EXPERIMENTAL STUDY

# Effects of thiamine treatment on oxidative stress in experimental diabetes

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**ABSTRACT**

AIM: Hyperglycemia, oxidative stress and hyperlipidemia are features of diabetes mellitus. Thiamine has beneficial effects on carbohydrate metabolism and it was proposed that this vitamin has antihyperlipidemic and antioxidant effects. Our aim was to investigate the effects of thiamine on oxidative stress and metabolic changes in streptozotocin (STZ) induced diabetic rats.

METHOD: Diabetes was induced by a single intraperitoneal injection of STZ. Thiamine (6 mg/kg) was added to drinking water for five weeks. The rats were divided into four groups: control rats; thiamine treated control rats; diabetic rats; thiamine treated diabetic rats. Plasma and tissue malondialdehyde (MDA) levels were measured by high-performance liquid chromatography and spectrophotometry, respectively. Paraoxonase (PON) and arylesterase (AE) activities were measured with spectrophotometric methods, and erythrocyte superoxide dismutase (SOD) and blood glutathione peroxidase (GSH-Px) activities were determined using commercial kits.

RESULTS: Thiamine treatment reduced plasma and tissue MDA levels, serum glucose, total cholesterol and triglyceride levels, and increased serum high density lipoprotein- cholesterol and insulin levels, serum PON and AE, erythrocyte SOD and blood GSH-Px activities.

CONCLUSION: Thiamine significantly improves oxidative stress and has hyperinsulinemic and antihyperlipidemic effects so we suggest that thiamine might be used as a supportive therapeutic agent in diabetes *(Tab. 2, Fig. 3, Ref. 53)*. Text in PDF *www.elis.sk*.

KEY WORDS: streptozotocin, diabetes mellitus, thiamine, oxidative stress, paraoxonase.

## Introduction

Oxidative stress is the imbalance between oxidant and antioxidant systems in favor of the former and has been widely accepted to be involved in the pathogenesis of various diseases, including diabetes mellitus and atherosclerotic vascular diseases. Diabetic patients are exposed to atherosclerotic micro- and macro-vascular complications and oxidative stress is believed to play a key role in the pathogenesis of these complications (1).

Hyperglycemia is a defining feature of diabetes mellitus and in the hyperglycemic state glucose induces lipid peroxidation. Moreover, the mitochondrial electron transport chain, the main source of free radicals in human metabolism, is one of the first targets of glucose and has been associated with enhanced free radical formation (2). In the hyperglycemic state plenty of molecules including proteins, enzymes, nucleic acid material and lipoproteins may be glycosylated which results in deactivation and deformation of those molecules (2, 3).

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Hyperglycemia, oxidative stress and hyperlipidemia are features of diabetes mellitus which are situations affecting each other in a vicious circle and it is well known that these physiopathological situations result in atherosclerotic vascular disease. Several investigators suggested that treatment of hyperglycemia and hyperlipidemia and improvement in oxidative stress may be beneficial in diminishing the complications of diabetes mellitus (1–3).

Thiamine, vitamin B1, is a nutritional factor of interest, particularly in the context of glucose metabolism and insulin action (4–6). Thiamine has been shown to be deficient in diabetic patients because of malabsorption and enhanced urinary excretion and its deficiency was also proposed to be a marker of microvascular diabetic complications (6–8). This vitamin is a coenzyme for transketolase, pyruvate dehydrogenase, and alpha-ketoglutarate dehydrogenase complex (7, 9). In addition to its beneficial effects on carbohydrate metabolism, it was proposed that thiamine has antihyperlipidemic and antioxidant effects (5–7, 10, 11). It was suggested that thiamine interacts with free radicals and hydroperoxides and inhibits lipid peroxidation (12). But, data about the effects of thiamine on the oxidant and antioxidant balance are limited and controversial. Lukienko et al (12) showed that thiamine decreased lipid peroxidation due to a direct interaction with free radicals and hydroperoxides in the rat liver microsomes. However, Schmid et al (13) reported that thiamine did not prevent oxidative stress and did not show a direct antioxidant action. So

the attitude towards the usage of thiamine as a supportive agent is ambiguous.

