Protective Effects of Moderate Exercise With Dietary Vitamin C and E on Blood Antioxidative Defense Mechanism in Rats With Streptozotocin-Induced Diabetes

Mustafa Naziroğlu1,* and Peter J. Butterworth2

Abstract /Résumé

Daily moderate exercise and supplementation of vitamins C and E (VCE) can be beneficial in diabetes by ameliorating the effects of free radical production. The present study sought to analyze the effect of moderate exercise accompanying VCE supplementation on lipid peroxidation (LP) and antioxidative systems in the blood of streptozotocin-induced diabetic rats. Forty female Wistar rats were randomly divided 4 groups. The 1st and 2nd groups served as the control and diabetic groups, respectively. The 3rd group was the diabetic-exercise group. The 4th group, also diabetic-exercise rats, received VCE-supplemented feed. Animals in the exercised groups were moderately exercised on a treadmill 5 days a week for 3 weeks. Diabetes was induced on Day 0 of the exercise. Plasma and red blood cell (RBC) samples were taken from all animals on Day 20. Glutathione peroxidase, catalase, and reduced glutathione levels in plasma and RBCs, and vitamins A, E, and β-carotene in plasma were lower in diabetic rats than in control animals, whereas there was a significant increase in platelet counts in both plasma and RBC LP levels. The decreased antioxidant enzymes and vitamins, and the increased LP levels and WBC counts, did improve through
exercise only, although their levels were mostly increased by exercise + VCE supplementation. There were no significant changes in the hemoglobin and hematocrit values in the 4 groups. In conclusion, these data demonstrate an increase in LP in the blood of diabetic animals whereas there was a decrease in the antioxidant vitamins and enzymes. However, dietary VCE with moderate exercise may strengthen the antioxidant defense system by decreasing reactive oxygen species.

La pratique journalière d’activité physique modérée en combinaison avec des suppléments de vitamines C et E (VCE) semblent bénéfiques aux diabétiques à cause de l’amélioration des effets de la production des radicaux libres. Le but de cette étude est d’analyser l’effet d’un exercice modéré doublé d’un supplément de VCE sur la peroxydation lipidique (LP) et sur les systèmes antioxydants du sang de rats rendus diabétiques par la streptozotocine. Quarante rats Wistar sont répartis aléatoirement en 4 groupes. Le premier sert de groupe témoin et le deuxième de groupe diabétique; le troisième est un groupe diabétique soumis à l’exercice, et le quatrième est un groupe diabétique soumis à l’exercice et recevant en plus un supplément de VCE. Les 2 groupes d’animaux soumis à l’effort physique le sont modérément, sur tapis roulant et à raison de 5 jours par semaine durant 3 semaines. Les rats ciblés sont rendus diabétiques au jour zéro de la période d’entraînement. Des échantillons de sang (plasma et globules rouges, RBC) sont prélevés chez tous les animaux au jour 20. Le taux de glutathion peroxydase, de catalase, et de glutathion réduit dans le plasma et dans les globules rouges de même que la quantité de vitamines A, E, et β-carotène plasmatiques sont plus faibles chez les diabétiques que chez les normaux; il y a une augmentation significative de la numération plaquettaire et des taux plasmatiques et érythrocytaires de LP. La diminution des enzymes et des vitamines antioxydantes et l’augmentation des taux de LP et de globules blancs (WBC) se sont manifestées à l’effort seulement et leur niveau s’est surtout amélioré par l’exercice combiné au supplément VCE. Dans les 4 groupes, on n’observa aucune modification du taux d’hémoglobine et de l’hématocrite. En conclusion, cette étude révèle une augmentation de LP dans le sang des animaux diabétiques et une diminution des enzymes et des vitamines antioxydantes. Cependant, un supplément alimentaire de VCE combiné à l’exercice modéré peut améliorer le système de défense antioxydant en réduisant les espèces réactives de l’oxygène.

