals and experimental design

Unless otherwise stated, all chemicals used were purchased from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany). All antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA). Omega-3E contains 70% pure omega-3, and natural vitamin E was purchased from FreeFlow, Vesteralens Naturprodukter, Sortland, Norway, and its dosage for rats, as used in here, was calculated to the suggestion of the company as two capsules each day for adult humans. Ingredients per capsule (692 mg) contain 477 mg total fish oil (nutrients include 15 mg natural vitamin E, and minimal 160 mg eikosapentaenacid, 105 mg dokosaheksaenacid and 215 mg other omega-3 fatty acids) and 192 mg antioxidant (D-alpha tocopherol), gelatin and glycerol.

Male Wistar rats (200–250 g) were used. Diabetes was induced as described previously [4]. A week after injection of STZ (50 mg/kg body weight), blood (whole blood) glucose concentration was measured and rats with threefold higher (at least) levels of blood glucose than preinjection levels were used in the experiments. Rats were divided into four groups. Two control groups consist of nondiabetic (C group) and diabetic rats (DM group) which received daily saline in an identical fashion to the treatment groups for 4 weeks. The third and fourth groups were DM groups, which received (intragastrically) either sodium selenate (0.3 mg/kg of body weight, daily; DMS group) or omega-3E (50 mg/kg of body weight, daily; DMFA group) for 4 weeks. All rats had free access to water and food (dietary composition of rat diet contained (as percentage): torula yeast 30.0, corn oil 2.0, sucrose 59.0, DL-methionine 0.3 and AIN-76 TM mineral mixture 5.0 and AIN-76 TM vitamin mixture 1.0 with digestible energy 12.59 MJ/kg from Horland Tekland, Madison, WI, USA) during the experimental protocol. Rat care and experimental procedures were in accordance with Ankara University Animal Ethics Guidelines (No: 94-2461).

2.2. Isolated Langendorff-perfused hearts

At the end of the 4 week treatment period hearts were isolated and perfused as described previously [27]. The left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and the rates of changes in developed pressure (±dP/dt) of isolated hearts were measured. For comparison, time to peak developed pressure (TP) and time to half-relaxation of the developed pressure (DT_{50}) were also measured, as described previously, without any modifications [27].

2.3. Preparation of heart homogenates

Frozen hearts were pulverized at liquid nitrogen temperature and then homogenized [6]. Protein content in homogenates was analyzed using the Bradford method (Bio-Rad). Bovine serum albumin was used as a protein standard.

2.4. Gelatin zymography

Gelatin zymography for MMP-2 and MMP-9 activities was performed as described previously [28]. To quantify the activities, zymograms were imaged by a Raytest camera attached to a computer with AIDA software (Straubenhardt, Germany). The intensities of the bands were analyzed using SigmaGel (Jandel) and reported as normalized to control group. Conditioned medium from untreated HT1080 cells was used as a MMP-2 reference standard.

2.5. Western blotting

MMP-2, tissue inhibitor of matrix metalloproteinase-4 (TIMP-4), TnI and α-actinin protein levels were determined by Western blot analysis. Briefly, equal amount of proteins from samples were loaded and separated on polyacrylamide sodium dodecyl sulfate-polyacrylamide gel electrophoresis (0.08–0.15) gels under reducing conditions. After electrophoresis (150 V, 20°C), samples were electroblotted onto a polyvinylidene fluoride membrane. Immunoreactive protein bands were visualized using a using the ECL plus detection system (Santa Cruz).

2.6. Determination of protein sulfhydryl concentration

Protein sulfhydryl (SH) concentration was measured spectrophotometrically in the heart homogenates as described previously [4]. Absorbance of the supernatants was read at 412 nm. To determine the free SH concentration, 0.7 ml aliquots of lysates were mixed with 0.35 ml of containing 20% trichloroacetic acid (TCA) and then centrifuged at 13 000 g for 10 min. The precipitates were then washed with 0.2 ml of TCA in a similar manner. Later, the supernatants were adjusted to pH 8.0 with NaOH. Free SH content of the supernatants was measured as described for total SH measurements.

