Effects of Vitamin E Supplementation on Oxidative Stress in Streptozotocin Induced Diabetic Rats: Investigation of Liver and Plasma

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This experimental study was designed to investigate the effects of vitamin E supplementation, especially on lipid peroxidation and antioxidant status elements¾ namely, glutathione (GSH), CuZn superoxide dismutase (CuZn SOD), and glutathione peroxidase (GSH Px), both in blood and liver tissues of streptozotocin (STZ) diabetic rats. The extent to which blood can be used to reflect the oxidative stress of the liver is also investigated. In diabetic rats, plasma lipid peroxide values were not significantly different, from control, whereas erythrocyte CuZn SOD ($p<0.01$), GSH Px ($p<0.001$) activities and plasma vitamin E levels ($p<0.001$), were significantly more elevated than controls. Vitamin E supplementation caused significant decreases of erythrocyte GSH level ($p<0.01$) in control rats and of erythrocyte GSH Px activity ($p<0.05$) in diabetic rats. Liver findings revealed significantly higher lipid peroxide ($p<0.001$) and vitamin E ($p<0.01$) levels and lower GSH ($p<0.001$), CuZn SOD ($p<0.001$) and GSH Px ($p<0.01$) levels in diabetic rats. A decreased hepatic lipid peroxide level ($p<0.01$) and increased vitamin E/lipid peroxide ratio ($p<0.001$) were observed in vitamin E supplemented, diabetic rats. A vitamin E supplementation level which did not cause any increase in the concentration of the vitamin in the liver or blood, was sufficient to lower lipid peroxidation in the liver. Vitamin E/lipid peroxide ratio is suggested as an appropriate index to evaluate the efficiency of vitamin E activity, independent of tissue lipid values. Further, the antioxidant components GSH, GSH Px and CuZn SOD and the relationships among them, were affected differently in the liver and blood by diabetes or vitamin E supplementation.

Key Words: Peroxidation, glutathione, glutathione peroxidase, superoxide dismutase, vitamin E, diabetes, liver

INTRODUCTION

Diabetes is widely known to induce metabolic derangement leading to oxidant-antioxidant imbalance.[1] Propagative lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress, often with cytopathological consequences.[2] Eukaryotic cells are equipped with a variety of primary and secondary defenses against the deleterious effects of oxidative stress. As lethal injury can occur when these defenses are overwhelmed, supplementation with antioxidant vitamins may prove beneficial.[2] Vitamin E is a hydrophobic antioxidant found in lipoproteins and membranes and can afford primary, as well as secondary stage protection. It is the most efficient scavenger of lipid peroxyl radicals.[3] So far, contradictory findings on plasma vitamin E levels in experimental or clinical diabetes mellitus, have been reported in the literature; some studies showing an increase; others showing a decrease.[4-7]

In this experimental study of STZ induced diabetes mellitus, the effects of vitamin E supplementation on lipid peroxidation and antioxidant defenses were investigated simultaneously in the liver and blood. It was also of interest to examine the extent to which blood can be used to provide an indication of the magnitude of alterations in the liver. The liver is an organ of central metabolic importance and is known to undergo oxygen free radicals (OFRs) mediated

injury in diabetes mellitus.

MATERIALS AND METHODS

Animal treatment

40 male Wistar rats weighing 150-200g were used in this study. The Guide for the Care and Use of Laboratory Animals was followed.[8] The rats received standard chow diet (20-30g/rat/day) and water ad libitum during the experimental period and were maintained at an environmental temperature of 22 ± 2°C with a12-h light/12 h dark cycle.

The rats were divided into two groups as control (n=20) and diabetic (n=20). Each group was further divided into 2 subgroups consisting of 10 rats each for Vit. E supplementation. Diabetes was induced, in overnight-fasted rats, by intraperitoneal injection of a single dose of STZ (60 mg/kg body weight) (Sigma-Aldrich, St. Quantin Fallavier, France) freshly dissolved in citrate buffer (pH 4.5). Control rats received an equivalent amount of the buffer. Only those animals with a blood glucose level >18 mM three days after injection were regarded as diabetic.

