# Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan-induced diabetic rats

**C.V. Anuradha and Ramasamy Selvam**

*Department of Medical Biochemistry, Dr. A.L. Mudaliar Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, India*

*Supplementation of thiol compounds has been suggested to protect against the toxic effects of reduced oxygen species by contributing to the thiol pool of the cell. The present study was designed to determine whether supplementation of methionine in the diet of diabetic animals protected against the oxidative stress in diabetic pathology. Oral methionine was administered at a dosage of 330 mg/100 g feed to diabetic rats. The effect was compared with the effect of insulin administration. Levels of lipid peroxides and antioxidants were measured in liver, kidney, and pancreas. A diabetic condition was associated with increased lipid peroxidation and depletion in antioxidants levels. Methionine did not lower the lipid peroxide content or the release of lipid peroxides from tissues in presence of promoters or restore the levels of α-tocopherol and ascorbic acid in tissues. Administration of insulin lowered the tissue lipid peroxide levels and restored the antioxidants levels.*

**Keywords:** methionine; diabetes mellitus; lipid peroxidation; antioxidants; insulin

## Introduction

The natural history of diabetes mellitus is characterized by a series of complications that affect many organs of the body and also play a detrimental role in the final outcome of the disease. A growing body of evidence is emerging that suggests that reactive oxygen-derived radicals play a crucial role in the pathogenesis of diabetes mellitus. For example, the activity of antioxidant enzymes in pancreas is low relative to the situation in other tissues, making it particularly susceptible to oxygen radical attack.[1] The role of oxygen radicals in myocardial ischemia and atherosclerosis have been firmly established.[2] Ischemia occurs to a more marked degree in diabetics than in nondiabetics. Besides these, other complications of diabetics such as retinal damage[3] and renal injury[4] occur through oxygen radical-related processes.

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems.[5,6] The levels of these defense mechanisms are altered in diabetes[7] and therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue injury.

Thiol compounds are well known for their free radical scavenging property.[8] Dietary supplementation of sulphur-containing aminoacids has been reported to improve the tissue antioxidant status of rats,[9] and methionine has been shown to provide a significant source of sulphur incorporated into intracellular glutathione.[10] Furthermore, the administration of supplementary methionine to rats treated with lanthanum chloride and neodymium chloride offers a protective effect against metal intoxication.[11]

Our earlier studies on the effect of dietary methionine on the lipid peroxidation reactions in diabetes mellitus showed reduction in the level of lipid peroxide content in blood.[12] In this study we administered methionine to diabetic rats and determined the role of feeding methionine on tissue lipid peroxidation and antioxidant levels. The effect was compared with that of untreated and insulin-treated diabetic rats.

## Materials and methods

Adult male albino rats of Wistar strain with an average body weight of 150–200 g were used for the study. Diabetes was

Address reprint requests to Dr. R. Selvam, Professor, Department of Medical Biochemistry, Dr. A.L. Mudaliar Post-Graduate Institute of Basic Medical Sciences, Taramani, Madras 600 113, India.
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induced by the intraperitoneal injection of alloxan monohydrate (100 mg/kg body weight) dissolved in physiological saline. Three days after alloxan injection, the diabetic rats were divided into three groups. One group was left untreated, the second group was treated with protamine zinc insulin, and the final group was treated with oral methionine for a period of 15 days. Accordingly, five groups of six animals each were maintained as follows: Group I: normal rats, injected with 0.5 mL of physiological saline. Group IIa: diabetic rats maintained on the commercial rat chow for 15 days. Group IIb: diabetic rats maintained on the commercial rat chow and injected with protamine zinc insulin (2 units/100 g body weight) daily, intraperitoneally for a period of 15 days from the third day of alloxan injection. Group IIc: diabetic rats maintained on the commercial rat chow supplemented with L-methionine (330 mg/100 g feed) for a period of 15 days. Group III: normal rats maintained on the commercial rat chow supplemented with L-methionine (300 mg/100 g feed) for a period of 15 days.

At the end of 15 days, the animals were fasted overnight then killed by cervical dislocation. The abdomen was cut open and the liver, kidney, and pancreas were dissected out and used for investigations.