2.7. Determination of nitrite concentration

Nitric oxide degradation products (nitrite and nitrate) were as previously described [5]. Spectrophotometric determination of nitrite using the Greiss reagent is sensitive for nitrite but does not measure nitrate, causing a possible underestimation of nitric oxide. In order to eliminate this underestimation, powdered cadmium metal was used for the chemical reduction of nitrate to nitrite. In acid solution, nitrite is converted to nitrous acid which diazotizes sulfanilamide. This sulfanilamide-diazonium salt is then reacted with *N*-(1-naphthyl)-ethylenediamine to produce a chromophore which is measured at 540 nm.

2.8. Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality assumption within groups and it was found that LVEDP and ±dP/dt of the DM group were not normally distributed except the other variances of the control group ($P<.05$). Furthermore, the Levene test was used to test for the homogeneity of variances amongst groups. If the data were not normally distributed and/or in the situation where the variances were non-homogenous, the Kruskal–Wallis one-way analysis of variance (ANOVA) test was used. Otherwise, if the data were distributed normally or variances were homogeneneous, one-way ANOVA with the Bonferroni test for post hoc analysis was used (Table 1). As a result, when a significant difference was found the Mann–Whitney *U* test was used to determine which group differs from each others. To control for Type I error rate, $\alpha_{cor}=\alpha/6=.008$ was taken as the corrected α value. To compare any changes in data values over time (the values measured in the beginning ("initial") and in the end ("final") of the experiments in each group, a two-way repeated-measures ANOVA test was carried out for the body weight and blood glucose values. The change in the variances for each group showed significant differences between the initial and final values ($P<.001$).

Pearson correlations were calculated for MMP-2, TIMP-4, TnI and α-actinin in heart samples with (i) LVEDP; (ii) LVDP; (iii) ±dP/dt; (iv) TP; (v) DT_{50}. $P<.05$ was considered to be an indication of a significant association.

3. Results

3.1. General characteristics of the rats

One week after initiation of control treatment or induction of diabetes by STZ injection, there was no difference in body weight or blood glucose concentration within this first week in the C ($n=28$), DM ($n=32$), DMS ($n=33$) and DMFA ($n=27$) groups. Rats in these four groups weighed 235.4±19.7 g, 241.3±20.8 g, 233.4±17.2 g and 228.5±11.5 g, respectively, while their blood glucose concentrations were 9.9±2.2 mmol/L, 10.0±3.3 mmol/L, 10.0±2.9 mmol/L and 10.0 ±3.7 mmol/L, respectively.

At the end of the experimental treatment (4 weeks) period, the final body weight of DM and DMS groups were smaller than their initial values ($P<.001$), while the final body weight of the C group was higher than its initial value ($P<.001$). The final body weight of the

Table 1
Test of homogeneity of variances for variables

Variables	Levene statistics	Significance level
Free SH	0.590	.626
Total SH	1.065	.380
Nitrite	0.779	.516
LVEDP	3.301	.032
LVDP	4.427	.010
+dP/dt	2.622	.069
−dP/dt	2.690	.063
TP	3.066	.045
DT_{50}	4.305	.015

Abbreviations: LVEDP, left ventricular end diastolic pressure; LVDP, left ventricular developed pressure; +dP/dt, rate of increase in the developed pressure; -dP/dt, rate of decay in the developed pressure; TP, time to peak of the developed pressure; DT50, time to half of decay of the developed pressure.

DMFA group was higher than those of DM ($P<.001$) and DMS groups ($P<.001$), while this was also less than that of C group ($P<.001$). The final blood glucose level of DM, DMS and DMFA groups were higher than their initial (first week) values ($P<.001$), while both DMS and DMFA groups had lower blood glucose level than the DM group ($P<.001$) (Table 2).