Vitamin E (DL-α-tocopherol acetate, Roche Inc., Basel, Switzerland) was administrated intraperitoneally at a dose of 500 mg/kg on days 1, 4, 7, 11, 14, 21, 24 and 27 to both the control and diabetic subgroups (n=10). Mortality was recorded daily. After 30 days blood glucose concentrations of diabetic rats was 13 mM and their body weight was about 130-170 gr. The rats were then anesthetized by intramuscular injection of Vetalar at 1 ml/kg (ketamine 100 mg/ml, Sigma chemical Co., St. Louise, MO, USA).

For erythrocyte lysate preparation, heparinized blood samples were obtained by heart puncture. After centrifugation at 2500 × g for 5 min, the plasma was removed and erythrocytes were washed 3 times in 5 ml saline, hemolyzed by diluting four-fold with water and stored at -80°C until biochemical analysis.

For homogenate preparation of subcellular fractions, liver tissues were promptly excised, weighed and chilled in ice-cold 0.15 M KCl (Sigma chemical Co., St. Louise, MO, USA). STZ-diabetic

rats had lower gross liver weights than those of normal controls (8.5 + 1.3 gm vs 11.8 + 2.7 gm). However, the liver weights of the STZ-diabetic rats were heavier than the controls when liver weights were normalized to body weights. Furthermore, the livers of the STZ-diabetic rats appeared swollen, yellowish in color and softer than those of the controls. For the determination of lipid peroxide and GSH; 10% (w/v) liver tissue homogenate was prepared in 0.15 M cold KCl (pH=7) and then centrifuged at 1000 × g for 15 min. Liver tissues were homogenized in 50 mM Tris-0,1 mM EDTA (pH=7.4) (Sigma chemical Co., St.Louise, USA) at 4°C for 30 sec (2 × 15 sec with 15 sec cooling intervals) with a polytron homogenizer. Cytosolic fractions prepared by centrifugation at 105 000 × g for 15 min were used for the measurement of GSH Px and CuZn SOD activities.

Lipid peroxide

Lipid peroxides were measured in plasma using the methodology of Buege & Aust.[9] One volume of plasma was mixed thoroughly with 2 volumes of a stock solution of 15% w/v trichloroacetic acid (Riedel-de Haen AG., Germany), 0.375% w/v thiobarbituric acid (Sigma chemical Co., St. Louise, MO, USA) and 0.25 N hydrochloric acid (Carlo Erba Reagent, Italy). The mixture was heated for 30 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 × g for 10 min. The light absorbance of the sample was determined at 535 nm.

Hepatic lipid peroxide levels were assayed according to the method of Ohkawa et al.[10] The reaction mixture contained 0.2 ml of sample, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS) (Sigma chemical Co., St. Louise, MO, USA), 1.5 ml of 20% acetic acid (pH: 3.5). (Merck AG., Darmstadt, Germany) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). The mixture was made up to 4 ml with distilled water and heated at 95°C for 60 min. After cooling, 1 ml distilled water and 5 ml of a mixture of n-butanol (Merck AG, Darmstadt, Germany) and pyridine (Sigma chemical Co., St. Louise, USA) (15:1, v:v) were added and the mixture was shaken vigorously. After centrifugation at 1500 × g for 10 min the

light absorbance of the organic layer was measured at 532 nm. 1,1,3,3-tetraethoxypropane (Sigma chemical Co., St. Louise, USA) was used as a standard.

Assay of GSH[11]

GSH content was determined by measuring the change in light absorbance (412 nm) occurring when GSH reduces 5,5'-dithiobis (2-nitrobenzoic acid) (Sigma chemical Co., St. Louise, USA).

Assay of CuZn SOD[12]

CuZn SOD activity was measured with an indirect inhibition assay, in which xanthine (Sigma chemical Co., St. Louise, USA) and xanthine oxidase (Sigma chemical Co., St. Louise, USA) serve as a superoxide generator and nitroblue tetrazolium (NBT) (Sigma chemical Co., St. Louise, USA) as a superoxide indicator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

Assay of GSH Px activity[13]

GSH Px activity was determined from the oxidation of NADPH to NADP+ in the presence of H_2O_2 used as a substrate and monitored spectrophotometrically at 340 nm for 3 min. GSH Px activity was expressed as (mol of NADPH/min /g protein using an extinction coefficient (6.22 mM-1 cm-1) for NADPH.

Assay of vitamin E[14]

Plasma vitamin E concentration was determined according to the method of Quaife et al. and Hashim et al.[15] The hydrogenation step, which obviates interference due to carotene, has been omitted since it was not found feasible to hydrogenate small samples without loss of tocopherol.

