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Effect of Vitamin E and Selenium Supplement on Paraoxonase-1 Activity, Oxidized Low Density Lipoprotein and Antioxidant Defense in Diabetic Rats

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ABSTRACT

Introduction: The aim of the present study was to assess the effects of vitamin E and selenium supplementation on serum paraoxonase (PON1) activity, lipid peroxidation and antioxidant defense in streptozotocin-induced diabetic rats. ***Methods:*** Thirty two female Sprague Dawley rats were divided into 3 groups: the control group (n=8) received a standard diet; streptozotocin (STZ)-induced diabetic rats (n=12), received corn oil and physiological solution; and vitamin E and selenium supplemented diabetic rats (n=12) were treated with oral administration of vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) once a day for 4 weeks. ***Results:*** Significantly lower total antioxidant status (TAS), PON1and erythrocyte SOD activities and a higher fasting plasma glucose level were observed in the diabetic rats compared to the control. A significant increase in SOD and GPX activities in vitamin E and selenium supplemented diabetic group was observed after 5 weeks of the experiment. Compared to the normal rats, malondialdehyde (MDA) and oxidized LDL (Ox-LDL) levels were higher in the diabetic animals; however, these values reduced significantly following vitamin E and selenium supplementation. ***Conclusion:*** Vitamin E and selenium supplementation in diabetic rats has hypolipidemic, hypoglycemic and antioxidative effects and may slow down the progression of diabetic complications through its protective effect on PON1 activity and lipoproteins oxidation.

Introduction

Human serum paraoxonase (aryldialkylphosphatase, EC 3.1.8.1; PON1) is a high-density lipoprotein cholesterol (HDL-C) related enzyme that may be an important modulator of cardiovascular disease risk (Heijmans *et al*. 2000; Durrington *et al*. 2001; Bergmeier *et al*. 2004).The studies have shown an inverse correlation between the serum concentration of HDL-C and the development of atherosclerosis, however, the exact mechanism(s) for this association continues to be the subject of considerable debate (Heinecke, 2008). According to the recent studies, there could be more than one single explanation for the observed association (Nofer, 2002).The ability of HDL-C to prevent the oxidation of low-density lipoprotein cholesterol (LDL-C) is one of these mechanisms that received the most attention in the literature. The susceptibility of LDL-C is a pivotal event in the initiation and progression of atherosclerosis (Chait *et al*. 1993; Aviram *et al*. 1999). This antioxidant capability of HDL-C has been attributed, at least in part, to its enzymes, primarily PON1 (Mackness *et al*. 1996). Further studies have indicated that PON1 is able to protect lipoproteins against oxidation and prevent lipid peroxide accumulation on LDL-C *in vitro* and *in vivo* (Mackness *et al*. 1993; Aviram *et al*. 1998). The enzyme can also hydrolyze and inactivate homocysteinethiolactone which is a risk factor for atherosclerotic vascular disease (Jakubowski, 2000).

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Serum PON1 activity reduces in cardiovascular disease, hypercholesterolemia and diabetes and it is more likely that oxidative stress plays a central role in the pathology of these diseases (Ikeda *et al*. 1998, Mackness *et al*. 1991; Feldman, 2003). According to several studies, there is an increase in the levels of circulating markers of free radical-induced damage and a decrease in antioxidant defences including circulating levels of vitamins in diabetes (Folmer *et al*. 2002; Ramakrishna and Jailkhani, 2007; Will *et al*. 1999; Sargeant *et al*. 2000). Depletion of antioxidative defence system can lead to an increase in reactive oxygen species (ROS) generation (King and Loeken, 2004). The increased oxidative status in the diabetic patients (Maritim *et al*. 2003) and subsequent inactivation of PON1 together with low PON1 expression could serve as major factors in the observed reduced PON1 activity in diabetes (Rozenberg and Aviram, 2006; Rozenberg *et al*. 2008). Taking into account the involvement of oxidative stress in the development of diabetes and also in the PON1 activity/inactivation, it is possible that the administration of antioxidants could protect PON1 inactivation arising from ROS and consequently ameliorate diabetes.

Vitamin E and selenium are two micronutrients with antioxidant properties that work co-dependently to protect the body from the harmful and destructive effects of free radicals. However, to our best knowledge, the effects of vitamin E and selenium supplementation on PON1 activity in diabetes have not covered in the literatures.