Introduction

Diabetes mellitus is characterized by a series of complications that affect blood and tissues (Atalay and Laaksonen, 2002; Naziroğlu and Cay, 2001). The disease is characterized by abnormal insulin secretion and derangement of carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia. In both insulin dependent (IDDM) and non-insulin-dependent diabetes there is increased oxidative stress (Naziroğlu et al., 2004a; 2004b; Upritchard et al., 2000). It is accepted that oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism’s antioxidant potential. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and with heavy oxidative stress (Atalay and Laaksonen, 2002; Upritchard et al., 2000; Naziroğlu and Cay, 2001; Naziroğlu et al., 2004a). Due to an increase in the formation of free radicals in diabetes, the balance normally present in cells between radical formation and protection against them is disturbed (Lee et al.,
This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids (Halliwell and Gutteridge, 1999). A variety of antioxidants scavenge reactive oxygen species (ROS) and prevent the occurrence of oxidative damage to biological structures (Cederberg et al., 2001). The primary defense against oxidative stress in the cell rests with antioxidants, including vitamins C and E (VCE), reduced glutathione (GSH), and glutathione peroxidase (GSH-Px) (Halliwell and Gutteridge, 1999). A number of studies have reported VCE deficiency in diabetic patients (Naziroğlu et al., 2004a; Upritchard et al., 2000) and experimental animals (Damasceno et al., 2002; Naziroğlu, 2003; Naziroğlu et al., 2004b), although contradictory results have been reported for blood levels during experimental diabetes (Meydani et al., 1993; Sun et al., 1999). The VCE treatment has been found to be beneficial in preventing or reducing some complications of IDDM such as infertility and fetal death (Naziroğlu, 2003; Naziroğlu et al., 2004b).

The most frequently studied antioxidant vitamins are C and E. These vitamins have been administered separately in studies in which they were given orally to diabetic rats, yielding a decrease in oxidative stress (Packer and Landvik, 1990; Young et al., 1992). Vitamin E is a lipophilic antioxidant that interferes with the chain reaction of lipid peroxidations (Packer and Landvik, 1990). Vitamin C is a hydrophilic molecule that can scavenge radicals, among them the hydroxyl radical (Czernichow and Hercberg, 2001). It is likely that vitamins C and E act in a synergistic manner, vitamin E primarily being oxidized to the tocopheroxyl radical and then being reduced back to tocopherol by vitamin C and glutathione (Czernichow and Hercberg, 2001; Packer and Landvik, 1990). In contrast to vitamin A, the VCE combination can also be safely used in high doses to help prevent diabetes and cardiovascular disease (Czernichow and Hercberg, 2001; Naziroğlu et al., 2004a).
In light of this we decided to use the lipophilic antioxidant vitamin E and the hydrophilic antioxidant vitamin C together as antioxidative protection in our rat model for STZ-induced radical production.

During exercise, free radicals may be produced in excess of the body’s natural defence (Radak et al., 1999). There are a few studies, in whole animals, demonstrating a two- to threefold increase in free radicals due to exercising the animal to exhaustion (Gul et al., 2002; Ji et al., 1988; Mastaloudis et al., 2001; Naziroğlu et al., 2004b). The biochemical mechanisms by which regular exercise has beneficial effects are not well understood. Reports of increased production during exercise and any protection afforded by dietary antioxidant supplements are also conflicting. Although exercise may cause lipid peroxide production in cells, it has been suggested that moderate exercise is beneficial in the management of diabetes mellitus (Atalay and Laaksonen, 2002; Naziroğlu et al., 2004b), presumably by improving the individual’s long-term metabolic control. However, whether moderate exercise confers lipid peroxide in diabetic rats is unknown and warrants further study.

If diabetes increases lipid peroxidation (LP), increased amounts of enzymatic and nonenzymatic antioxidants should be oxidized and their levels in the blood should be diminished. To test this hypothesis, we studied diabetic rats after regular moderate exercise in order to check whether exercise in diabetic rats decreases LP, and the rate of plasma and RBC enzymatic and nonenzymatic antioxidant disappearance. Our second aim was to test the moderate dose of VCE and its possible beneficial effect on the antioxidative defense system by evaluating LP and scavenging enzyme activity in diabetic and exercised animals.

Materials and Methods

CHEMICALS

All chemicals were obtained from Sigma Chemical (St. Louis, MO) and all organic solvents from Merck Chemical (Darmstadt, Germany). The dietary forms of vitamin C (ascorbic acid) and vitamin E (dl-α-tocopheryl acetate) were obtained from F. Hoffman La Roche (Istanbul, Turkey). STZ was obtained from SERVA GmbH (Heidelberg, Germany). All reagents were of analytical grade and all except the phosphate buffers were prepared each day and stored in a refrigerator at 4 °C. The reagents were equilibrated at room temperature for 0.5 hrs before use when the analysis was initiated or reagent containers were refilled. Phosphate buffers were stable at 4 °C for 1 month.