After the proteins in the plasma or serum were precipitated by an equal volume of absolute ethanol, the whole mixture was subjected to extraction by an equal volume of xylene. The α, α'- dipyridyl was added to an aliquot of the upper layer to estimate the principal interfering substance, (carotene at 460 nm. At this time ferric

chloride solution ($FeCl_3$) was added to the system to produce the color obtained by the Emmeric-Engel procedure which is measured at 520 nm.

Hb concentration was analyzed by the cyanmethemoglobin method.[16] Liver protein concentration was measured by the method of Lowry et al.[17]

Statistical analysis

The data was given as means ± SD. Analysis of variance (ANOVA) was used to assess the significance for each parameter among the experimental groups. The Tukey test was employed to determine the "p" values. The level of statistical significance was taken as $p < 0.05$.

RESULTS

The values and statistical comparisons of the analyzed parameters in the blood and liver tissues of the experimental groups are shown in Tables 1 and 2 respectively.

Lipid peroxidation, as reflected by plasma lipid peroxide values, was higher in diabetic rats compared to controls, however the difference was not a significant amount.Erythrocyte GSH Px ($p < 0.001$) and CuZn SOD ($p < 0.01$) activities and plasma vitamin E levels ($p < 0.001$) were significantly higher in diabetic rats.

Vitamin E supplementation caused significant decreases in GSH level ($p < 0.01$) in control rats and in GSH Px activity ($p < 0.05$) in supplemented diabetic rats vs. non supplemented diabetic rats.

Comparison of the vitamin E supplemented diabetic rats with the control group revealed significantly higher CuZn SOD ($p < 0.01$) and vitamin E ($p < 0.001$) levels and vitamin E/lipid peroxide ratio ($p < 0.01$) and a significantly lower GSH level ($p < 0.05$) in the former group.

The evaluation of liver tissue findings revealed significantly higher lipid peroxide levels ($p < 0.001$) in STZ induced diabetic rats. As to antioxidant defense parameters; liver tissue GSH level ($p < 0.001$) and CuZn SOD ($p < 0.001$) and GSH Px ($p < 0.01$) activities were significantly lower than the controls; whereas the vitamin E level was significantly higher ($p < 0.01$) than the controls.

Table 1. Values and Statistical Comparison of the Analyzed Parameters, in Blood Tissues, of the Experimental Groups (mean ± SD)

	Control group (n=10)	Control+Vitamin E group (n=10)	Diabetic group (n=10)	Diabetic+Vitamin E group (n=10)
Lipid peroxide (mM)	6.27 ± 1.49	6.09 ± 1.84	7.90 ± 1.77	6.62 ± 1.32
CuZn SOD (mg/L)	227.29 ± 37.56	248.16 ± 28.55	297.05 ± 41.59[a,†]	303.59 ± 42.31[a,†]
GSH Px (U/gHb)	23.92 ± 3.23	29.47 ± 4.76	46.14 ± 4.53[a,‡]	35.22 ± 7.04[b,*]
GSH (mg/g Hb)	5.92 ± 0.91	3.57 ± 1.29[a,†]	4.26 ± 1.71	3.61 ± 1.18[a,*]
Vitamin E (mM)	4.88 ± 0.94	7.12 ± 0.63	8.82 ± 1.00[a,†]	9.97 ± 0.60[a,‡]
Vitamin E/lipid peroxide	0.83 ± 0.33	1.28 ± 0.47	1.17 ± 0.39	1.54 ± 0.34[a,†]

[a] vs control.
[b] vs diabetic, $*p < 0.05$, $†p < 0.01$, $‡p < 0.001$.

Table 2. Values and Statistical Comparison of the Analyzed Parameters, in Liver Tissues, of the Experimental Groups (mean ± SD)