There is also a doubt in the ability of selenium administration in the prevention of diabetes development. Blevs *et al*. (2007) have found a positive association between high serum selenium levels and the prevalence of diabetes among US adults. It is also reported that selenium supplementation not only may not prevent type 2diabetes, but also may increase the risk for the disease (Stranges *et al*. 2007). Recently, antioxidant vitamin supplements have been considered in the publication of the Guidelines of the American Heart Association among therapies not recommended for cardiovascular disease prevention, because of their usefulness and possible harmful effects (Mosca *et al*. 2007). Therefore, it seems that despite published results, further studies are required to elucidate the contribution of oxidative stress to diabetes development and also the function and role of vitamin antioxidants in those cases that ROS and its consequential oxidative damage is a factor to consider in the dietary or nutraceutical management of diabetes (Rozenberg *et al*. 2008).

In the present study, the effect of vitamin E and selenium supplementation on antioxidant defence in diabetic rats with focus on PON1 activity has been investigated.

Materials and methods

Experimental design

Thirty two female Sprague Dawley rats weighing 200±30 g (about 2-3 months of age), were kept in the animal house at temperatures of 23±2°C and relative humidity of 50% under a lighting cycle of 07.00-19.00 h light and 19.00-07.00 h dark. They were fed with a standard laboratory diet and allowed food and water ad libitum for an acclimatization period of 1 week prior to the experiment. The animals were divided into three groups of (I) control rats, C (n=8), (II) streptozotocin (STZ)-induced diabetic rats, DM (n=12) and (III) diabetic rats with orally administered vitamin E and selenium, DM + S (n=12). The body weights of all the animals were determined every week, and water consumed was recorded daily.

Diabetes induction

The experimental type 1 diabetes was induced in groups II and III by a single intraperitoneal injection of 60 mg/kg STZ (Sigma), freshly dissolved in physiological serum. STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality (Ramesh and Pugalendi, 2006). Control rats received an injection of physiological serum. Six days after STZ injection, rats with blood glucose levels above 300 mg/dL estimated with enzymatic method, were considered as diabetic and used for the study (Mahay *et al*. 2004).

Vitamin E and selenium treatment

On the seventh day following STZ injection (first day of treatment), vitamin E as α-tocopherol acetate(Merck) dissolved in corn oil and selenium as sodium selenite (Merck) dissolved in water were administrated to group III at concentrations of 300 and 0.5 mg/kg/day, respectively, by oral gavage once a day for four weeks. The concentrations of vitamin E and selenium were chosen based on previous reports (Haidara *et al*. 2009; Yanardag *et al*. 2007). Rats of group II received 0.5 ml corn oil and 0.5 ml physiological solution and were used as diabetic control group.

Sample preparation

At the beginning and the end of the experimental period, blood samples were collected from the oculo-orbital vein in EDTA-containing tubes under light ether anesthesia after 12 hours of fasting. Plasma samples were prepared by 10 min centrifugation of blood at 3000 g at 4°C and were stored at −76°C for further studies. A part of whole blood was frozen for GPX determination. Erythrocytes for SOD determination were washed three times by 0.9% saline and frozen after hemolysis.

Measurement of glucose and lipid profile

Plasma glucose, total cholesterol (TC) and triglyceride (TG) levels were measured by enzymatic colorimetric

methods (Pars Azmune, Tehran, Iran), using an Auto analyzer Model Alcyon. Non-HDL-C lipoproteins in the plasma were precipitated by phosphotungstic acid and magnesium chloride (Burstein *et al*. 1970) and the content of HDL-C was determined using an enzymatic colorimetric method (Pars Azmune, Tehran, Iran). The concentration of LDL-C was calculated according to the Friedewald formula for lessthan400 mg/dL TG containing samples (Fridewald *et al*. 1972).

Analysis of PON1 activities

PON1 activity toward paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate; Sigma Chemical Co.) was determined by adding plasma to Tris/HCl buffer (0.1 mol/L, pH 8.0) containing paraoxon (2 mmol/L) and CaCl2 (2 mmol/L). The rate of hydrolysis of paraoxon was measured spectrometrically (UV 1250, Shimadzu, Japan) by monitoring p-nitrophenol libration at 412 nm. The amount of generated p-nitrophenol was calculated from the molar absorptivity of 18,290 mol-1 cm-1 (Jafarnejad *et al*. 2008).