The activity of lipid peroxidation was determined by measuring the content of thiobarbituric acid reactive substances (TBARS) in the tissue homogenates following the procedure of Hogberg et al.[13] The ascorbate-induced system in a total volume of 2.0 mL contained 0.2 mL of tissue homogenate, 50 μmol/L $FeSO_4$, 1 mmol/L $KH_2PO_4$, and 0.2 mmol/L ascorbic acid in 0.15 M Tris-HCl buffer, pH 7.4. For others, the assay contained 0.2 mL tissue homogenate, either 0.1 mmol/L t-butyl hydroperoxide, lauryl peroxide, or $H_2O_2$ in 0.15 M Tris-HCl buffer, pH 7.4 in a total volume of 2.0 mL. The release of thiobarbituric acid reactive substance (TBARS) was determined after incubation at 37° C for 20 min. Lipid hydroperoxides were assayed by the iodometric procedure described by Buege and Aust.[14] This method is based on the ability of the iodide ion to reduce hydroperoxides in an acid medium. Iodide ion is oxidized to elemental iodine, which is measured at 353 nm. Diene conjugates were estimated by the method of Klein.[15] Aliquots of lipid extracts were evaporated to dryness and suspended in methanol and the absorbance at 215 and 233 nm were measured against a solvent blank. Conjugated diene content was determined by computing the ratio of absorbance at 233 and 215 nm. The formation of a hydroxyl radical ion in tissue was assayed by measuring the generation of formaldehyde from dimethyl sulfoxide (DMSO) as described by Puntarulo and Cederbaum.[16] Briefly, to 1.0 mL of the tissue homogenate, 0.2 mL of phosphate buffer (0.05 M, pH 7.4), 0.1 mL each of $MgCl_2$ (0.1 M), $NADPH_2$ (0.004 M), sodium azide (0.01 M), and dimethyl sulfoxide (0.33 M) were added, and mixed well. The reaction mixture was incubated at 37° C for 10 min and then terminated by the addition of 0.4 mL of 20% TCA. The tubes were centrifuged. To the supernatants 5.0 mL of chromotropic acid (0.2% in 19 N $H_2SO_4$) was added, shaken well, and kept in a boiling water bath for 30 min. A blank containing 2.0 mL of water and a range of standard formaldehyde (2–10 μg) in 2.0 mL was treated similarly as test. The concentration of hydroxyl radical was expressed as μmoles of formaldehyde formed/min/mg protein. The enzymic antioxidants superoxidedismutase (SOD),[17] catalase,[18] and glutathione peroxidase[19] were assayed. The levels of nonenzymic antioxidants α-tocopherol[20] and ascorbic acid[21] were determined. Total and nonprotein thiol contents were determined following the method of Sedlack and Lindsay.[22] Protein content of tissue homogenates was measured by the method of Lowry et al.[23] using bovine serum albumin as standard. Student $t$ test was used to determine statistically significant differences between control and treated groups.

## Results

Several chemical tests have been used for the assessment of lipid peroxidation products such as fluorometry of lipofuscin-like substances; spectrometry of conjugated dienes; gas chromatography of ethane, pentane, and other alkanes; and colorimetric measurement of malondialdehyde (MDA) through TBARS. Measurement of TBARS is a widely accepted method for the measure of lipid peroxidation reaction in tissues.

Measurement of MDA-TBA adduct was not influenced in the presence of high concentration of glucose (10 mmol/L) in the reaction medium. However, in the presence of added plasma glucose (5–10 mmol/L) stimulated the formation of TBARS (data not shown) suggesting that the observed increased lipid peroxidation product was due to glucose-promoted reaction and not due to the direct glucose effect on the MDA-TBA measurement. Hence, we used this method for measuring the lipid peroxidation reaction in tissues where one could expect very low free glucose concentration in diabetes. Four different oxidative stresses were used to study whether there was any difference in the degree of stimulation among the experimental tissues.

The levels of lipid peroxides were determined in tissue homogenates in the presence and absence of initiators. *Table 1* gives the activity of lipid peroxidation in liver, kidney, and pancreas. The level of TBARS was significantly higher in diabetic animals than in control animals under basal condition and also in the presence of the ascorbate-induced system. In the presence of peroxides, the amount of TBARS was low when compared with that of the ascorbate system. Insulin treatment restored the peroxidation reaction promoted by ascorbate and the t-BH system in both liver and kidney, while it had no effect on $H_2O_2$-induced lipid peroxidation on both tissues. Furthermore, it is significant to note that methionine feeding was not effective in normalizing the diabetic liver and kidney.