	Control group (n=10)	Control+Vitamin E group (n=10)	Diabetic group (n=10)	Diabetic+Vitamin E group (n=10)
Lipid peroxide (nmol/mgprotein)	0.92 ± 0.07	0.88 ± 0.06	1.52 ± 0.15[a,†]	0.95 ± 0.04[b,*]
CuZn SOD (U/mg protein)	175.46 ± 14.51	190.34 ± 21.76	96.90 ± 5.98[a,†]	140.77 ± 16.58
GSH Px (μmol/NADPH/min/g wet weight)	30.75 ± 7.76	28.69 ± 5.80	18.85 ± 6.49[a,*]	25.27 ± 5.58
GSH (mmol/g wet weight)	6.53 ± 0.97	5.43 ± 0.69	4.17 ± 0.54[a,†]	3.74 ± 0.64[a,†]
Vitamin E (nmol/g liver)	24.50 ± 15.58	28.47 ± 5.70	44.63 ± 5.87[a,*]	55.58 ± 3.39[a,†]
Vitamin E/Lipid peroxide	26.70 ± 6.05	32.35 ± 6.84	29.36 ± 3.16	58.81 ± 4.66[a,†,b,†]

[a] vs control.
[b] vs diabetic $*p < 0.01$, $†p < 0.001$.

Vitamin E supplementation to diabetic rats caused a significant decrease in hepatic lipid peroxide levels ($p < 0.01$) and a significant increase in vitamin E/lipid peroxide ratio ($p < 0.001$), whereas no significant change was observed in antioxidant parameters.

Correlation analysis of blood findings revealed a significant positive correlation between lipid peroxide and GSH Px (r=0,78, $p < 0.001$) in the control group and a significant negative correlation between vitamin E and GSH (r=-0,90, $p < 0.001$) in vitamin E supplemented diabetic rats.

As to liver tissue findings; in the control rats GSH was found to be negatively correlated to GSH Px and vitamin E (r=-0,83, r=-0,81, $p < 0.001$ respectively) and GSH Px positively correlated to vitamin E (r=0,91, $p < 0.001$). In the diabetic rats CuZn SOD was positively correlated to GSH and

GSH Px (r=0,65, $0.05 < p < 0.02$; r=0,75, $0.02 < p < 0.01$ respectively). In vitamin E supplemented diabetic rats a positive correlation was observed between lipid peroxide and GSH (r=0,64, $p=0.05$) levels.

DISCUSSION

This experimental study of STZ-induced diabetes mellitus reveals that lipid peroxidation, which is one of the deleterious effects of oxidative stress, increased substantially in the liver and that this increase was not reflected in the blood. Further, vitamin E supplementation lowered lipid peroxides in the livers of diabetic rats to levels nonsignificantly different from controls.

Hepatic lipid peroxides are reported to be

increased in STZ diabetic rats in most of the current literature[18-20] and unchanged in some.[22] Glucose auto-oxidation, protein glycation and the interaction of advanced glycation end-products with their specific receptors on macrophages are the main mechanisms of increased production of OFRs in diabetes.[6,21-24] The impaired regeneration of protective antioxidants such as glutathione, vitamins C and E, also contribute to oxidative stress. Although STZ is an agent which causes the generation of oxygen free radicals, the increase in oxidative stress over the 4-week period of our study cannot be due to the effects of STZ solely but also to diabetes because firstly, STZ acts selectively in pancreatic beta islet cells and secondly, it is readily metabolized with a half life of 6 hours.[25]

Depending on the extent of increase, the cells respond to lipid peroxidation by antioxidant induction, by death program induction (apoptosis) or by membrane lysis (necrosis).[2] In our study, none of the endogeneous antioxidant components studied appeared to be induced in the liver due to diabetes, instead they displayed significant decreases. Thus,supplementation of diabetic rats with vitamin E was of major importance in the prevention of apoptosis or necrosis in the liver. In this study we supplemented rats intraperitoneally, every 3 days, with 500 mg/kg body weight of DL-α tocopherol acetate. Previously a high dose of 400 mg/kg body weight of vitamin E acetate had been shown to be effective in inhibiting lipid peroxidation in diabetic rats.[26] Antioxidant treatment that was initiated after the establishment of diabetes had no effect on the subsequent course of diabetes as monitored by serum glucose. In response to supplementation we observed a significant decrease in hepatic lipid peroxides in diabetic rats. Vannuchi H et al.[27] has reported similar findings. Upon vitamin E supplementation, the activities of both of the antioxidant enzymes showed a clear tendency to increase and GSH; a tendency to decrease, although not significantly, in the livers of the diabetic rats in this study.