In the human biological system, antioxidant activity is achieved by several enzymes and antioxidant molecules (vitamins A, C, E and uric acid, bilirubin etc.). Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are the frontline defense that prevents oxidative damage to biological molecules and tissues (14). Apart from these enzymes, paraoxonase is a high density lipoprotein (HDL)-associated antioxidant enzyme carried on apolipoprotein A1 (15). Paraoxonase exerts both paraoxonase (PON) and arylesterase (AE) activities, since the enzyme hydrolyzes organophosphates (such as paraoxon) and aromatic esters (such as phenyl acetate). AE activity born by paraoxonase can be considered as an index of actual protein concentration (16). PON activity protects lipoproteins and tissues against oxidative modification and it is well known that lipoprotein oxidation is one of the key mechanisms involved in the initiation and progression of atherosclerosis (17). We and others have previously reported that PON activity was reduced in diabetes mellitus (18–20). Decreased serum PON activity might be related to glycation and/or oxidative modification of HDL and the enzyme paraoxonase (21). To our knowledge this is the first study investigating the effects of thiamine treatment on PON and AE activities.

Malondialdehyde (MDA) is the most widely used measure of lipid peroxidation, which is produced when polyunsaturated fatty acids are attacked by oxygen derived free radicals (22). We examined plasma and tissue MDA levels as an indicator of oxidative stress. Until now, there is no study that investigated tissue MDA levels after thiamine treatment in diabetes mellitus.

In the present study, our aim was to investigate the effects of thiamine on the oxidative and the antioxidative systems in streptozotocin (STZ) induced diabetic rats. For this purpose, we determined serum PON and AE activities, erythrocyte SOD and blood GSH-Px activities to evaluate the antioxidative mechanisms, and we measured plasma and tissue MDA levels to evaluate lipid peroxidation status.

**Materials and methods**

*Animals*

The experiments were performed with 40 male Wistar strain rats (age: 4 months) weighing approximately 300–350 g. Rats were given free access to standard laboratory chow (carbohydrates 35 %, proteins 25 %, lipids 7 % and vitamins 3 %) and tap water for one week before the experiment. Ten rats were housed per cage. Thiamine 6 mg/kg was prepared daily and administered in drinking water to thiamine supplemented control and diabetic rats for five weeks after STZ injection. The study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of Uludag University, Bursa.

*Experimental design*

The rats were divided into four groups of ten rats each: Group 1: Normal control rats (C), Group 2: Control rats with orally administered thiamine (CT), Group 3: STZ induced diabetic rats (D), Group 4: Diabetic rats with orally administered thiamine (DT).

*Diabetes induction*

The type 1 diabetes was induced with a single intraperitoneal injection of 65 mg/kg STZ (Sigma, St. Louis, MO) freshly dissolved in sodium citrate buffer (pH 4.5). Control rats received an injection of citrate buffer. Blood glucose level was measured 48 h after STZ injection. Rats with blood glucose level > 11.1 mmol/L were considered as diabetic and were included in the study. STZ injection may result in fatal hypoglycemia related to massive insulin release. To prevent hypoglycemia, rats were kept on a 5% glucose solution diet for 24 h after STZ injection.

*Sample preparation*

At the end of the experimental period, blood samples were obtained by cardiac puncture under light ether anesthesia following 10–12 h of fasting. Liver, kidney, heart and skeletal muscle (m. gastrocnemius) tissues were removed immediately after blood collection, rinsed with cold saline, blotted with gauze and stored at -20 °C until analysis. Blood samples were drawn in heparin-coated, EDTA-containing and non-additive tubes. A part of whole blood was frozen for GSH-Px determination. Erythrocytes for SOD determination were washed by saline and frozen after hemolysis.