Measurement of GPX and SOD activities

The hemoglobin content of the erythrocytes was determined using the cyanmethaemoglobin method (Drabkin, 1965). Erythrocyte GPX and SOD activities were determined using Randox kits (Randox Laboratories). GPX activity levels were measured using the method of Paglia and Valentine, (1967), in which GPX activity was coupled with the oxidation of NADPH by glutathione reductase. The oxidation of NADPH was followed spectrophotometrically at 340 nm. The reaction mixture comprised 50 mmol/L potassium phosphate buffer (pH 7.2), 4.3 mmol/L EDTA, 0.18 mmol/L cumenehydroperoxide, 0.34 mmol/L NADPH, 4 mmol/L glutathione and 0.5 U/L of glutathione reductase. The absorbance at 340 nm was recorded for 5 min and the activity calculated from the slope of the line as micromoles of NADPH oxidized per minute. The results were expressed as units per gram of haemoglobin.

The determination of SOD activity was based on the production of O2- anions by the xanthine/xanthine oxidase system (Fairbanks and Klee, 1994). O2-react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The SOD activity was measured by the degree of inhibition of this reaction in 505 nm. The results were expressed as units per gram of haemoglobin.

Measurement of plasma total antioxidant status (TAS)

TAS was measured in plasma by means of a commercial kit (Randox Laboratories). The assay is based on the incubation of 2,2'-azino-di-(3-ethylbenzthiazolinesulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogenperoxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm (Miller *et al*. 1993).The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L).

Measurement of plasma malondialdehyde (MDA)

Plasma MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL plasma (Ohkawa *et al*. 1979). Briefly, 0.5 ml serum was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. Then, 1ml of 0.67 % TBA was added to the mixture, shaken, and warmed for 60 min in a boiling water bath followed by rapid cooling. It was, then, shaken into a 4 ml of n-butanol layer in a separation tube and MDA content in the serum was determined at 532 nm by spectrophotometer against n-butanol. The standards of 0.1 to 20 mol/L tetramethoxypropane (TMP) were used to obtain the calibration curve.

Measurement of Ox-LDL

The level of Ox-LDL was measured by using a Mercodia Oxidized LDL ELISA kit (Lot No. 15904; Mercodia, Uppsala, Sweden). Mercodia Ox-LDL Competitive ELISA is based on the monoclonal antibody 4E6.The Ox-LDL in the sample competes with a fixed amount of Ox-LDL bound to the microtiter well for the binding of the biotin-labelled specific antibodies. After a washing step that removes non-reactive plasma components, the biotin-labelled antibody bound to the well is detected by HRP-conjugated streptavidin. After a second incubation, unbound enzyme-labelled antibody is removed by a simple washing step and the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. Then, the reaction is stopped by addition of acid and the microtitration strips are read spectrophotometrically at 450 nm. The results are calculated using the computerized data reduction of absorbance for the standards versus the log concentration using a cubic spline regression and expressed as U/l.

Statistical analyses

All data were expressed as mean ± SEM. The statistical comparison between the experimental groups was performed using one-way analysis of variance (ANOVA) and Tukey HSD. The relationship between plasma PON1 activity and other parameters was estimated by Pearson correlation analysis. A value of $p<0.05$ was considered statistically significant. Statistical analyses were carried out by SPSS version 15.0 program.

Results

As it was expected, the plasma glucose level was significantly ($p<0.01$) elevated in diabetic rats one and five weeks after injection of STZ compared to the

normal control group (Table 1). Vitamin E and selenium administration for four weeks caused a significant decrease in the glucose level of the treated diabetic rats in comparison to the diabetic group without treatment. Plasma TC, TG and LDL-C levels were significantly increased in diabetic animals compared with the normal control group, whereas a significant reduction was observed in HDL-C. After four weeks, plasma triglyceride levels significantly increased in the treated diabetic group compared with the normal control group, where as the plasma levels of HDL-C, LDL-C and TC were not significantly changed following vitamin E and selenium administration (Table 1).

Table 1. Plasma glucose and lipids levels in the control group, streptozotocin (STZ)-induced diabetic rats, DM group, and diabetic rats with orally administered vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) supplementation, DM + S group, after 1 and 5 weeks of STZ treatment

	control group	DM group (n = 12)		DM + S group (n = 12)	
	(n = 8)	One week	Five weeks	One week	Five weeks
Glucose (mg/dL)	87.3±3.4	404.9±11.7^{a**}	396.4±15.4^{a**}	384.1±17.9^{a**}	317.9±17.8^{b*,a**}
Triglycerides (mg/dL)	67.1±4.0	73.6±1.4	108.6±1.2^{a**,b**}	79.4±2.7	93.4±3.9^{a**}
Total cholesterol (mg/dL)	60.1±2.0	62.4±2.6	79.1±1.9^{b**,a**}	63.0±1.0	67.0±1.4
HDL-C (mg/dL)	16.6±0.7	14.1±0.6	11.3±1.2^{a**}	12.7±0.8^{a*}	13.5±0.7
LDL-C (mg/dL)	30.0±2.5	33.6±2.6	46.0±3.3^{a**,b**}	34.4±1.7	34.8±1.9