ANIMALS AND DIETS

The medical faculty experimentation ethics committee of our university approved the procedures of the study. Animals that died due to diabetes were removed from the study. We began with 40 Wistar rats bred in our laboratory and housed them at 22–24 °C, with light from 08.00 to 20.00 hrs and free access to water. At the start of the experiment the rats were 12 weeks old and weighed 145–160 g. They were randomly distributed into 4 groups and housed individually in stainless steel cages in a pathogen-free university laboratory animal research facility. All animals were fed a commercial diet (Elaziğ Feed Factory, Elaziğ, Turkey) during the experi-
ment, including the ingredients shown in Table 1. VCE-supplemented and unsupplemented feed compositions were homogenized by using a mixer, and pellets were prepared in our laboratory by heating below 45 °C for 2 days. The VCE-supplemented diet contained an ascorbic acid (1 g) and dl-α-tocopheryl acetate (600 mg) combination per kg feed.

Table 1  Diet Composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>25.1 %</td>
</tr>
<tr>
<td>Barley</td>
<td>20.0 %</td>
</tr>
<tr>
<td>Soybean</td>
<td>36.0 %</td>
</tr>
<tr>
<td>Wheat</td>
<td>9.7 %</td>
</tr>
<tr>
<td>Fish flour</td>
<td>3.2 %</td>
</tr>
<tr>
<td>Meat-bone flour</td>
<td>2.4 %</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>1.7 %</td>
</tr>
<tr>
<td>Salt</td>
<td>1.1 %</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Vitamin/mineral mix*</td>
<td>0.2 %</td>
</tr>
</tbody>
</table>

*In per kg mixture: vitamin A = 12,000,000 IU; vitamin C = 50 mg; vitamin D₃ = 400,000 IU; vitamin E = 30 mg; vitamin K₃ = 2.5 mg; vitamins B₁, B₂, B₆, and B₁₂ = 3, 7, 4, and 15 mg; nicotinamide 4 mg; calcium-D pantothenate 8 mg; folic acid 1 mg; biotin 45 mg; Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; I 0.4 mg; Co 0.1 mg; Se 0.15 mg; and antioxidant (butyhydroxytoluol) 10 mg.

INDUCTION OF DIABETES

After 2 weeks acclimatization, diabetes was induced in the female rats with STZ using a previously described method (Naziroğlu, 2003; Naziroğlu and Cay, 2001). STZ was intraperitoneally (i.p.) administered at a dose of 40 mg/kg body weight dissolved in citrate buffer (0.1 M, pH 4.5). Control rats received i.p. citrate buffer. Blood glucose was measured 14 days after the induction of diabetes. The diabetic state was confirmed when glucose concentration exceeded 20 mmol/L.

EXERCISE PROGRAM

To induce diabetes, we had the STZ-administered rats run on a treadmill at a speed of 16.1 m/min for 15 min duration for 14 days. This time period served as an adaptation to exercise. By Day 14 the speed was held constant at 16.1 m/min and the animals ran 45 min/day as described by Uriu-Hare et al. (1989). This level of exercise was established on the first day of the study and was maintained until the end of the 20 days. This exercise intensity provides mild to moderate aerobic stress.
for animals (Uriu-Hare et al., 1989). Animals not complying with the adaptation protocol were removed from the study, and other animals were added to the groups (10 per group). The nonexercising groups remained sedentary during this period but were exposed to the same environmental conditions as the exercise groups during the training sessions. All exercise sessions were conducted during each animal’s dark cycle so as to exclude the effects of sunlight on lipid peroxidation production. The sedentary groups had no access to food or water during the time period corresponding to the exercise bouts.

STUDY GROUPS AND SAMPLING

Of the four groups in this study, the first was used as the control group; the second was the diabetic (D) group; the third was the diabetic-exercise (DEx) group; the fourth group received VCE supplementation and served as the diabetic-exercise (DExCE) group. On Day 20 of the study the rats were anaesthetized with ether for 5 minutes, and 5-ml blood samples were obtained by cardiac puncture and placed into tubes containing sodium EDTA. Animals were rested and fasted for 12 hrs after the last bout of exercise before being sacrificed.