*Analyses*

Blood glucose concentration was measured with a glucostix strip test in a glucometer (Abbott Glucometer Medisense Products, USA). Serum insulin level was measured by radioimmunoassay kit for rats (Millipore Corporation, Billerica, MA, USA). Total cholesterol (TC), triglyceride (TG) and HDL-C levels were measured using an auto analyzer (Aeroset, Abbott Laboratories, Diagnostic Division, IL, USA). PON activity was determined as described by Eckerson et al (23). The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm at 25 °C. PON activity is expressed in U/L serum and defined as 1 μmol p-nitro phenol generated per minute under the above conditions. AE activity was determined by using phenyl acetate as the substrate. The reaction mixture contained 1.0 mM phenyl acetate and 0.9 mM calcium chloride in 9.0 mM Tris–HCl buffer, pH 8.0. One unit of AE activity is defined as 1 mmol phenol generated per minute under the above conditions and expressed as kU/L serum (24). Erythrocyte SOD and whole blood GSH-Px activities were determined using commercial kits (Randox Laboratories Antrim, UK). Briefly, the determination of SOD activity was based on the production of superoxide anions by the xanthine/xanthine oxidase system. GSH-Px catalyzes the oxidation of reduced glutathione (GSH) in the presence of cumene hydroperoxide. The generation of nicotinamide adenine dinucleotide phosphate (NADP) was monitored spectrophotometrically at 340 nm. The activity of GSH-Px was expressed as U/mL (25) Tissue MDA levels were determined by the thiobarbituric acid method and expressed as nmol MDA/mg tissue (26). Plasma MDA concentrations were determined with the high-performance liquid chromatography (Shimadzu LC-10AT) procedure of Young and Trimble (27). A calibration curve was pre-

**Tab. 1. Body weight, food and water consumption and serum glucose and insulin levels and lipid profile of the study groups.**

| Group | C | CT | D | DT |
|---|---|---|---|---|
| Food intake (g/24 h) | 15±1 | 13±1 | 31±5 [b*] | 23±3 [c*] |
| Fluid intake (mL/24 h) | 33±1 | 32±1 | 182±10 [b**] | 125±19 [c**] |
| Final body weight (g) | 373±5 | 353±6 | 291±19 [b**] | 275±13 [c*] |
| Glucose (mmol/L) | 6.87±0.01 | 6.75±0.01 | 21.15±0.58 [b**] | 16.68±0.83 [c*] |
| Insuline (mIU/mL) | 2.00±0.05 | 2.11±0.07 | 1.68±0.27 [b*] | 2.19±0.05 [c**] |
| TC (mmol/L) | 2.51±0.14 | 2.04±0.10 [a*] | 3.23±0.12 [b**] | 2.18±0.12 [c**] |
| TG (mmol/L) | 0.77±0.04 | 0.69±0.07 | 3.09±0.18 [b**] | 2.77±0.17 [c*] |
| HDL-C (mmol/L) | 1.30±0.01 | 1.33±0.02 | 1.20±0.02 [b*] | 1.29±0.01 [c*] |

TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, C: Normal control rats, CT: Control rats with orally administered thiamine, D: Streptozotocin induced diabetic rats, DT: Diabetic rats with orally administered thiamine, Values are expressed as mean ± SEM (standard error of mean) for ten rats in each group, Statistical comparison: [a] C vs CT, [b] C vs D, [c] D *vs* DT, Statistical significance, * p < 0.05, ** p < 0.01

**Tab. 2. Serum paraoxonase, arylesterase, erythrocyte superoxide dismutase, blood glutathione peroxidase activities and thiamine levels in rats.**

| Group | C | CT | D | DT |
|---|---|---|---|---|
| PON (U/L) | 122.1±7.1 | 134.0±6.0 | 57.2±3.4 [b**] | 96.4±5.6 [c**] |
| AE (kU/L) | 145.4±8.0 | 147.2±8.6 | 75.0±6.7 [b**] | 154.4±9.3 [c**] |
| PON/HDL-C | 93.9±14.4 | 100.7±21.2 | 47.7±4.8 [b**] | 66.9±5.7 [c**] |
| SOD (U/mL) | 64.3±4.1 | 87.2±5.7 [a**] | 99.6±3.8 [b**] | 123.1±5.6 [c*] |
| GSH-Px (U/mL) | 10.6±1.1 | 15.3±1.3 [a**] | 9.8±1.1 | 18.4±1.0 [c**] |
| Thiamine (µg/L) | 1262± 63 | 1364±79 [a*] | 1116±51 [b*] | 1471±41 [c*] |