Statistical comparison: [a] Experimental group vs. control; [b] Week 1 vs. Week 5.
Statistical significance: $^{*}p < 0.05$; $^{**}p < 0.01$.

Plasma TAS levels significantly decreased in the diabetic group 5 weeks after STZ injection compared with the control group, however, vitamin E and selenium administration increased this value almost to the control value indicating the efficiency of this treatment in the improvement of TAS. Although STZ-induced diabetes did not affect the blood GPX activity, the level of this enzyme in treated diabetic group was found to be significantly higher than the control and diabetic animals after 5 weeks (Table 2). At the first week of treatment, a significant reduction was observed in erythrocyte SOD activity in both diabetic and treated diabetic groups, however, the enzyme activity increased to almost the control value after four weeks treatment with vitamin E and selenium.

Table 2. Plasma total antioxidant status (TAS), blood glutathione peroxidase (GPX) and erythrocyte superoxide dismutase (SOD) activities after 1 and 5 weeks of experiment in the control group, streptozotocin (STZ)-induced diabetic rats, DM group, and diabetic rats with orally administered vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) supplementation, DM + S group

	control group	DM group (n = 8)		DM + S group (n = 8)	
	(n = 8)	One week	Five weeks	One week	Five weeks
TAS (mmol/L)	0.74±0.03	0.67±0.04	0.31±0.05^{a**,b**}	0.63±0.04	0.67±0.03
GPX (U/grHb)	140.9±4.1	137.2±3.0	131.9±3.7	136.9±3.5	151.1±2.1^{a*}
SOD (U/grHb)	2012.2±49.4	1751.1±78.3	1708.4±67.1 ^{b*}	1707.7±81.0^{b*}	1984.8±40.1 ^{a*}

Statistical comparison: [a] Week 1 vs. Week 4; [b] Experimental group vs. Control
Statistical significance: $^{*}p<0.05$; $^{**}p<0.01$

Plasma MDA and Ox-LDL levels have been presented in Fig. 1. STZ-induction of diabetes caused a dramatic elevation in the plasma MDA levels. Vitamin E and selenium supplementation could only reduce the elevated level of MDA by 5-fold compared to the diabetic animals. The level of MDA in diabetic rats was still 5-times higher than the corresponding value in the control group after five weeks. Almost similar results were observed in the plasma Ox-LDL levels of the studied groups (Fig. 1).

After five weeks, plasmaPON1 activity decreased by two-fold in diabetic animals, but this decrease was compensated by vitamin E and selenium supplementation, although the activity did not reach to the control value (Fig. 2).

A very strong positive correlation was found between plasma PON1 activity and either TAS or SOD activity in diabetic rats (Table 3). There was also a positive correlation between PON1 and TAS in diabetic supplemented rats (Table 3). The PON1 activity was inversely correlated with Ox-LDL and TC levels in DM group. However, no significant relationship was observed between the enzyme activity with serum LDL-C and TC concentrations.