3.2. Rats from the DMS and the DMFA groups show improved cardiac function compared to the DM group

Diabetes caused a significant depression in the LVDP measurement in the isolated hearts which was abolished both in the DMS and the DMFA groups ($P<.001$) (Fig. 1A, right). In addition, the mean value of the LVDP of the DMS group was greater than that of the DMFA group ($P<.001$). Furthermore, the LVDP of the DMS group was also greater than that of the C group ($P<.001$). The mean values of both +dP/dt and –dP/dt were smaller in the DM group compared to the C group ($P<.001$), while these values in both the DMS and the DMFA groups were greater than that of the DM group ($P<.001$) (Fig. 1B, left and right). No significant increase or decrease was observed in the LVEDP of the DM group compared to the C group at the end of experimental period (Fig. 1A, left). These values were also similar between the C group and DM groups after the first week prior to the initiation of the experimental treatments (data not shown). Moreover, we did not observe any significant change in the LVEDP in both the DMS and the DMFA groups compared to the C and the DM groups (Fig. 1A, left).

We also tested the baseline cardiac contractile parameters in hearts from all of the groups. Representative LVDP records are given in Fig. 2A. The TP and the DT_{50} values of the DM group were greater than that of the C group (Fig. 2B, right and left, respectively) ($P<.001$). The values of the DMS and the DMFA groups were not different from the values of the C group while they were smaller than that of the DM group ($P<.001$) (Fig. 2B).

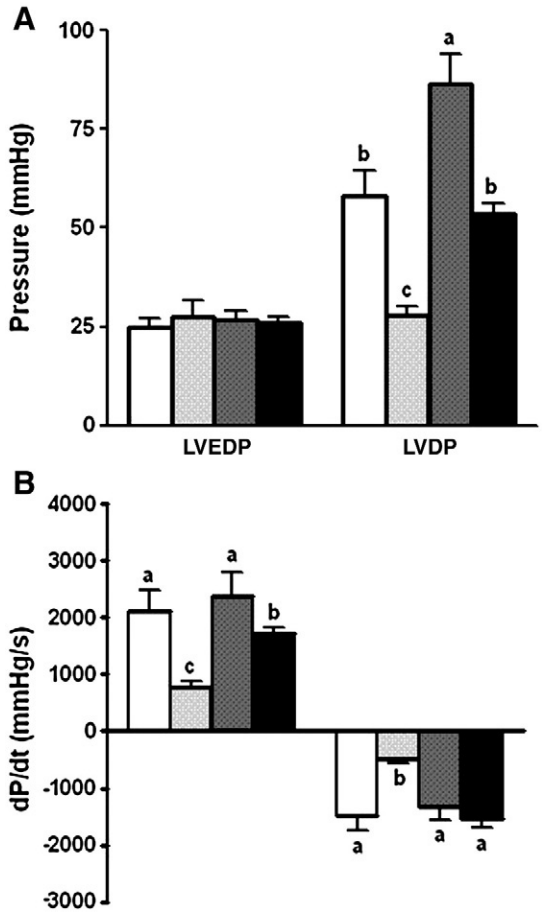

Fig. 1. Effects of antioxidant treatments on the mechanical activity of the isolated hearts from diabetic rats. Bars represent mean±S.E.M. The mean values of LVDP and LVEDP are presented in (A). White, light grey, dark grey and black bars represent the C ($n=10$), DM ($n=12$), DMS ($n=12$) and DMFA ($n=10$) groups, respectively. ±dP/dt are given in (B). Means on bars with superscripts without a common letter differ, $P<.001$.

3.3. MMP activity and protein level of in heart homogenates

The activity and the protein level of MMP-2 of the DM group were lower than that of the C group ($P<.001$) (Fig. 3A and B, respectively). Values in both the DMS and the DMFA groups were higher than those of the DM group, while the increase in the protein of MMP-2 of the DMS group was higher than that of the DMFA group ($P<.001$). No MMP-9 activity was seen by gelatin zymography in any heart group (data not shown). The protein level of myocardial TIMP-4 in the DM group was lower than that of the C group. This value was normalized in the DMS and the DMFA groups compared to the C group ($P<.001$) (Fig. 3C).