PON: Paraoxonase, AE: Arylesterase, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, Values are expressed as mean ± SEM (standard error of mean) for ten rats in each group, Statistical comparison: [a] C vs CT, [b] C vs D, [c] D *vs* DT, Statistical significance, * p < 0.05, ** p < 0.01, for abbreviations of study groups see Table 1.

pared for each day by using 1,1',3,3'-tetramethoxypropane as the standard. Plasma thiamine concentrations were determined with the high-performance liquid chromatography (Thermo Finnigan Spectra Systems, USA), as described procedure of Sander et al (28).

*Statistical analysis*

Statistical analyses were carried out by SPSS 20.0 program for Windows (SPSS, Chicago, IL). Data are presented as mean ± SEM (standard error of mean). For statistical analysis, Kruskal–Wallis
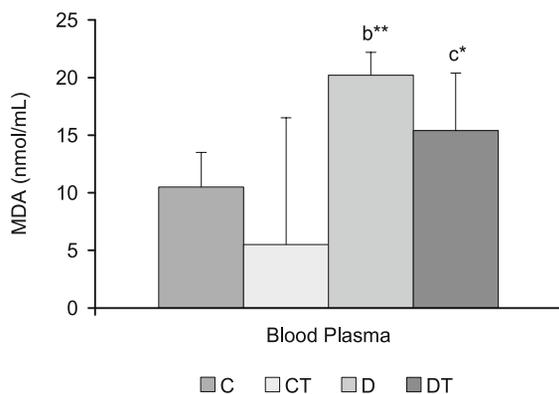
test was used, followed by the Mann–Whitney U test. A level of p < 0.05 was accepted as statistically significant.

**Results**

Food and fluid intake, body weight and metabolic changes can be seen in Table 1. Compared with the C group, food and fluid consumption, serum glucose, TC and TG levels were significantly increased, whereas body weight, insulin and HDL-C levels were significantly decreased in the D group of rats. Food and fluid intake and body weight in the DT group were lower than those of the D group. However, food and fluid intake and body weight were not different between the C and CT groups. In the CT group, serum TC levels were significantly reduced compared with the C group. Significant reductions were observed in serum glucose, TC and TG levels and significant increments were observed in insulin and HDL-C levels in the DT group, compared with those of the D group (Tab. 1).

Compared with the C group, serum PON and AE activities and PON/HDL-C ratio were significantly decreased in the D group of rats. Serum PON and AE activities and PON/HDL-C ratio were not different between the C and CT groups. However, serum PON and AE activities and PON/HDL-C ratio in the DT group were significantly higher than that of the D group (Tab. 2). While erythrocyte SOD activity was significantly increased in the D group compared with the C group, blood GSH-Px activity did not show any difference between the groups. However, we found that both control and diabetic rats receiving thiamine (CT and DT groups, respectively) exhibited higher SOD and GSH-Px activity than the rats not receiving thiamine (C and D groups, respectively). Plasma thiamine levels were found lower in the D group