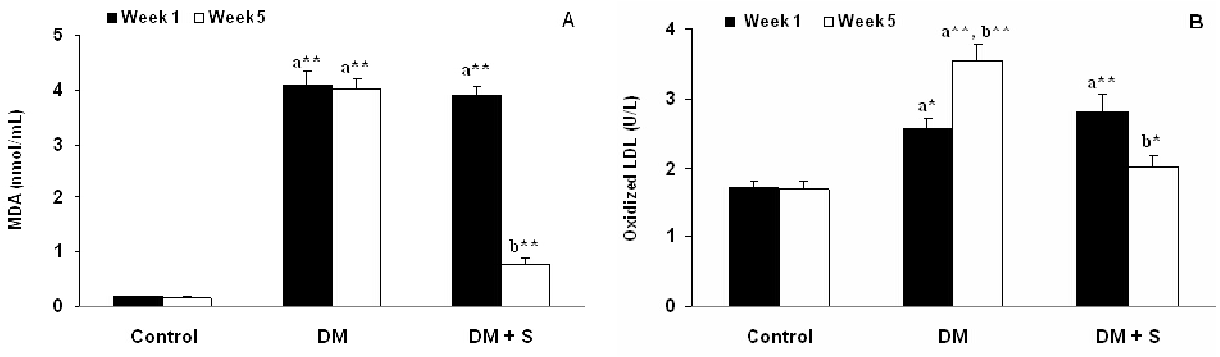

Fig. 1. Plasma MDA levels (A) and Ox-LDL concentrations (B) after 1 and 5 weeks of STZ treatment in the control group, streptozotocin (STZ)-induced diabetic rats, DM group, and diabetic rats with orally administered vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) supplementation, DM + S group.
Statistical comparison: [a] Week 1 vs. Week 5; [b] Experimental group vs. Control
Statistical significance: $^{*}p<0.05$; $^{**}p<0.01$

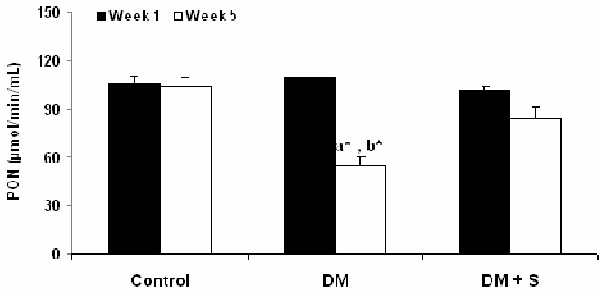

Fig. 2. Plasma paraoxonase activity in the control group, streptozotocin (STZ)-induced diabetic rats, DM group, and diabetic rats with orally administered vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) supplementation, DM + S group after 1 and 5 weeks of STZ treatment.
Statistical comparison: [a] Week 1 vs. Week 5; [b] Experimental group vs. Control; Statistical significance: $^{*}p<0.01$

Discussion

The possible involvement of oxidative stress and ROS in the pathogenesis of diabetes and diabetic complications has been extensively studied by many authors and is still subject for more studies. According to some animal studies (Jafarnejad *et al*. 2008), diabetes is accompanied with increased level of lipid peroxides and ROS and a decreased level of antioxidant enzymes such as PON1. Therefore, the administration of antioxidants could be effective in the diabetes prevention or improvement of diabetic complications probably through protection of PON1 inactivation as one of the involved mechanisms. However, the results obtained from clinical studies are not unequivocal and the usefulness of antioxidant supplementation in diabetic patients is not convincing (Stranges *et al*. 2007; Mosca *et al*. 2007; Lukacinova *et al*. 2008).

Table 3. The correlation of PON1 activity with Glucose, TG, TC, HDL-C, LDL-C, GPX, SOD, TAS, MDA, Ox- LDL levels in the control group, streptozotocin (STZ)-induced diabetic rats, DM group, and diabetic rats with orally administered vitamin E (300 mg/kg) and sodium selenite (0.5 mg/kg) supplementation, DM+S group

	Eq	r	P value
Control			
Glucose	Y= 0.5411x + 55.8436	0.33	0.21
TG	Y= 0.0964x + 96.5377	0.07	0.79
TC	Y= -0.5791x +137.3000	-0.23	0.39
HDL-C	Y= -2.01498x +136.3873	-0.29	0.27
LDL-C	Y= -0.2796x +111.1746	-0.13	0.64
GPX	Y= 0.5625x + 23.9532	0.43	0.09
SOD	Y= -0.0070x +117.0195	-0.08	0.78
TAS	Y= -36.9743x +130.3585	-0.27	0.31
MDA	Y= -0.0016x + 0.3381	-0.28	0.29
Ox- LDL	Y= 0.0003x + 1.6509	0.00	0.9
DM			
Glucose	Y= 0.4297x + -90.3117	0.40	0.12
TG	Y= -1.0145x + 174.3129	-0.56	0.02
TC	Y= -1.5655x + 192.5889	-0.42	0.10
HDL-C	Y= 4.2355x + 27.9615	0.34	0.19
LDL-C	Y= -1.16428x + 128.1842	-0.31	0.25
GPX	Y= 1.4970x + -119.6016	0.37	0.16
SOD	Y= 0.0433x + 6.8662	0.22	0.41
TAS	Y= 117.0683x + 24.1751	0.64	0.008
MDA	Y= -0.0035x + 4.3778	-0.24	0.37
Ox- LDL	Y= -0.0108x + 3.9464	-0.59	0.016
DM+S			
Glucose	Y= 0.0817x + 64.2751	0.28	0.26
TG	Y= -0.3740x + 125.2861	-0.25	0.31
TC	Y= -2.3836x + 247.8925	-0.55	0.02
HDL-C	Y= 0.7799x + 82.75609	0.10	0.69
LDL-C	Y= -1.2385x + 135.8464	-0.37	0.13
GPX	Y= -0.4347x + 155.5721	-0.27	0.28
SOD	Y= 0.0442x + 11.3835	0.57	0.01
TAS	Y= -0.4838x + 93.2739	0.00	0.99
MDA	Y= 0.0504x + -2.3040	0.54	0.021
Ox- LDL	Y= 0.0167x + 0.8683	0.40	0.094