As stated above, lipid peroxides did not increase in plasma due to diabetes. As is known, lipid peroxides measured in plasma represents the amount of lipid peroxides released from the

tissues, to the circulation, with erythrocytes making the major contribution.[28] The induction of both of the antioxidant enzymes in the erythrocytes in diabetic rats might have been protective against lipid peroxidation The method we used for quantification of lipid peroxides measures various aldehydes derived from lipid hydroperoxides.[29] In addition to the fact that the detection of these aldehydes are used to assess the extent of the damage, these aldehydes,generated endogenously, have some oxidative stress associated, pathophysiological effects within cells and tissues.[29] Unlike reactive free radicals, aldehydes are rather long lived and can, therefore, diffuse from their site of origin and reach and attack distant targets; they circulate either freely in blood or incorporated into lipoproteins.[29]

As significantly higher values for vitamin E have been measured both in the liver and plasma of diabetic rats; in comparison to controls, and, as neither the liver nor the blood demostrated observable increases in response to vitamin E supplementation, the rational for the supplementation could be questioned. Since the majority of α-tocopherol in plasma is carried by lipoproteins, the increase in the plasma level of vitamin E, due to diabetes, is more likely due to hyperlipoproteinemia accompaning diabetes. The increase in the liver could be due to concentration of the vitamin in the increasingly synthesized lipids.[18,30] Unfortunately, lipid values are lacking in this study. In accordance with these findings, it was reported that feeding high dietary vitamin E had little effect on plasma vitamin E levels in diabetic rats.[31] However,seperate from this study, an increase in liver vitamin E content was observed.[31] In a previous study the vitamin E/lipid peroxide ratio of LDL (low density lipoprotein) was used to evaluate the susceptibility of LDL to oxidative modification in NIDDM (non- insulin dependent diabetes mellitus) patients.[32] This ratio might also be used to evaluate the efficiency of vitamin E. Similarly to vitamin E, as lipid peroxides are also lipid dependent compounds, the use of this ratio also serves for the correction for lipids. Cholesterol, on the other hand, besides being transported with vitamin E in lipoproteins, has been shown to affect the efficiency of vitamin E. It has been shown that vitamin E becomes less efficient as an

antioxidant when plasma cholesterol increases.[33] Thus,using this ratio would also serve to eliminate the differences in the antioxidant activity of vitamin E ascribed to the differences in plasma levels of cholesterol.Vitamin E/lipid peroxide ratio showed a substantial increase in livers of supplemented diabetic rats but not in control rats. However, no increase was noted due to diabetes. Thus, our data reevaluated by vitamin E/lipid peroxide ratio clearly reveals that vitamin E supplementation was beneficial in diabetic rats.

Our data shows that the alterations in lipid peroxidation and antioxidant status in livers of rats due to STZ-diabetes mellitus and/or vitamin E supplementation are not reflected in blood. Among the various tissues, the liver was observed to be supplied with the most potent antioxidant defenses such as GSHPx, SOD and GSH.[34] Due to diabetes, there was an observed decrease in antioxidant defenses in the liver, whereas there was an increase in the erythrocytes. A compensatory adaptive response to oxidative stress was observed in erythrocytes. Increased CuZnSOD and GSHPx activities in erythrocytes may be due to induction or mutually protective interactions. Especially; CuZn SOD, a constituent present at low levels only but highly inducible under oxidative stress.[35,36] On the other hand, the decreased activities of GSH Px and CuZn SOD in livers of diabetic rats, may be due to oxidative stress induced inactivation and/or exhaustion.[37] In diabetes, insulin deficiency promotes β oxidation of fatty acids, resulting in H_2O_2 formation. In the presence of decreased GSHPx activity H_2O_2 accumulates in the liver which in turn inactivates CuZn SOD. The glycation of CuZn SOD which occurs in the diabetic state also causes inactivation of CuZn SOD.Under these conditions, the increased concentration of causes the inactivation of GSH Px.[32,34,38,39]