Table 2
Body weight, blood glucose concentration, protein SH and nitrite concentrations

Parameters	C group	DM group	DMS group	DMFA group
Body weight, *g*	251.6 ± 23.9^{a} (258.2)	192.1 ± 20.2^{c} (194.9)	193.5 ± 22.2^{c} (192.3)	230.3 ± 16.8^{b} (230.6)
Blood glucose concentration, *mmol/L*	$10.3\pm2\ .8^{c}$ (10.2)	50.6 ± 3.1^{a} (51.0)	45.3 ± 3.5^{b} (45.7)	43.8 ± 2.1^{b} (44.1)
Free SH, μmol/mg protein	2.03 ± 0.68^{b} (2.02)	1.15 ± 0.81^{c} (1.14)	3.03 ± 0.84^{a} (3.03)	2.84 ± 0.13^{a} (2.83)
Total SH, μmol/mg protein	4.07 ± 0.13^{b} (4.09)	1.82 ± 0.11^{c} (1.82)	4.31 ± 0.14^{a} (4.28)	4.10 ± 0.17^{b} (4.11)
Nitrite, nmol/mg protein	11.56 ± 0.31^{b} (11.62)	14.36 ± 0.32^{a} (14.43)	10.97 ± 0.48^{b} (11.03)	10.83 ± 0.30^{b} (10.84)

Abbreviations: C, control group; DM, diabetic group; DMS, the sodium selenate treated diabetic group; DMFA, pure omega-3 fish oil with antioxidant vitamin E (omega-3E) treated diabetic group.
Values are expressed as mean ± SEM and the median of each parameter is given in the bracket. Numbers of rats used in biochemical analysis are 5-7 for each measurement of each group. Means in a row with superscripts without a common letter differ, $P<.001$.

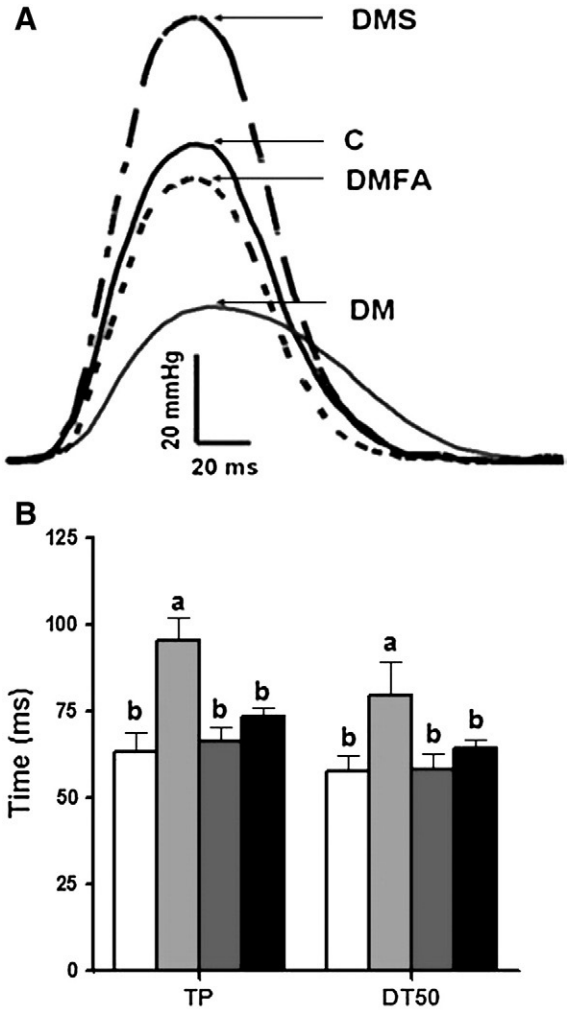

Fig. 2. Effects of antioxidant treatments on the parameters of baseline mechanics of LVDP measured in diabetic hearts. (A) Representative LVDP records and times related with baseline mechanics. (B) Bars represent mean±S.E.M. TP (right) and time DT_{50} (left) of LVDP measured in the C (white), DM (light grey), DMS (dark grey) and DMFA (black) groups. Means on bars with superscripts without a common letter differ, $P<.001$.

3.4. Protein levels of TnI and α-actinin in the heart homogenates

The protein levels of both TnI and α-actinin in the hearts of the DM group were lower compared to that of the C group ($P<.001$). The values of both the DMS and the DMFA groups were equivalent compared to the C group ($P<.001$) (Fig. 4A and B). There was a significant inverse correlation between α-actinin and +dP/dt ($r=-0.6$, $P=.01$) (data not shown).