BLOOD COLLECTION AND PREPARATION OF BLOOD SAMPLES

One ml of noncoagulated blood was used for hematological analysis. The remaining blood sample was separated into plasma and RBCs by centrifugation at 1500 × g for 10 min at 4 °C. The RBC samples were washed three times in cold isotonic saline (0.9%, v/w), then hemolyzed with a nine-fold volume of phosphate buffer (50 mM, pH 7.4). After the addition of butylhydroxytoluol (4 µl per ml), the hemolyzed RBC and plasma samples were stored at –30 °C for <3 months pending measurement of enzymatic activity. The remaining hemolyzed RBC and plasma samples were used for immediate lipid peroxidation and vitamin assay. Blood hematological parameters were measured within 6 hrs following blood draw.

LIPID PEROXIDATION (LP) ASSAY

Lipid peroxidation (as malondialdehyde, MDA) levels in plasma and hemolyzed RBC were measured with the thiobarbituric acid reaction by the method of Placer et al. (1966) as described in previous studies (Naziroğlu, 2003; Naziroğlu et al., 2004b). The quantification of thiobarbituric acid reactive substances was determined by reference to a standard curve of malondialdehyde equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. The values of MDA were expressed as nmol/ml for plasma or nmol/ mg protein for RBC. Every sample was assayed in duplicate, and the assay coefficients of variation for MDA were less than 3%.

PROTEIN, GSH-PX, CATALASE, AND GSH ASSAY

We used the methods of Goth (1991, 1992) to determine catalase activities in hemolyzed RBC and plasma samples. The yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against a blank using a spectrophotometer (Shimadzu 2R/UV, Kyoto, Japan). The GSH content of the hemolyzed RBC and
plasma was measured at 412 nm using the method of Sedlak and Lindsay (1968) as described in our previous studies (Naziroğlu, 2003; Naziroğlu et al., 2004b). GSH-Px activity of plasma and hemolyzed RBC was spectrophotometrically measured at 37 °C and 412 nm according to Lawrence and Burk (1976). The protein content in the plasma and hemolyzed RBC was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

**PLASMA VITAMINS A AND E AND β-CAROTENE ANALYSES**

Vitamins A (retinol) and E (α-tocopherol) were determined in the plasma samples by a modification of the method described by Desai (1984). We saponified 200 microliters of plasma samples by adding 0.3 ml 60% (w/v in water) potassium hydroxide and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70 °C for 30 min. After the samples were cooled on ice, 2 ml of water and 1 ml of n-hexane were added and mixed with the samples, which were then rested for 10 min to allow phase separation. The n-hexane extract was dried under nitrogen at 40 °C and redissolved using 0.2 ml methanol. Twenty-microliter portions of the methanol extracts were chromatographed on high-performance liquid chromatography. Fluorimetric detection of vitamin A used excitation and emission wavelengths of 330 and 480 nm, respectively. The relevant wavelengths for α-tocopherol detection were 292 and 330 nm. Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in methanol.

The levels of β-carotene in plasma samples were determined according to the method of Suzuki and Katoh (1990). Two milliliters of hexane were mixed with 0.25 ml plasma and the absorbance of hexane was measured at 453 nm in the spectrophotometer.

White blood cells (WBC), RBC, and platelet counts, and hematocrit and hemoglobin values were determined using an automated blood counter (Beckman Coulter, Miami, FL).

**STATISTICAL ANALYSES**

All results are expressed as means ± SD. To determine the effect of treatment, we analyzed the data using one-way ANOVA repeated measures; *p* values of less than 0.05 were regarded as significant. Significant values were assessed with Tukey’s multiple range test. Data were analyzed using the SPSS statistical program (version 10.0 software, SPSS Inc., Chicago, IL).

**Results**

**CHANGES IN VITAMIN CONCENTRATIONS**

The changes in vitamin E and β-carotene in plasma are shown in Table 2. The results revealed that the plasma vitamin E and β-carotene concentrations in the D group were significantly (*p* < 0.01) lower than in the control group. Therefore we observed that STZ administration induced decreases in vitamin E and β-carotene in the plasma. Vitamin E and β-carotene concentrations were significantly increased in the exercised and VCE-supplemented groups. Vitamin E and β-carotene concentrations were found to be significantly higher (*p* < 0.01 and *p* < 0.05, respec-
Effects of Exercise in Rats With Diabetes

Vitamin E and β-carotene are increased by exercise and VCE supplementation. Diabetes, exercise, and VCE supplementation were also associated with a change in plasma vitamin A levels. Plasma vitamin A concentrations were significantly ($p < 0.01$) lower in the D group than in the control. Its concentration in plasma was increased by exercise and VCE supplementation. Vitamin A concentrations in DExCE were significantly ($p < 0.05$) higher than in DPEx groups.