Significantly high lipid peroxidation was observed in the diabetic pancreas compared with that of controls ($P < 0.001$). It is very interesting to note that neither insulin treatment nor methionine feeding could restore the lipid peroxidation reaction mediated in the presence of the ascorbate-induced system, lauryl peroxide, and hydrogen peroxide to normalcy. No significant difference was observed in ascorbic acid and t-butyl hydroperoxide-induced lipid peroxidation among the diabetic and treated conditions.

*Table 2* presents the concentration of hydroxyl radical, hydroperoxides, and diene conjugates in liver, kidney, and pancreas of control and diabetic animals. Two- and three-fold increases in hydroxyl ion concentrations were observed in the liver and pancreas respectively in diabetic animals ($P < 0.001$) when compared with controls. The kidney showed only a

**Table 1** Activity of lipid peroxidation in liver, kidney and pancreas of control and experimental rats in presence of promoters

| Stimulant | Control | Diabetic | Diabetic + insulin | Diabetic + methionine | Methionine |
|---|---|---|---|---|---|
| Liver | | | | | |
| Non-stimulated | 2.8 ± 0.21 | 3.6 ± 0.35$^{a***}$ | 2.5 ± 0.11$^{b***}$ | 3.7 ± 0.21$^{a***}$ | 2.7 ± 0.22 |
| Ascorbic acid-induced system | 15.6 ± 1.71 | 19.9 ± 1.91$^{a**}$ | 15.7 ± 1.59$^{b***}$ | 20.0 ± 1.52$^{a**}$ | 15.3 ± 1.01 |
| t-butyl hydroperoxide | 4.7 ± 0.68 | 5.0 ± 0.41 | 4.3 ± 0.66 | 5.1 ± 0.50 | 4.3 ± 0.45 |
| Lauryl peroxide | 3.5 ± 0.38 | 3.5 ± 0.22 | 3.3 ± 0.42 | 3.4 ± 0.63 | 3.5 ± 0.50 |
| Hydrogen peroxide | 3.1 ± 0.23 | 4.4 ± 0.03$^{a***}$ | 4.3 ± 0.02$^{a***}$ | 4.5 ± 0.03$^{a***}$ | 3.5 ± 0.03 |
| Kidney | | | | | |
| Non-stimulated | 1.1 ± 0.09 | 3.7 ± 0.37$^{a***}$ | 1.2 ± 0.18$^{b***}$ | 1.9 ± 0.16$^{a***}_{b***}$ | 1.8 ± 0.15 |
| Ascorbic acid-induced system | 12.2 ± 1.41 | 15.8 ± 1.10$^{a***}$ | 12.7 ± 1.20$^{b***}$ | 15.7 ± 1.53$^{a***}$ | 12.0 ± 1.25 |
| t-butyl hydroperoxide | 3.6 ± 0.68 | 4.9 ± 0.61$^{a***}$ | 3.0 ± 0.39$^{b***}$ | 4.2 ± 0.41$^{a***}$ | 3.2 ± 0.27 |
| Lauryl peroxide | 2.2 ± 0.31 | 3.2 ± 0.30$^{a***}$ | 2.6 ± 0.22$^{b***}$ | 3.2 ± 0.23$^{a***}$ | 2.6 ± 0.32 |
| Hydrogen peroxide | 2.6 ± 0.38 | 4.2 ± 0.53$^{a***}$ | 4.2 ± 0.24$^{a***}$ | 4.2 ± 0.35$^{a***}$ | 2.7 ± 0.24 |
| Pancreas | | | | | |
| Non-stimulated | 0.14 ± 0.011 | 0.25 ± 0.010$^{a***}$ | 0.11 ± 0.050$^{b***}$ | 0.20 ± 0.010$^{a***}$ | 0.12 ± 0.010 |
| Ascorbic acid-induced system | 0.32 ± 0.008 | 0.50 ± 0.008$^{a***}$ | 0.43 ± 0.010$^{a**}_{a**}$ | 0.43 ± 0.007$^{a**}$ | 0.36 ± 0.010 |
| t-butyl hydroperoxide | 0.11 ± 0.020 | 0.20 ± 0.011$^{a***}$ | 0.15 ± 0.020$^{b*}$ | 0.12 ± 0.020$^{b***}$ | 0.11 ± 0.010 |
| Lauryl peroxide | 0.11 ± 0.020 | 0.16 ± 0.012$^{a***}$ | 0.16 ± 0.010$^{a***}$ | 0.16 ± 0.080$^{a***}$ | 0.10 ± 0.010 |
| Hydrogen peroxide | 0.15 ± 0.040 | 0.17 ± 0.009$^{a***}$ | 0.17 ± 0.011$^{a***}$ | 0.17 ± 0.020$^{a***}$ | 0.15 ± 0.012 |