3.5. Beneficial effects of sodium selenate or omega-3E are accompanied by a reduction in oxidized protein SH and nitrite concentrations of the heart tissues

Total and free protein SH concentrations in the hearts of the DM group were lower compared to those of the C, the DMS and the DMFA groups (Table 2, $P<.001$). The values of total SH of the DMS group were higher than that of the DMFA group ($P<.001$) which were higher compared to that of the C group ($P<.001$). Free SH levels were similar in both DMS and DMFA groups which were higher than that of the C group (Table 2). Nitrite concentration of the heart tissue obtained from the DM group was higher compared to that of the C group ($P<.001$). This increase was prevented both in the DMS and the DMFA groups ($P<.001$) (Table 2).

4. Discussion

Diabetes is well known for its cardiovascular complications. In this study, we demonstrated that the myocardial levels of TnI and α-

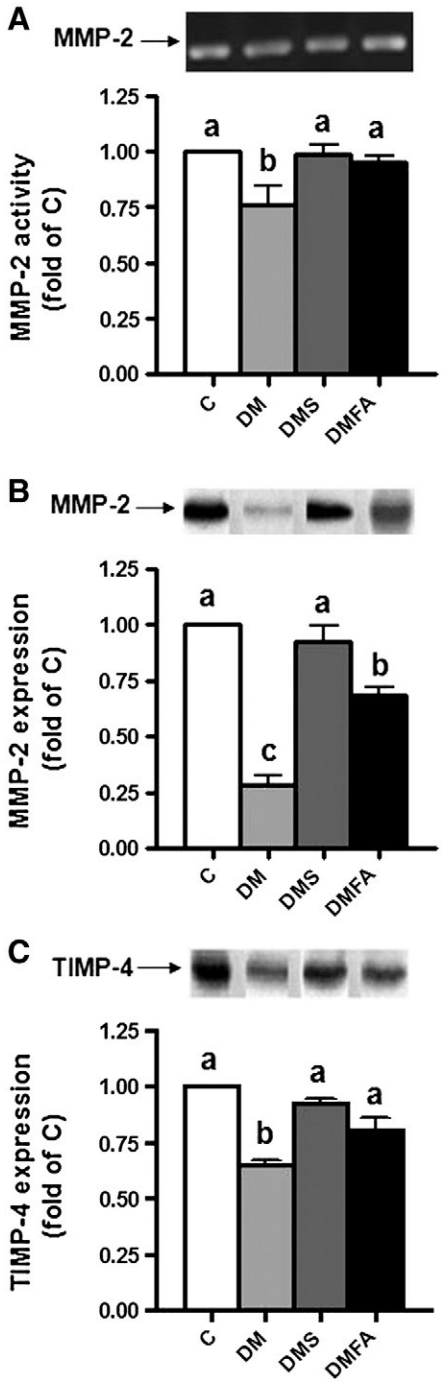

Fig. 3. Antioxidant treatments prevented the changes in MMP-2 activity and protein level of as well as TIMP-4 protein level in heart tissue from diabetic rats. Bars represent mean±S.E.M. Heart homogenates were assayed for MMP-2 activity (as fold of the C group) by gelatin zymography (A) or MMP-2 (B) and TIMP-4 (C) protein levels by Western blotting. Upper panels show representative gelatin zymogram (A) and Western blots (B–C) and bottom panels show the summary of the densitometric analysis. Means on bars with superscripts without a common letter differ, $P<.001$.