**Fig. 1. Malondialdehyde (MDA) levels in plasma of the rats. Values are expressed as mean ± SEM (standard error of mean) for ten rats in each group. Statistical comparison: [a] C vs CT, [b] C vs D, [c] D vs DT, Statistical significance, *p < 0.05, **p < 0.01, for abbreviations of study groups, see Table 1**
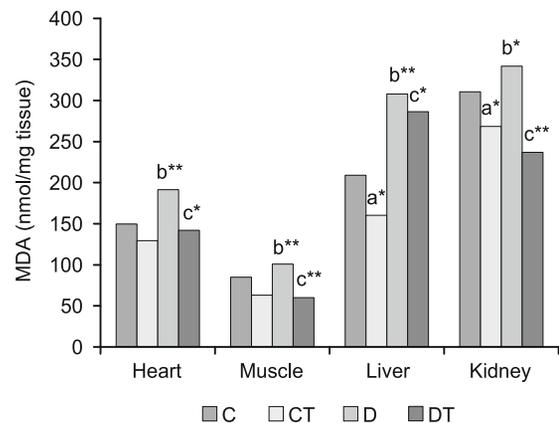


**Fig. 2. Malondialdehyde (MDA) levels in tissues of the rats. Values are expressed as mean ± SEM (standard error of mean) for ten rats in each group. Statistical comparison: [a] C vs CT, [b] C vs D, [c] D vs DT, Statistical significance, *p < 0.05, **p < 0.01, for abbreviations of study groups, see Table 1.**

compared with the C group. As expected, rats receiving thiamine had higher thiamine levels as compared to the rats not receiving thiamine (Tab. 2).

MDA levels in all tissues (liver, kidney, skeletal muscle and heart) and plasma were significantly increased in the D group compared with the C group. DT group had lower plasma and tissue MDA levels compared with the D group. CT group had lower liver and kidney tissue MDA levels compared with the C group (Figs 1 and 2).

## Discussion

In the present study, as well as in our previous ones (18, 19, 29–31), we demonstrated oxidative stress in STZ-induced diabetic rats. Oxidative stress was evident by the increased plasma and tissue MDA levels accompanying alterations in the activities of antioxidant enzymes. The reason for the increased plasma and tissue MDA levels, observed in this study, might partly be related to glycative and oxidative effects of glucose which are well reported (32). Furthermore, lipids are substrate for lipid peroxidation and increased TG and TC levels, observed in the D group, might be other contributing factors for the increased MDA levels.

We also found reduced levels of thiamine in the D group as parallel with the other studies (33–37). Thiamine was reported to have potential radical-scavenging activity (33) and free radical formation and lipid peroxidation may be enhanced in thiamine deficiency. Therefore reduced levels of thiamine, observed in the present study, might be one of the reasons for the increased plasma and tissue MDA levels. The reason for thiamine deficiency in diabetes is not well understood, however several mechanisms have been proposed (38–40). On the other hand, several authors

also suggested that thiamine deficiency causes a marked impairment in insulin synthesis and secretion (5, 6, 37, 39). Therefore it can be stated that insulin deficiency may exacerbate thiamine deficiency and vice versa.