The decreased hepatic GSH concentration in diabetic rats could be the result of decreased synthesis, increased consumption or increased loss through efflux. As the hexose monophosphate shunt is impaired in diabetes, NADPH availability is reduced and the ability to recycle GSSG to GSH is decreased.[40] Vitamin E supplementation did not appear to influence the GSH concentration in the liver of the experimental animals. However,

vitamin E supplementation caused a decrease in erythrocyte GSH concentration in control rats and a decrease in erythrocyte GSHPx activity in diabetic rats. GSH plays a common key role in cellular resistance to oxidative damage, as a free radical scavenger, as protein-bound glutathione and by regeneration of ascorbate or tocopherol in both erythrocytes and liver.[40-42] The liver is the major site of GSH synthesis for export to supply other tissues. The detoxification of a variety of xenobiotics in the liver also involves GSH. The liver, different from erytherocytes, has various metabolic functions and nearly all major biological processes involve the thiol redox state. Erythrocytes also possess an active machinery for GSH synthesis. We observed that the concentrations of vitamin E and GSH were negatively correlated (r=-0,81) in the livers of control rats.A similar correlation was observed in the blood of vitamin E supplemented diabetic rats. (r=-0,90), The coupled regeneration of α-tocopherol to oxidation of GSH and ascorbate may account for the negative correlation.[34] We observed a very strong correlation (r=0,91) between vitamin E concentration and GSH Px activity in the livers of control rats. An important feature of the antioxidant network is that its components act in synergy to destroy activated oxygen species. This correlation is the proof of the synergistic interaction between Vitamin E and GSH Px. Such synergistic interactions are reinforced by mutual protections of antioxidant enzymes.The positive correlation that we observed between CuZn SOD and GSH Px activity in the livers of diabetic rats (r=0,75), reflects this fact. The positive correlation of GSH concentration with CuZn SOD activity in livers of diabetic rats (r=0,65), implies the free radical scavenger role of GSH. On the other hand,the negative correlation of GSH concentration with GSH Px activity in the livers of control rats (r= -0,83), reflects the consumption of GSH by GSHPx. Interestingly, we observed that GSH and lipid peroxide concentrations were positively correlated in the blood of control rats (r=0,78); and livers of vitamin E supplemented diabetic rats (r=0,64).These latter findings suggest the potentation of the antioxidant defence by lipid peroxides. As evident from the foregoing, the complex nature of the alterations in oxidative stress related

parameters, cannot be readily explained by simple and uniform compensatory responses. Furthermore, depending on the particular time of analysis and the particular tissue examined, divergent results could be obtained.

To conclude, this experimental study of STZ induced diabetes mellitus reveals that the increase in lipid peroxidation observed in the liver, is efficiently lowered by vitamin E supplementation. The vitamin E/lipid peroxide ratio is suggested as an appropriate index to evaluate the efficiency of Vitamin E, independent of tissue lipid values.

Further to this, the antioxidant components GSH, GSH Px and CuZn SOD and the relationships among them were affected in different ways, in the liver and blood, by diabetes or Vitamin E supplementation.

REFERENCES

1. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405-12.
2. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res 1998;39:1529-42.
3. Chaudiere J, Ferrari-Iliou R. Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem Toxicol 1999;37:949-62.
4. Young IS, Torney JJ, Trimble ER. The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat. Free Radic Biol Med 1992;13:41-6.
5. Young IS, Tate S, Lightbody JH, McMaster D, Trimble ER. The effects of desferrioxamine and ascorbate on oxidative stress in the streptozotocin diabetic rat. Free Radic Biol Med 1995;18:833-40.
6. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin induced diabetes. J Clin Sci (Lond) 1998;94:623-32.
7. Thompson KH, Godin DV, Lee M. Tissue antioxidant status in streptozotocin-induced diabetes in rats. Effects of dietary manganese deficiency. Biol Trace Elem Res 1992;35:213-24.
8. Committee on Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. Washington, DC: Institute of Laboratory Animal Resources, National Research Council; 1985. p.83.
9. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
11. Beutler E, Durgn O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;51:882-8.
12. Sun, Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34: 497-500.
13. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxides. J Lab Clin Med 1967;70:158-69.
14. Quaife ML, Schrimshaw NS, Lowry OH. A micro method for assay of total tocopherols in blood serum. J Biol Chem 1949;180:1220-35.
15. Hashim SA, Schuttringer GR. Rapid determination of tocopherol in macro and microquantities of plasma. Result obtained in various nutrition and metabolic studies. Am J Clin Nutr 1966;19:137-45.
16. Fairbanks VF, Klee GG. Biochemical aspects of haematology. In: Tietz NM, editor. Textbook of Clinical Chemistry. Philadelphia: W.B. Saunders; 1986. p.1532-4.
17. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin reagent. J Biol Chem 1951;193:265-75.
18. Sun F, Iwaguchi K, Shudo R, Nagaki Y, Tanaka K, Ikeda K, et al. Change in tissue concentration of lipid hydroperoxides, vitamin C and vitamin E in rats with streptozotocin induced diabetes. Clin Sci (Lond) 1999; 96:185-90.
19. Bastar I, Seckin S, Uysal M, Aykac-Toker G. Effect of streptozotocin on glutathione and lipid peroxide levels in various tissues of rats. Res Commun Mol Pathol Pharmacol 1998;102:265-72.
20. Seckin S, Alptekin N, Kocak-Toker N, Uysal M. Lipid peroxide and glutathione levels in the liver and its subcellular fraction of alloxan diabetic rats. Horm Metab Res 1993;25:444-5.
21. Sukalski KA, Pinto KA, Berntson JL. Decreased susceptibility of liver mitochondria from diabetic rats to oxidative damage and associated increase in alpha-tocopherol. Free Radic Biol Med 1993;14:57-65.
22. Rungby J, Flyvbjerg A, Andersen HB, Nyborg K. Lipid peroxidation in early experimental diabetes in rats: effects of diabetes and insulin. Acta Endocrinol (Copenh) 1992;126:378-80.
23. Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, et al. Enhanced serum levels of thiobarbituric-acid-reactive substance in diabetes mellitus. Am J Med 1995;98:469-75.
24. Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull 1993;49:642-52.
25. Loven D, Schedl H, Wilson H, Daabees TT, Stegink LD, Diekus M, et al. Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozotocin induced diabetes. Diabetes 1986;35:503-7.
26. Morel DW, Chisolm GM. Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxi-