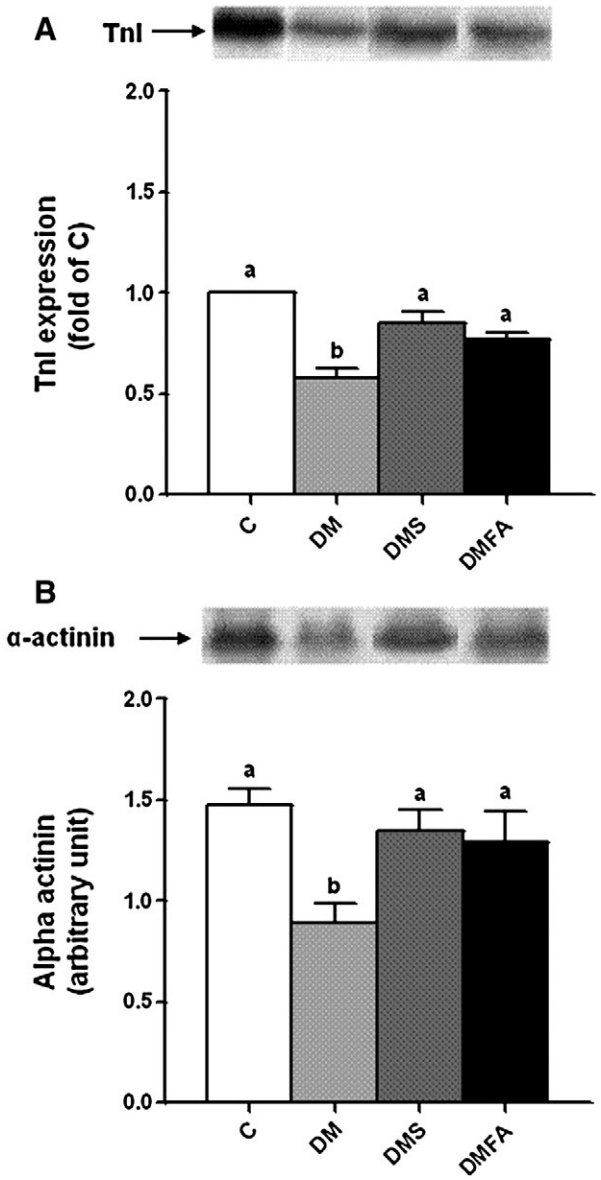

Fig. 4. Antioxidant treatments prevented diabetes-induced lost both in protein levels of TnI and α-actinin in the heart tissue. Bars represent mean±S.E.M. TnI protein level (A) and α-actinin protein level (B) in the heart homogenates. Upper panels show representative Western blots (A and B). Bottom panels show the summary of the densitometric analysis. Numbers of rats are five to seven for each group. Means on bars with superscripts without a common letter differ, $P<.001$.

actinin were reduced in STZ-induced diabetic rats. Treatment of diabetic rats with either an antioxidant sodium selenate or omega-3E (basically pure omega-3 fish oil enriched with vitamin E) protected hearts from mechanical dysfunction via reducing MMP-2 activation and therefore reducing the degradation of two of its target proteins in the cardiac myocyte, TnI [18,20] and α-actinin [12].

The data from the present and previous studies suggest that oxidative stress is involved in the etiology of diabetes-induced down-regulation of heart function and that there is a close relationship between impaired insulin signalling and alteration in heart function via depressed endogenous antioxidant defence mechanisms [18,29,32]. Previously published data have shown significant changes in the expression and activity of the MMPs (especially MMP-2) and their inhibitors in oxidative stress-related pathologies [33–35]. This activation contributes not only to the degradation of extracellular matrix but also to degradation of some intracellular proteins. Supporting this hypothesis, a detrimental role of MMP-2 in diabetic cardiomyopathy was demonstrated previously [20].

In our study, we used two dietary substances with unique mechanisms of action in the cell defence system: sodium selenate and omega-3E. While selenium gets incorporated into enzymes and proteins, (n-3) fatty acid get incorporated into lipid membranes. Therefore, the effectiveness of these agents may depend particularly on their mode of action and the compartment being affected by the disease state.

The mechanisms of antioxidant-mediated regulation of depressed cardiac function in diabetic rats are not fully understood. However, our hypothesis of glutathione (GSH) involvement, which was mentioned in previous studies [5,36], is supported by the present data. Although we did not measure GSH level in the present study, we and others have already shown that selenium supplement to diabetic rats prevented the diabetic cardiac dysfunction via a significant role in cell redox state, in part, due to its role in the insulin-signalling cascade [36,37]. Therefore, in here, our data present the possible essential role of antioxidants in controlling the intracellular redox state and the normal function of cardiomyocytes.