In the present study, we observed a reduced plasma PON1 activity in STZ-induced diabetic rats and an increased serum PON1 activity after four weeks of vitamin E and selenium supplementation. Antioxidants are able to protect lipoproteins and PON1against oxidation (Rozenberg *et al*. 2008).It has been shown that PON1 activity is inactivated *in vitro* by more than 80% under oxidative stress conditions and vitamin E can protect against this loss of activity (Maritim *et al*. 2003). Therefore, the improvement of the imbalance between oxidative and antioxidative mechanisms in diabetic rats by vitamin E and selenium administration may account for the observed variations in rat serum PON1 activity (Rozenberg *et al*. 2008). These results are consistent with those reports indicating the beneficial effect of vitamin E in the treatment of diabetes complication in humans (Wu *et al*. 2007) and STZ-treated rats (Al-Shamsi *et al*. 2006), as well as in delaying diabetes development in mice (Rozenberg *et al*. 2008).

It is also possible that the preserved PON1 activity in vitamin E and selenium supplemented diabetic rats observed in the present study results from the protective effect of the antioxidants against HDL-C modification in oxidative stress. The HDL-C is compositionally abnormal in diabetic rats and this may influence the binding of PON1 to HDL-C leading to a conformational change in PON1 (Heijmans *et al*. 2000). The protection of HDL-C from oxidative modification by vitamin E and selenium may prevent undesirable alteration of HDL-associated PON1 conformation.

Vitamin E and selenium may also exert their beneficial effects on serum PON1 activity through their antihyperglycemic and, thus, antioxidative actions. In the present study, antioxidative effect of vitamin E and selenium supplementation was reflected by an increase in the GPX and SOD activities in the diabetic animals treated with vitamin E and selenium for four weeks.

The plasma levels of GPX and SOD in the supplemented diabetic group increased significantly after four weeks of treatment. There was also a significant reduction in the plasma level of TAS in the diabetic rats, whereas, in the vitamin E and selenium supplemented diabetic animals the level of TAS was remained almost unchanged compared with the control group. These indicate that the administration of vitamin E and selenium has been able to prevent the animals against oxidative stress through elevation of the antioxidant enzymes and maintaining the plasma total antioxidant level. The reduction of TAS and SOD activity after five weeks of diabetes induction could be indicative of oxidative stress increase during diabetes.

In diabetic rats, significant reduction in the activities of antioxidant enzymes could be indicative of ongoing oxidative disturbances. Decline in the activities of these antioxidant enzymes might be due to their inactivation caused by excessive ROS production. It has been shown that SOD activity reduction can increase the level of superoxide, which is able to inactivate GPX. In addition, failure in the elimination of H2O2 by GPX may lead to the accumulation ofH2O2 which in turn can cause inactivation of SOD (Selvakumar *et al*. 2005). The consistent reduction in the activities of antioxidant enzymes and enhanced lipid peroxidation suggests that all of the organs are subjected to oxidative stress in STZ-induced diabetes. Bearing in mind the antioxidant property of vitamin E and the presence of selenium in the active site of GPX, it seems that vitamin E and selenium supplementation is able to reduce oxidative stress through elevation of the activity of antioxidant enzymes.

In the present study, ox-LDL levels in the diabetic rats were found to be significantly higher than its level in the supplemented diabetic group. It has been generally accepted that ox-LDL is produced *in vivo* and contributes to atherogenesis (Inoue *et al*. 2001). The association between diabetes and increased LDL oxidative susceptibility has been demonstrated in several studies (Girona *et al*. 2008). Kondo *et al*. (2002) have shown that treatment of diabetics with insulin can prevent LDL oxidation.