### CHANGES OF ANTIOXIDATIVE ENZYMES AND LP LEVELS

Changes in LP and GSH levels, catalase, and GSH-Px activities in plasma and RBC are shown in Tables 2 and 3. Compared to the control group, plasma and RBC LP levels were significantly ($p < 0.01$) higher in the D group. On the other hand, 20 days of exercise and VCE supplementation were associated with a decrease in LP levels in RBC. The LP levels in the RBC samples were found to be significantly ($p < 0.05$) lower in the DExCE than in the DEx group.

Diabetes and exercise were associated with changes in catalase, GSH-Px, and GSH levels in plasma and RBC samples. Catalase, GSH-Px, and GSH levels

### Table 2 Effects of Moderate Exercise ($\pm SD$) With Dietary Vitamins C and E on Lipid Peroxidation, Antioxidant Vitamins, Reduced Glutathione, Glutathione Peroxidase, and Catalase Levels in Plasma of Diabetic Rats

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>Study Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>($n = 10$)</td>
</tr>
<tr>
<td>GSH-Px (IU/g protein)</td>
<td>5.27 ±0.72</td>
</tr>
<tr>
<td>GSH (µmol/ml)</td>
<td>0.61 ±0.02</td>
</tr>
<tr>
<td>Catalase (kU/g protein)</td>
<td>1.37 ±0.32</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol/ml)</td>
<td>0.85 ±0.17</td>
</tr>
<tr>
<td>Vitamin A (µmol/L)</td>
<td>7.09 ±0.66</td>
</tr>
<tr>
<td>β-carotene (µmol/L)</td>
<td>1.71 ±0.23</td>
</tr>
<tr>
<td>Vitamin E (µmol/L)</td>
<td>9.33 ±2.39</td>
</tr>
</tbody>
</table>

$^b p < 0.01$ and $^c p < 0.001$ vs. Control; $^d p < 0.05$, $^e p < 0.01$, and $^f p < 0.001$ vs. D group; $^g p < 0.05$, $^h p < 0.01$, and $^i p < 0.001$ vs. DEx.
in plasma and RBC were significantly ($p < 0.05$ to $p < 0.001$) lower in the D and DEx groups than in the control group. Catalase, GSH-Px, and GSH levels in plasma and RBC were positively affected by exercise and VCE supplementation. Catalase and GSH-Px activities, and GSH levels in plasma and RBC, were significantly ($p < 0.05$ to $p < 0.01$) higher in the DExCE than in the DPEx group.

### CHANGES IN HEMATOLOGICAL VALUES

The WBC, RBC, and platelet counts, and hematocrit and hemoglobin values in the four groups are shown in Table 4. The RBC value was significantly ($p < 0.05$) lower in the D group than in the control group, although the platelet value was increased in the D group ($p < 0.01$). However, the WBC count was higher in the D group than in the control group, although platelet and WBC counts in the DExCE group were significantly ($p < 0.05$ and $p < 0.001$) decreased. Hematocrit and hemoglobin values were not affected by exercise or VCE supplementation.

### Discussion

Diabetes mellitus is a major worldwide health problem that predisposes to markedly increased oxidative stress. The link between diabetes and oxidative stress in the blood has been extensively discussed for years, but definitive evidence confirming its importance is still lacking. Different strategies have been proposed to inhibit diabetes-induced oxidative stress. Although exercise may acutely induce oxidative stress, moderate regular training with dietary VCE appears to enhance

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**Table 3** Effects of Moderate Exercise ($\pm SD$) and Vitamin C and E Supplementation on Lipid Peroxidation, Antioxidant Vitamins, Reduced Glutathione, Glutathione Peroxidase, and Catalase Levels in RBC of Diabetic Rats