Our previous data also demonstrated that administration of the MMP inhibitor doxycycline reduced diabetes-induced cardiac dysfunction as well as TnI degradation [20]. Here, we demonstrated that the levels of myocardial TnI and α-actinin are reduced during 5 weeks of diabetes. This loss in protein levels is reduced by administration of either sodium selenate or omega-3E. According to the present data, the activity and protein level of cardiac MMP-2 were reduced in STZ rats compared to control. This can be explained by hypothesizing that at the end of 5 wk-diabetes, most of the latent MMP-2 might have been activated and released from the tissue. In fact, a reduction in both MMP-2 and TIMP-4 level has been reported for the diabetic cardiomyopathy in a similar experimental setting [20,38]. This is also supported by the preservation of the MMP-2 level and activity in the treatment with either sodium selenate or omega-3E.

Although both sodium selenate and omega-3E provide significant protection against diabetes-induced depression in LVDP, the effect of sodium selenate seems to be more prominent compared to that of omega-3E. Our present study also demonstrated that both treatments reduced the diabetes-induced increase in myocardial oxidized protein SH concentration. Selenium is known to have an important role in the cellular antioxidant system [4,5,21,23,38]. It is incorporated into several important enzymes known as selenocysteines. Thioredoxin reductases, glutathione peroxidases and thyroid hormone deiodinases are well characterized selenoenzymes involved in redox regulation of intracellular signalling such as redox homeostasis [39]. We have previously published that either selenium compound or omega-3E treatment of diabetic rats has marked protection in the depressed antioxidant defence system and redox homeostasis of the heart [4,5,27]. Selenium's superior antioxidant capacity can be attributed to its participation in antioxidant enzyme expression and activity when compared with the omega-3E, whose effect is evident only after incorporation into lipid membranes [40–42]. An efficient antioxidant system, glutathione and α-tocopherol as well as the (n-3) polyunsaturated fatty acids are critical for effective functioning of the myocardium [29–32]. Consequently, it can be concluded that both types of supplements can protect the heart against diabetes-induced dysfunction, preserving not only antioxidant defence system but also preserving contractile proteins. Indeed, in an early study, an important role with altered level of free SH in diabetes-induced alterations in the heart had been reported [43]. According to that study, myofibrillar ATPase and SH activities were reduced in cardiomyocytes isolated from diabetic rats. Furthermore, increased ROS production caused significant reduction in free SH levels and caused contractile dysfunction of the isolated skinned cardiomyocytes due to an induced formation of disulfide bonds on the

contractile proteins [44]. In addition, it is also known that the generation of ROS may not only activate MMPs but also inhibit the TIMP causing an imbalance between the two favouring enhanced MMP activity [33,45,46] of which our data are in line with these already known processes.

Our data demonstrated that either sodium selenate or omega-3E treatment caused a slight but significant decrease (12%) in the elevated blood glucose level. This observation could be attributed to the reduction in protein SH oxidation which may result in better activity of glucose transporters [37,47–49]. Thus, when comparing the published data with the present findings, it seems likely that sodium selenate treatment affects the diabetes-induced inhibited heart function through its effect on cellular thiols, which, in turn, is closely related with the insulin-signalling cascade. However, other possible explanations for the relationship between the beneficial effects of sodium selenate and the (n-3) fatty acid metabolism in the diabetic rat heart cannot be excluded at this time. Bogdanov et al. [50] demonstrated the modulation of electrical activity of the heart with polyunsaturated fatty acids. Therefore, these two dietary supplements used in experimental diabetic animals may play an important role in controlling oxidative status and altered lipid metabolism, thereby maintaining favourable fatty acid distribution in cell membranes of tissues affected by diabetic complications [51].

In summary, present data demonstrated that treatment of diabetic rats with an antioxidant, sodium selenate or a pure omega-3 fish oil with antioxidant vitamin E may protect the heart from diabetes-induced dysfunction by preserving both contractile and cytoskeletal target proteins from MMP-2 mediated degradation.

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The authors declare no conflict of interest.

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