city. J Lipid Res 1989;30:1827-34.

27. Vannucchi H, Araujo WF, Bernardes MM, Jordao Junior AA. Effect of different vitamin E levels on lipid peroxidation in streptozotocin-induced diabetic rats. Int J Vitam Nutr Res 1999;69:250-4.

28. Draper HH, Polensek L, Hadley M, McGirr LG. Urinary malondialdehyde as an indicator of lipid peroxidation in the diet and in the tissues. Lipids 1984; 19:836-43.

29. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81-128.

30. Asayama K, Nakane T, Uchida N, Hayashibe H, Dobashi K, Nakazawa S. Serum antioxidant status in streptozotocin-induced diabetic rats. Horm Metab Res 1994;26:313-5.

31. Pritchard KA Jr, Patel ST, Karpen CW, Newman HA, Panganamala RV. Triglyceride-lowering effect of dietary vitamin E in streptozocin-induced diabetic rats. Increased lipoprotein lipase activity in livers of diabetic rats fed high dietary vitamin E. Diabetes 1986;35:278-81.

32. Yoshida H, Ishikawa T, Nakamura H. Vitamin E/lipid peroxide ratio and susceptibility of LDL to oxidative modification in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1997;17:1438-46.

33. Djahansouzi S, Braesen JH, Koenig K, Beisiegel U, Kontush A. The effect of pharmacological doses of different antioxidants on oxidation parameters and atherogenesis in hyperlipidaemic rabbits. Atherosclerosis 2001;154:387-98.

34. Wohaieb SA, Godin DV. Alterations in free radical

35. Shull S, Heintz NH, Periasamy M, Manohar M, Janssen YM, Marsh JP, et al. Differential regulation of antioxidant enzymes in response to oxidants. J Biol Chem 1991 25;266:24398-403.

36. Gonzalez E, Rosello-Catafau J, Jawerbaum A, Sinner D, Pustovrh C, Vela J, et al. Pancreatic nitric oxide and oxygen free radicals in the early stages of streptozotocin-induced diabetes mellitus in the rat. Braz J Med Biol Res 2000;33:1335-42.

37. Wohaieb SA, Godin DV. Starvation-related alterations in free radical tissue defense mechanisms in rats. Diabetes 1987;36:169-73.

38. Godin DV, Wohaieb SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 1988;84:223-31.

39. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Antioxidant defense system in diabetic kidney: a time course study. Life Sci 1997;60:667-79.

40. McLennan SV, Heffernan S, Wright L, Rae C, Fisher E, Yue DK, et al. Changes in hepatic glutathione metabolism in diabetes. Diabetes 1991;40:344-8.

41. Mak DH, Ip SP, Li PC, Poon MK, Ko KM. Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. Mol Cell Biochem 1996;20: 162, 153-8.

42. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J 1999;3: 1169-83.