Values are expressed as n moles malondialdehyde released/mg protein/20 minutes. Values are given as mean ± S.D for six animals in each group.
[a]Significantly different compared with control.
[b]Significantly different compared with diabetic.
Values are statistically significant when $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

**Table 2** Contents of hydroperoxides, diene conjugates, and hydroxyl free radical ion in liver, kidney, and pancreas of control and experimental animals

| Particulars | Control | Diabetic | Diabetic + insulin | Diabetic + methionine | Methionine |
|---|---|---|---|---|---|
| Hydroxyl free radical | | | | | |
| Liver | 3.85 ± 0.34 | 7.34 ± 0.36$^{a***}$ | 3.14 ± 0.52$^{b***}$ | 5.76 ± 0.79$^{a**}_{b*}$ | 3.67 ± 0.19 |
| Kidney | 2.09 ± 0.12 | 2.65 ± 0.48$^{a**}$ | 2.62 ± 0.12$^{a***}$ | 2.67 ± 0.80$^{a**}$ | 2.06 ± 0.14 |
| Pancreas | 2.07 ± 0.24 | 6.35 ± 0.52$^{a***}$ | 2.89 ± 0.11$^{b***}$ | 7.26 ± 0.18$^{a***}$ | 2.20 ± 0.22 |
| Hydroperoxides | | | | | |
| Liver | 2.30 ± 0.05 | 4.56 ± 0.04$^{a***}$ | 2.28 ± 0.01$^{b***}$ | 4.52 ± 0.02$^{a***}$ | 2.24 ± 0.01 |
| Kidney | 1.37 ± 0.02 | 3.21 ± 0.02$^{a***}$ | 1.52 ± 0.009$^{b***}$ | 3.69 ± 0.001$^{a***}$ | 1.49 ± 0.011 |
| Diene conjugates | | | | | |
| Liver | 1.08 ± 0.01 | 1.45 ± 0.02$^{a***}$ | 1.01 ± 0.002$^{b***}$ | 1.46 ± 0.01$^{a***}$ | 1.09 ± 0.02 |
| Kidney | 1.12 ± 0.002 | 1.56 ± 0.001$^{a***}$ | 1.12 ± 0.0015$^{b***}$ | 1.54 ± 0.005$^{a***}$ | 1.13 ± 0.01 |

The values are expressed in terms of μg of t-BH/mg tissue for hydroperoxides, as the ratio of absorbances $A_{233}/A_{213}$ for diene conjugates, as n moles of formaldehyde formed/10 min/mg protein for hydroxyl free radical ion. Values are given as mean ± S.D. for six animals in each group.
[a]Significantly different compared to control.
[b]Significantly different compared to diabetic.
Values are statistically significant when $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

marginal increase in the hydroxyl radical ion concentration. Insulin treatment of diabetic animals significantly reversed these changes to near normal levels in both liver and pancreas, while methionine feeding was ineffective. The hydroxyl radical level was still high in this group of animals. However, both insulin and methionine treatment did not restore the level in the kidney.

The hydroperoxide concentration was increased 2.0–2.5 fold in liver and kidney of diabetic animals compared with control animals. This increased level was found to be restored to normal by insulin treatment and not by methionine. These results suggest that the free radical production was increased in diabetes due to insulin deficiency, and supplementation of methionine did not decrease the production of free radicals. Similarly, the increased diene conjugates observed in diabetic rat liver and kidney was restored by insulin treatment and not by methionine treatment.