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>Controls ($n = 10$)</th>
<th>D ($n = 10$)</th>
<th>DEx ($n = 10$)</th>
<th>DExCE ($n = 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH-Px (IU/g protein)</td>
<td>32.91 ±6.86</td>
<td>16.19 ±2.36</td>
<td>18.47 ±2.80</td>
<td>26.98 ±5.30</td>
</tr>
<tr>
<td>GSH (µmol/g protein)</td>
<td>3.03 ±0.62</td>
<td>2.53 ±0.46</td>
<td>2.27 ±0.43</td>
<td>3.76 ±0.67</td>
</tr>
<tr>
<td>Catalase (kU/g protein)</td>
<td>15.56 ±3.15</td>
<td>6.26 ±0.94</td>
<td>10.32 ±1.68</td>
<td>13.77 ±1.52</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol/mg protein)</td>
<td>27.23 ±5.88</td>
<td>52.23 ±8.10</td>
<td>38.21 ±8.92</td>
<td>31.62 ±9.79</td>
</tr>
</tbody>
</table>

$^a p < 0.05$, $^b p < 0.01$, and $^c p < 0.001$ vs. Control; $^d p < 0.05$, $^e p < 0.01$, and $^f p < 0.001$ vs. D group; $^g p < 0.05$ vs. DEx.
antioxidant defences and decrease LP in normal nondiabetic rats (Radak et al., 1999). In this study we have shown for the first time that moderate exercise in conjunction with VCE up-regulates certain antioxidant enzymes and vitamins in the plasma and RBC of STZ-induced diabetic rat.

We used LP concentration as an index of oxidative stress status in diabetic rats. Our findings show that LP levels in the plasma and RBC in the D group were higher than in the control group (Tables 2 and 3). A significant increase of LP concentration in the plasma and tissues of diabetic animals was demonstrated. Our results are consistent with other reports of an increase of lipid hydroperoxides in plasma, liver, testis, and RBC of animals with experimental diabetes (Cederberg et al., 2001; Damasceno et al., 2002; Naziroğlu, 2003; Naziroğlu et al., 2004a; 2004b).

A possible explanation for the enhancement of LP concentration may be the decreased formation of antioxidants in STZ-induced tissues and blood cells, which in view of augmented activity of ROS allows a consequent increase in LP production. LP in plasma and RBC was normalized by the dose used in our treatment, thus indicating that a combination of VCE treatment was efficacious in containing LP levels of the samples. Similar to our LP results, numerous studies (Naziroğlu, 2003; Naziroğlu et al., 2004a; 2004b; Upritchard et al., 2000) have demonstrated that antioxidant vitamin supplementation can help lower the markers indicative of oxidant stress and lipid peroxidation in diabetic subjects and animals.

GSH-Px and catalase are the main enzymes of the enzymatic antioxidant defense system responsible for protection against an increase in ROS production.

Table 4  Effects of Moderate Exercise (±SD) With Dietary Vitamin C and E Combination on Hemoglobin, Hematocrit, Platelet, and Red and White Blood Cell Values in Blood of Diabetic Rats

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>Controls (n = 10)</th>
<th>D (n = 10)</th>
<th>DEx (n = 10)</th>
<th>DExCE (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>14.10 ± 1.64</td>
<td>12.20 ± 3.70</td>
<td>14.40 ± 1.71</td>
<td>14.00 ± 0.95</td>
</tr>
<tr>
<td>RBC (×10^6/µl)</td>
<td>6.96 ± 0.34</td>
<td>5.57 ± 0.33^a</td>
<td>6.43 ± 1.26</td>
<td>6.95 ± 0.93</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>38.00 ± 1.73</td>
<td>34.80 ± 5.35</td>
<td>36.80 ± 6.97</td>
<td>39.70 ± 4.21</td>
</tr>
<tr>
<td>WBC (×10^3/µl)</td>
<td>6.63 ± 1.37</td>
<td>7.11 ± 3.41</td>
<td>16.56 ± 1.23^d</td>
<td>13.50 ± 3.53^h</td>
</tr>
<tr>
<td>Platelet (×10^3/µl)</td>
<td>420 ± 112</td>
<td>669 ± 162^b</td>
<td>445 ± 126^i</td>
<td>264 ± 104^h</td>
</tr>
</tbody>
</table>

^a p < 0.05 and ^b p < 0.01 vs. Control; ^d p < 0.05 and ^i p < 0.001 vs. D group; ^f p < 0.01 vs. DEx.
Hydrogen peroxide, formed by the catalytic reaction of superoxide dismutase, is both a reactive form of oxygen and a normal cellular metabolite, and it is further detoxified by GSH-Px and catalase (Halliwell and Gutteridge, 1999). Catalase and GSH-Px activities were reduced in the D and DEx groups. The reduced activities of GSH-Px and catalase could be due to its depletion or inhibition as a result of the increased production of free radicals. Hypoinsulinemia increases the activity of the fatty acyl-CoA oxidase that initiates β-oxidation of fatty acids, resulting in the production of hydrogen peroxide, which detoxifies catalase and GSH-Px (Viana et al., 2003). However, VCE supplementation caused an increase in GSH-Px activity in the RBC and plasma of the DExCE group. The protein molecule of antioxidant enzymes may have been exposed to less ROS attack in the groups with higher VCE concentrations.