In the present study, we observed that metabolic disturbances, hyperglycemia, hyperlipidemia, reduced insulin and thiamine levels, were improved with thiamine treatment in the DT group. Serum glucose, TG and TC levels were decreased in the DT group which might be contributing factors for reduced plasma and tissue MDA levels, observed in the DT group. Furthermore, liver and kidney tissue MDA levels were significantly reduced in the CT group compared with the C group which suggested a possible direct antioxidant effect of thiamine. TC levels were also lower in the CT group compared with the C group which also suggested a possible direct antihyperlipidemic effect of thiamine (10, 11). Thiamine deficiency was reported to decrease the activity of transketolase and thus slow down the pentose phosphate pathway (PPP) (Fig. 3A). In this case, excess glucose may be directed to hexosamine pathway (Fig. 3B). Increase in glucosamine 6 phosphate levels inhibits glucose 6 phosphate dehydrogenase (G6PDH) activity (41) which may contribute to slow down the PPP (Fig. 3C). Furthermore, inhibition of glyceraldehyde 3 phosphate dehydrogenase activity by increased levels of reactive oxygen species (ROS) may contribute to decreased rate of glycolysis and may be another factor that increases the rate of hexosamine pathway (Fig. 3D). Increased hexosamine pathway intermediates stimulation of lipogenic enzymes in the liver (Fig. 3E) (5, 10, 11). Furthermore, it was reported that glutamine: fructose 6-phosphate amidotransferase (GFA), the rate – limiting enzyme of the hexosamine pathway, was overexpressed in the hyperlipidemic state (Fig. 3F) (11) which might be another contributing factor for the dyslipidemia observed in diabetes. Thia-
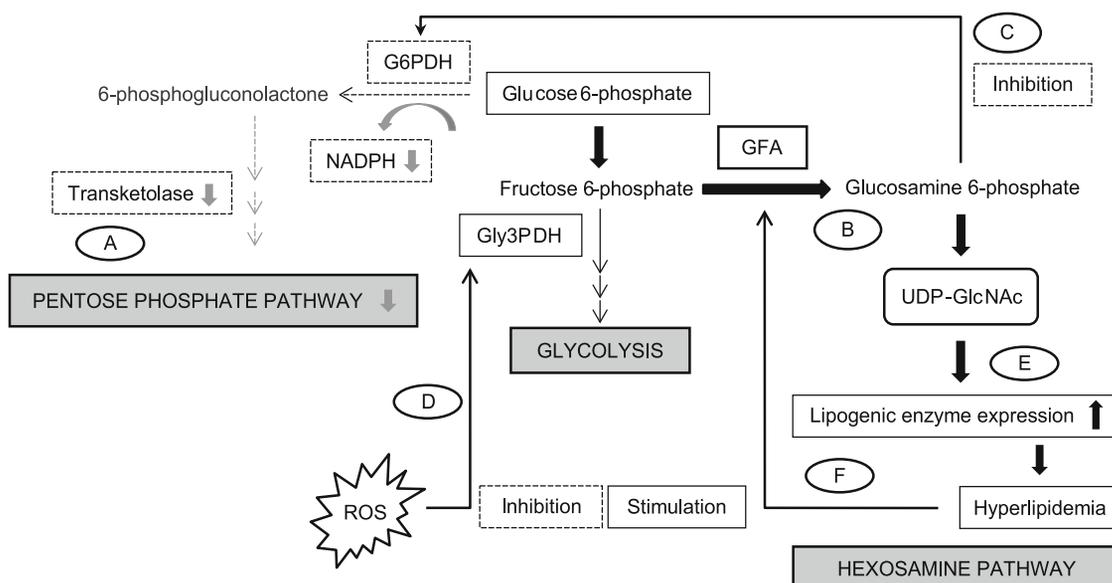


**Fig. 3. Metabolic changes in thiamine deficiency (adapted from 11). G6PDH: Glucose 6 phosphate dehydrogenase, GFA: Glutamine: fructose 6-phosphate amidotransferase, Gly3PDH: Glyceraldehyde 3 phosphate dehydrogenase, NADPH: Reduced nicotinamide adenine dinucleotide phosphate, UDP-GlcNAc: Uridine diphosphate N-acetylglucosamine, ROS: Reactive oxygen species.**

mine supplementation increases the activity of transketolase and activates the PPP, thereby diverts metabolic flux of glucose away from the hexosamine pathway (6).