A marked decline in ox-LDL concentration in the treated animals with vitamin E and selenium is indicative of direct effect of these two antioxidants on lipids and lipoproteins oxidation. This is in agreement with the elevated level of MDA in the diabetic rats and its reduction in the supplemented animals observed in this study. As MDA reflects the sensitivity of lipoproteins to oxidation, a lower level of MDA in the supplemented diabetic rats compared with untreated diabetic animals would be indicative of the beneficial effect of vitamin E and selenium on the oxidizability of lipoproteins in diabetes. It, thus, seems that vitamin E and selenium supplementation can reduce ox-LDL concentration through the reduction of glucose level, elevation of the antioxidant enzymes and the improvement of antioxidant defence mechanisms.

Ethical issues

All animals were handled with humane care in accordance with the National Institutes of Health guidelines and the experimental procedure of the study was approved by the ethics committee of Tabriz University of Medical Sciences.

Conflict of interests

Authors declare no conflict of interest.

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References

Al-Shamsi M, Amin A and Adeghate E. **2006**. Vitamin E ameliorates some biochemical parameters in normal and diabetic rats. *Ann N Y Acad Sci*, 1084, 411–31.

Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier CL, et al. **1999**. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved byantioxidants. *Free Radic Biol Med*, 26(7-8), 892–904.

Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL and La Du BN. **1998**. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its function: a possible peroxidative role for paraoxonase. *J Clin Invest*, 101(8), 1581-90.

Bergmeier C, Siekmeier R and Gross W. **2004**. Distribution spectrum of paraoxonase activity in HDL fractions. *Clin Chem*, 50(12), 2309-15.

Blevs J, Navas-Acien A and Guallar E. **2007**. Serum selenium and diabetes in U.S. adults. *Diabetes Care*, 30(4), 829-34.

Burstein M, Scholnick HR and Morfin R. **1970**. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res*, 11(6), 583–95.

Chait A, Brazg RL, Tribble DL and Krauss RM. **1993**.Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med*, 94(4), 350-6.

Drabkin DL. **1965**. The molecular weight of haemoglobin, its iron and nitrogen content and optical properties - their relevance in the problem of a reference standard for haemoglobin measurement. *Bibl Haematol*, 21, 33-42.

Durrington PN, Mackness B and Mackness MI. **2001**. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol*, 21(4), 473-80.

Fairbanks V and Klee GG. **1994**.Biochemical aspects of haematology. In: *Tietz Textbook of Clinical Chemistry* (Burtis CA, AshwoodER, eds.) WB Saunders, Philadelphia; p. 2020-1.

Feldman EL. **2003**. Oxidative stress and diabetic neuropathy: a new understanding of an old problem. *J Clin Invest*, 111(4), 431–3.

Folmer V, Soares JC and Rocha JB. **2002**. Oxidative stress in mice is dependent on the free glucose content of the diet. *Int J Biochem Cell Biol*, 34(10), 1279–85.

Fridewald WJ, Levy RJ and Fredrikson DS. **1972**. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem*, 18(6), 499–502

Girona J, Manzanares JM, Marimon F, Cabre A, Heras M, Guardiola M, et al. **2008**. Oxidized to non-oxidized lipoprotein ratios are associated with arteriosclerosis and the metabolic syndrome in diabetic patients. *Nutr Metab Cardiovasc Dis*, 18(5), 380-7.

Haidara MA, Mikhailidis DP, Rateb MA, Ahmed ZA, Yassin HZ, Ibrahim IM, et al. **2009**. Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rats with streptozotocin-induced Type 1 diabetes. *J Diabetes Complications*, 23(2), 130-6.

Heijmans BT, Westendorp RGJ, Lagaay AM, Knook DL, Kluft C and Slagboom PE. **2000**. Common paraoxonase gene variants, mortality risk and fatal cardiovascular events in elderly subjects. *Atherosclerosis*, 149(1), 91-7.

Heinecke JW. **2009**. The HDL proteome: a marker and perhaps mediator of coronary artery disease. *J Lipid Res*, 50, 167–71.

Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arii K, et al. **1998**. Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. *Metabolism*, 47(5), 598-602.

Inoue T, Uchida T, Kamishirado H, Takayanagi K, Hayashi T and Morooka S. **2001**. Clinical significance of antibody against oxidizedlow density lipoprotein in patients with atheroscleroticcoronaryartery disease. *J Am Coll Cardiol*, 37(3), 775-9.

Jafarnejad A, Bathaie SZ, Nakhjavani M andHassan MZ. **2008**. Effect of spermine on lipid profile and HDL functionality in the streptozotocin-induced diabetic rat model. *Life Sciences*, 82(5-6), 301–7.