*Table 3* presents the antioxidant enzyme activities in liver, kidney, and pancreas of control and experimental animals. Diabetic animals showed a significant reduction in SOD activity in all three tissues. Insulin treatment restored the activity in all tissues, and me-

**Table 3** Activities of superoxide dismutase, catalase, and glutathione peroxidase in liver and kidney of control and experimental animals

| Parameters | Control | Diabetic | Diabetic + insulin | Diabetic + methionine | Methionine |
|---|---|---|---|---|---|
| Liver | | | | | |
| Superoxide dismutase | 9.15 ± 0.84 | 5.16 ± 1.60[a**] | 9.13 ± 1.04[b***] | 9.09 ± 0.19[b***] | 9.18 ± 1.50 |
| Catalase | 66.6 ± 3.50 | 52.8 ± 7.80[a**] | 65.6 ± 12.05[b**] | 66.1 ± 10.50[b*] | 66.4 ± 4.20 |
| Glutathione peroxidase | 5.58 ± 1.28 | 3.25 ± 1.05[a**] | 5.47 ± 1.09[b*] | 5.25 ± 1.08[b*] | 5.56 ± 1.20 |
| Kidney | | | | | |
| Superoxide dismutase | 5.28 ± 1.50 | 3.72 ± 0.90[a*] | 5.23 ± 1.20[b*] | 5.39 ± 1.45[b**] | 5.16 ± 0.80 |
| Catalase | 50.1 ± 9.50 | 42.5 ± 5.80[a*] | 49.4 ± 5.20[b*] | 48.2 ± 6.20[b*] | 50.9 ± 5.90 |
| Glutathione peroxidase | 3.28 ± 0.91 | 5.13 ± 1.80[a*] | 3.61 ± 0.95 | 3.29 ± 0.11[b*] | 3.15 ± 1.20 |
| Pancreas | | | | | |
| Superoxide dismutase | 1.25 ± 0.13 | 0.36 ± 0.29[a***] | 1.15 ± 0.18[b***] | 0.48 ± 0.20[a***] | 1.28 ± 0.15 |
| Catalase | 33.3 ± 5.60 | 49.9 ± 7.20[a**] | 33.4 ± 7.20[b**] | 48.2 ± 9.80[a**] | 34.5 ± 8.50 |
| Glutathione peroxidase | 2.18 ± 0.25 | 4.28 ± 0.15[a***] | 2.02 ± 0.32[b***] | 4.13 ± 0.22[a***] | 2.15 ± 0.35 |

Values are expressed as units for superoxide dismutase, μmoles $H_2O_2$ utilized/min/mg protein for catalase and μg glutathione utilized/min/mg protein for glutathione peroxidase. Values are given as mean ± S.D. for six animals in each group.
[a]Significantly different compared to control.
[b]Significantly different compared to diabetic.
Values are statistically significant when $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

**Table 4** Levels of total and non-protein thiols, ascorbic acid, and α-tocopherol in liver and kidney of control and experimental animals

| Parameters | Control | Diabetic | Diabetic + insulin | Diabetic + methionine | Methionine |
|---|---|---|---|---|---|
| Liver | | | | | |
| Total thiols | 15.3 ± 1.20 | 13.1 ± 1.50[a*] | 15.1 ± 1.20[b*] | 15.4 ± 1.20[b*] | 15.1 ± 2.10 |
| Non-protein thiols | 3.28 ± 0.70 | 2.12 ± 0.80[a*] | 3.19 ± 0.80[b*] | 3.25 ± 0.90[b*] | 3.42 ± 0.12 |
| Ascorbic acid | 0.85 ± 0.20 | 0.61 ± 0.15[a*] | 0.79 ± 0.20[b*] | 0.60 ± 0.10[a*] | 0.89 ± 0.20 |
| α-tocopherol | 5.50 ± 0.92 | 3.20 ± 0.05[a***] | 5.05 ± 1.20[b***] | 3.22 ± 0.25[a**] | 5.61 ± 0.12 |
| Kidney | | | | | |
| Total thiols | 10.6 ± 2.20 | 8.22 ± 1.50[a*] | 10.3 ± 2.10[b*] | 10.5 ± 2.10[b*] | 10.6 ± 3.20 |
| Non-protein thiols | 2.56 ± 0.80 | 1.25 ± 0.01[a**] | 2.72 ± 0.80[b**] | 2.62 ± 0.70[b**] | 2.93 ± 0.72 |
| Ascorbic acid | 0.63 ± 0.03 | 0.42 ± 0.03[a***] | 0.65 ± 0.009[b**] | 0.39 ± 0.10[a***] | 0.61 ± 0.05 |
| α-tocopherol | 3.00 ± 0.92 | 1.22 ± 0.09[a***] | 3.19 ± 0.03[b***] | 1.55 ± 0.09[a**] | 3.24 ± 0.92 |

Values are expressed as μg/mg protein. Values are given as mean ± S.D for six animals in each group.
[a]Significantly different compared to control.
[b]Significantly different compared to diabetic.
Values are statistically significant when $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

thionine treatment restored the activity only in liver and kidney but not in pancreas.