One of the most important intracellular antioxidant systems is the glutathione redox cycle. Glutathione is an essential compound for maintaining cell integrity because of its reducing properties and participation in cell metabolism (Menegola et al., 1996). The exact mechanisms of the diabetes-induced changes in blood GSH concentration are not completely clear. Glutathione may modulate metal reduction, and the thiol portion is very reactive with several chemical compounds, mainly with alkylating agents such as STZ (Menegola et al., 1996; Naziroglu and Cay, 2001). The GSH concentration in plasma and RBC was also lower in the D group than in the control group (Tables 2 and 3). These findings are similar to those of other researchers studying GSH in relation to risk factors in diabetic subjects and animals (Damasceno et al., 2002; Menegola et al., 1996; Naziroglu and Cay, 2001; Naziroglu et al., 2004b).

Vitamin C is the strongest physiological antioxidant acting in the organism’s aqueous environment (Czernichow and Hercberg, 2001). It has been shown to be an important antioxidant, to regenerate vitamin E through redox cycling, and to raise intracellular glutathione levels (Menegola et al., 1996). Thus vitamin C plays an important role in protein thiol group protection against oxidation. The GSH levels in plasma and RBC were significantly higher in the DExCE than in the DEx group. Given the data here, the observed increased concentration of GSH in the plasma and RBC of the DExCE group may play an essential role in the prevention of GSH in diabetes via vitamin C supplementation.

Vitamin E is an important antioxidant with lipophilic properties acting in cellular membranes (Packer and Landvik, 1990). The β-carotene also has antioxidant activity, particularly against singlet oxygen, and it can be metabolised to vitamin A (retinal) (Czernichow and Hercberg, 2001). There have been reports on the effect of exercise on vitamin E and β-carotene concentrations. While Meydani et al. (1993) and Mastaloudis et al. (2001) have reported increased vitamin E concentrations during or shortly after exercise, many studies on human and animal models have shown decreased serum and muscle vitamin E concentration in extreme endurance exercise (Bowles et al., 1991). In addition, Schroder et al. (2001) reported that supplementation with α-tocopherol, β-carotene, and ascorbic acid could partially account for the hormonal and enzymatic stress marker profile observed during habitual training activity of professional basketball players.

The decrease in muscle vitamin E concentration in animals during exercise has been attributed to the generation of free radicals and LP (Bowles et al., 1991).
In the present experimental model, significantly depleted plasma vitamin E concentrations via diabetes were increased in the DEx groups due to exercise. Our results are in accordance with Palmer et al. (1998), who noted that the increased vitamin A, E, and β-carotene demand of diabetic tissues of diabetic rats is supplied from plasma.

After the exercise protocol, significant elevations in RBC counts were observed and this was interpreted as the effect of hemoconcentration (Table 4). Some studies have shown that a short period of exercise caused WBC demarginalisation, leading to elevated WBC counts in peripheral blood (Akova et al., 2001). Also, in vitro and in vivo studies have suggested that ROS are implicated in such WBC and platelet aggregation (Akova et al., 2001; Palmer et al., 1998). In the current study, elevated WBC and platelet counts in the DEx group may be explained by this mechanism and the antioxidant effects of VCE. That scavenging ROS may prevent WBC and platelet demarginalisation in turn would seem to be responsible for unchanged WBC counts among the DExCE group.

In conclusion, we have shown that experimental diabetes increased lipid peroxidation levels in rat blood. We therefore suggest that oxidative stress is a cause of diabetes-induced pathophysiology. Simultaneously, we have studied the co-administration of VCE as an approach to ameliorating STZ-induced oxidative damage. These agents may protect against diabetes by inhibiting the inactivation of glutathione and the antioxidant system by STZ, and by up-regulating GSH-Px levels and vitamin E in the plasma and RBC. Moderate exercise training and VCE supplementation may help prevent the formation of diabetes-induced oxidative stress in diabetic animals.

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References


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