Jakubowski H. **2000**. Calcium-dependent human serum homocysteinethiolactonehydrolase: a protective mechanism against protein N-homocysteinylation. *J BiolChem*, 275(6), 3957–62.

King GL and Loeken MR. **2004**. Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem Cell Biol*, 122(4), 333-8.

Kondo A, Manabe M, Saito K, Maekawa M and Kanno T. **2002**. Insulin treatment prevents LDL from accelerated oxidation in patients with diabetes. *J Atheroscler Thromb*, 9(6), 280-7.

Lukacinova A, Mojzis J, BenackaR, Keller J, Maguth T, Kurila P, et al. **2008**. Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. *Acta Vet Brno*, 77, 175-82.

Mackness MI, Arrol S, Abbott C and Durrington PN. **1993**. Protection of low-density lipoprotein againstoxidativemodification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*, 104(1-2), 129-35.

Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, et al. **1991**. Serum paraoxonaseactivity in familial hypercholesterolemia and insulin-dependent diabetes mellitus.*Atherosclerosis*, 86(2-3), 193-9.

Mackness MI, Mackness B, Durrington PN, Connelly PW and Hegele RA. **1996**. Paraoxonase:biochemistry, genetics and relationship to plasma lipoproteins. *Curr Opin Lipidol*, 7(2), 69-76.

Mahay S, Adeghate E, Lindley M, Rolph C and Singh J. **2004**. Streptozotocin-induced type 1 diabetes mellitus alters the morphology, secretary function and acyl lipid contents in the isolated rat parotid salivary gland. *Mol Cell Biochem*, 261(1-2), 175-81.

Maritim AC, Sanders RA and Watkins JB. **2003**. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*, 17(1), 24-38.

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V and Milner A. **1993**. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* (Lond), 84(4), 407-12.

Mosca L, Banka CL, Benjamin EJ, Berra K, Bushnell C, Dolor RJ, et al. **2007**. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. *Circulation*,115(11), 1481–501.

Nofer J. **2002**. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis*, 161(1), 1-16.

Ohkawa H, Ohishi N andYagi K. **1979**. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95(2), 351-8.

Paglia DE and Valentine WN. **1967**. Studies on the quantitative and qualitative charachtristication of erythrocyte GPX. *J Lab Clin Med*,70(1), 158-69.

Ramakrishna V and Jailkhani R. **2007**. Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. *Diagn Pathol*, 2, 22.

Ramesh B and Pugalendi KV. **2006**. Antihyperglycemic Effect of Umbelliferone in Streptozotocin-Diabetic Rats. *J Med Food*, 9(4), 562–6.

Rozenberg O and Aviram M. **2006**. S-Glutathionylation regulates HDL-associated paraoxonase 1 (PON1) activity. *Biochem Biophys Res Commun*, 351(2), 492-8.

Rozenberg O, Shiner M, Aviram M and Hayek T. **2008**. Paraoxonase 1 (PON1) attenuates diabetes development in mice through its antioxidative properties. *Free Radic Biol Med*, 44(11), 1951-9.

Sargeant LA, Wareham NJ, Bingham S, Day NE, Luben RN, Oakes S, et al. **2000**. Vitamin C and hyperglycemia in the European Prospective Investigation into Cancer--Norfolk (EPIC-Norfolk) study: a population-based study. *Diabetes Care*, 23(6), 726-32.

Selvakumar E, Prahalathan C, Mythili Y and Varalakshmi P. **2005**. Beneficial effects of DL-alpha-lipoic acid on cyclophosphamide-induced oxidative stress in mitochondrial fractions of rat testis. *Chem Biol Interact*, 152(1), 59-66.

Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, et al. **2007**. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: A randomized trial. *Ann Intern Med*, 147(4), 217-23.

Will JC, Ford ES and Bowman BA. **1999**. Serum vitamin C concentrations and diabetes: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*, 70(1), 49-52.

Wu JH, Ward NC, Indrawan AP, Almeida CA, Hodgson JM, Proudfoot JM, et al. **2007**. Effects of alpha-tocopherol and mixed tocopherol supplementation on markers of oxidative stress and inflammation in type 2 diabetes. *Clin Chem*, 53(3), 511–9.

Yanardag R, Ozsoy-Sacan O, Ozdil S and Bolkent S. **2007**. Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats. *Int J Toxicol*, 26(6), 513-23.