In diabetes, catalase activity was found to be significantly decreased in liver ($P < 0.01$) and kidney ($P < 0.05$), while in pancreas the activity was significantly increased ($P < 0.01$). Insulin treatment normalized the activities, and methionine treatment restored the activity only in liver and kidney.

Glutathione peroxidase activity was significantly increased in kidney (50%) and pancreas (200%) and significantly decreased in liver (35%). Treatment with insulin or methionine normalized the activity in liver and kidney, but only insulin was effective in pancreas.

The nonenzymatic antioxidant status of the tissues of normal and diabetic animals is shown in *Table 4*. It is evident that diabetic animals showed a significant reduction of total thiol compounds, nonprotein thiols, and ascorbic acid in both the liver and kidney when compared with that of control animals. A significant normalization was noted in total and nonprotein thiols in insulin- and methionine-treated animals in both tissues when compared with diabetic animals. Ascorbic acid levels remained low in methionine-treated diabetic liver and kidney, while significant normalization ($P < 0.001$) was seen in insulin-treated diabetic animals. Statistically significant reduction in α-tocopherol was observed in liver and kidney of diabetic animals ($P < 0.01$). Normalization occurred with insulin treatment, while methionine treatment did not raise the levels when compared with untreated diabetic animals.

## Discussion

A marked increase in the concentration of TBA-reactive substances and elevation in the production of hydroxyl radicals, hydroperoxides, and diene conjugates are observed in the tissues of diabetic rats. The observed increased susceptibility of the tissues of diabetic animals to lipid peroxidation may be due to that effect. Tissue lipid peroxide levels are significantly

increased in the diabetic condition in the presence of stimulants. This suggests that diabetic tissues are less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate, organic peroxides such as t-butyl hydroperoxide, lauryl peroxides, and hydrogen peroxide. Increase in lipid peroxide concentration in the liver and kidney of animals dosed with alloxan has been observed.[24] Lipid peroxides may not only play an important role in the genesis and progress of arteriosclerosis, but also evoke adverse effects on the normal organs into which they are distributed.[25]

Insulin treatment is found to restore the changes observed, while methionine is found to be ineffective.

Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Alteration in antioxidant levels, both enzymic and nonenzymic, are observed in the present study. A significant decrease in SOD activity is observed in the tissues, which is brought back to a normal level by both insulin and methionine treatment. Reduction in SOD activity is observed in tissues of the liver, kidney, spleen, heart, testis, pancreas, skeletal muscle, and erythrocytes in rats with alloxan-induced diabetes of 2 months duration when compared with untreated control animals.[26] Similar results have been obtained in renal cortex and large and small bowel mucosa of rats with streptozotocin-induced diabetes of 9–10 days duration by Loven et al.[27] The activity of the other antioxidant enzyme catalase is found to be decreased in liver and kidney. Catalase has been shown to be responsible for the detoxification of significant amounts of hydrogen peroxide.[28] Reactive oxygen radicals can themselves reduce the activity of the enzymes.[29] Depression in glutathione peroxidase activity is observed only in the liver, while the kidney has restored a significant increase in activity. Glutathione peroxidase has been shown to be an important adaptive response to conditions of increased peroxidative stress.[26]

The total thiol content includes nonprotein thiols (predominantly GSH) and protein thiols. The levels of total and nonprotein thiols are also observed to decline during diabetes. Glutathione is known to protect the cellular system against the toxic effects of lipid peroxidation.[30] The total glutathione concentrations decline in livers of diabetic rats[31] and hepatic glutathione levels decrease by 30% in male rats with streptozotocin-induced diabetes.[32] Lowered levels of liver glutathione represent increased utilization due to oxidative stress. Insulin- and methionine-treated animals show significant reversal of glutathione content in the liver and kidney.

Reduced α-tocopherol levels in liver and kidney were observed in diabetic animals in our study. Insulin treatment tends to bring the α-tocopherol levels to near normal values. Higuchi[33] observed a decrease in hepatic vitamin E in rats with streptozotocin-induced diabetes. The result suggests that the demand for the antioxidant vitamin E is increased due to the disruption in free radical metabolism in diabetes. Asayama et al.[34] observed that in rats maintained on vitamin E-deficient diets, insulin secretory capacity is markedly reduced, and glucose intolerance develops in those animals with combined deficiency of vitamin E and selenium.

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