Mitochondrion is the main source of superoxide radicals and once formed, superoxide will be readily dismutated by SOD into hydrogen peroxide ($H_2O_2$). GSH-Px is very important to prevent oxidative stress and is responsible for the degradation of $H_2O_2$ into $H_2O$ and $O_2$ avoiding the formation of the harmful hydroxyl radicals. NADPH, provided by the PPP, is crucial for the activity of GSH-Px and for maintenance of GSH (42). It was proposed that when SOD activity increases in response to increased free radical formation, GSH-Px activity can fall behind to degraded increased amounts of $H_2O_2$ since NADPH supply may be inadequate (42, 43). As shown in Figure 3, when thiamine levels are reduced, increase in glucosamine 6 phosphate inhibits glucose 6 phosphate dehydrogenase activity (41) which may be another contributing factor for the inadequate NADPH supply. The findings of various studies on the antioxidant activity in diabetes mellitus are contradictory. There are studies indicating SOD and GSH-Px activities as increased (19, 20, 31), decreased (44, 45) or unaltered (46). We found that D group exhibited higher SOD and unchanged GSH-Px activity compared to the C group. Increased SOD activity may be a protective mechanism against increased free radical production and unchanged GSH-Px activity might be related to the inadequate NADPH supply, as mentioned above. Since thiamine is a coenzyme of transketolase in the PPP, its deficiency may lead to a decrease in NADPH levels that eventually causes insufficiency of GSH-Px activity (45). It appears that increased free radical production and thiamine deficiency may result in insufficient GSH-Px activity. SOD and GSH-Px activities were significantly increased both in the CT and DT groups compared to those of C and D groups, respectively. These findings may suggest both indirect (via transketolase by providing NADPH) and direct antioxidative supportive effects of thiamine on these enzymes.

In the present study serum PON and AE activities were significantly reduced in the diabetic rats, which is consistent with our previous studies (18, 19, 29–31, 47, 48) and others (49, 50). Decreased serum PON and AE activities could be related to oxidative stress and hyperglycemia since the enzyme activity and/or synthesis might be inhibited by glycation or increased lipid peroxidation products (21, 51). It is suggested that AE activity reflects the mass of the enzyme and reduction in the enzyme protein synthesis could be the reason or a contributing factor for the decreased PON activity observed in the D group. Since PON is an HDL-associated enzyme, decreased HDL-C levels, found in the present study, may be another factor resulting in reduced serum PON activity. For this purpose, in the present study, HDL-C standardized enzyme activity (PON/HDL-C ratio) was investigated to assess whether the altered PON activity was associated with the reduction in HDL-C levels. Because PON/HDL-C ratio was significantly reduced in the D group compared with the C group, we can state that decreased serum PON activity is not related only to decreased HDL-C levels, but also to oxidative stress and hyperglycemia as well. Moreover, Abbott et al. (50) reported that diabetic HDL was compositionally abnormal, which might

affect binding of paraoxonase to HDL and lead to a conformational change in paraoxonase. Currently, in contrast to considerable literature describing the relation between PON activity and diabetes (19–21, 47–50), there are no reports investigating PON activity in diabetic rats treated with thiamine. In the present study, the reduced serum PON activity observed in diabetic rats was significantly increased with thiamine supplementation. Because PON inhibits lipid peroxidation in lipoproteins and tissues, this might be another contributing factor for the decreased tissue and plasma MDA levels, observed in thiamine receiving rats (CT and DT groups).

In the present study, we found that insulin and HDL-C levels increased and blood glucose, TC and TG levels decreased in the DT group compared with those of the D group. These findings suggest that thiamine acted as an insulin secretagogue and thiamine treatment exerts beneficial effects on the blood lipid levels in diabetic rats. Parallel to our results, Naveed et al. (10) and Babaei-Jadidi et al. (11) reported that thiamine therapy reduced TC and TG levels in experimental diabetes.

We observed that fluid and food intake was decreased in diabetic rats receiving thiamine. Decreased diuresis and fluid intake was suggested to be associated with reversal of diabetes-induced activation of protein kinase C by thiamine and consequent reversal of the inhibition of water re-uptake (52). It was suggested that thiamine metabolites, thiamine pyrophosphate and thiamine triphosphate, might affect dopamine signaling in the brain related to sensory-specific satiety (53).

## Conclusion

The authors suggest that thiamine significantly improves oxidative stress as evident by reduced levels of lipid peroxide end product MDA and by increased antioxidant enzyme activities. We observed that thiamine therapy has hyperinsulinemic and antihyperlipidemic effects. Moreover, taking the role of paraoxonase into consideration (preventing lipoproteins from being oxidized), thiamine treatment might particularly be important for inhibiting the progression of atherosclerotic vascular complications. So, we suggest that thiamine might be used as a supportive therapeutic agent in diabetes.

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