Lipoic acid restores antioxidant system in tissues of hyperinsulinaemic rats

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Background & objectives: Feeding rats with high fructose induces insulin resistance, hyperinsulinaemia, elevation of blood glucose level and impaired glucose tolerance. Oxidative stress plays a vital role in pathology associated with insulin resistance. The present study was to investigate the effects of α-lipoic acid (LA) on the oxidant-antioxidant balance in liver and kidney of high fructose-fed rats.

Methods: Male Wistar rats (170-180 g) were divided into six groups. The control group received diet containing starch; the fructose group was given a high fructose diet (>60% of total calories); the third and fourth groups were given fructose diet and administered with two different doses of lipoic acid as low dose (35mg/kg body weight) and high dose (70mg/kg bw) intraperitoneally using olive oil as vehicle; the fifth group received control diet and was administered with lipoic acid (70 mg/kg bw); the sixth group received the control diet and olive oil. The rats were maintained in their respective dietary regimen for 20 days. Lipid peroxidation indices and antioxidant status in liver and kidney were quantitated.

Results: The rats fed fructose showed increased levels of lipid hydroperoxides, thiobarbituric acid reactive substances (TBARS), conjugated dienes, and impaired antioxidant defence potential as evidenced by a decrease in the levels of non-enzymatic and enzymatic antioxidants. Treatment with LA to the fructose-fed rats mitigated these alterations and LA was effective uniformly at both the doses. Increased lipid peroxidation and inadequate antioxidant system are observed in the high dose fructose-fed rats.

Interpretation & conclusion: LA administration restored the antioxidant potential and lowered lipid peroxidation. These findings strengthen the utility of LA in the management of insulin resistance and associated pathology.

Key words Antioxidants - fructose - kidney - lipid peroxidation - liver - α-lipoic acid

Feeding rats with high fructose as the sole source of carbohydrate (>60% calories in diet) can produce insulin resistance, compensatory hyperinsulinaemia, hyperglycaemia, hypertriglyceridaemia and hypertension\(^1\). Fructose fed rats provide a useful rodent model of insulin resistance. High fructose diet affects the liver, skeletal muscle and adipose tissue of the animal\(^2,3\).

Currently there is a growing interest among researchers to study the relationship between oxidative stress and insulin resistance. It has been suggested that oxidative stress can impair insulin action\(^4\). Oxidative stress resulting from increased production of reactive oxygen species (ROS) plays a key role in the pathogenesis of late diabetic complications\(^5\). We have earlier demonstrated increased lipid peroxidation in aorta...
of high fructose-fed rats. In addition an increase in ROS, a decline in important cellular antioxidant defense mechanisms, including the glutathione redox system, vitamin C-vitamin E cycle and the LA (DHLA) redox pair significantly increase the susceptibility to oxidative stress. Attempts have been made to reduce the complications due to insulin resistance by supplementing the naturally occurring antioxidants especially vitamin E, vitamin C and LA. Supplementation of α-tocopherol to high fructose-fed rats improves antioxidant potential and insulin action.

α-lipoic acid (1,2-dithiolane-3-pentanoic acid) forms a part of several multienzyme complexes, such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and the glycine cleavage system. Mammalian tissues contain 5-25 nmol/g of lipoic acid. Four distinct antioxidant actions of LA have been observed including reactive oxygen species scavenging activity, capacity to regenerate endogenous antioxidants such as glutathione, vitamins C and E, metal chelating activity and repair of oxidized proteins. Beneficial effects of LA have been documented in metal ion toxicity and in carcinogenesis in experimental animals.

Though LA has shown to be beneficial in various pathogenic conditions, its role in lipid peroxidation and antioxidant levels in the insulin resistant state has not been elucidated clearly. The present study was undertaken to study the effects of LA on lipid peroxides and antioxidants in liver and kidney of rats fed with high fructose diet, which exhibit the characteristic features of insulin resistance.

**Material & Methods**

**Chemicals:** DL-α-lipoic acid was purchased from Sigma Chemical Company; St. Louis, Mo, USA. All other chemicals used obtained from S.D. Fine Laboratories, Mumbai and were of analytical grade.

**Animals:** Male adult Wistar rats of body weight 170-190 g were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. The animals were kept under controlled conditions on a 12 h light/12 h dark cycle. They all received a standard pellet diet (Karnataka State Agro Corporation Ltd, Agro Feeds Division, Bangalore, India) and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee.

After acclimatisation the animals were divided into six groups consisting of 6 rats each. Group 1 (Control): received control diet and water ad libitum; Group 2 (Fructose): received a fructose enriched diet containing 61 per cent fructose, 20 per cent casein, 0.7 per cent methionine, 5 per cent groundnut oil, 9.7 per cent wheat bran and 3.5 per cent salt mixture and water ad libitum. Vitamin mixture of 0.2 ml was added per kg feed. [Vitamin A concentrate I.P.; 2500 IU; vitamin D₃, cholecalciferol, 200 IU; thiamine hydrochloride, 0.5 mg; riboflavin, 0.5 mg; pyridoxine, 0.5 mg; sodium pantothenate, 1.5 mg; nicotinamide, 5 mg; ascorbic acid, 25 mg. (ABDEC multi vitamin drops, Pharmapak Pvt. Ltd., Mumbai)]. The diet was prepared fresh daily; Group 3 [FR+LA(LD)]: received the fructose diet and administered with lipoic acid (35 mg/kg b.w) dissolved in olive oil by intraperitoneal injection; Group 4 [FR+LA (HD)]: received the fructose diet and were administered lipoic acid (70 mg/kg b.w) dissolved in olive oil by intraperitoneal injection; Group 5 (Control+LA): received the control diet and were given lipoic acid (70 mg/kg b.w) in olive oil by intraperitoneal injection; and Group 6 (Control+Oliv): received the control diet and were given 0.2 ml of olive oil intraperitoneally.

The animals were maintained in their respective groups for 20 days. Body weight changes were measured weekly. At the end of the experimental period the rats were sacrificed by cervical decapitation and the liver and kidneys were removed immediately and stored in ice-cold saline. Homogenates were prepared from the tissues and were used to measure the lipid peroxidation and antioxidant status.

**Analytical procedures:** TBARS and lipid hydroperoxides levels were determined in tissue homogenates. Conjugated dienes were measured by the method of Rao and Recknagel. The absorbance of tissue lipid extracts dissolved in cyclohexane was determined at 233 nm. An extinction co-efficient of 2.52 x 10⁴/M was used to calculate the concentration of conjugated dienes. Tissue protein was measured by the method of Lowry et al. The activities of superoxide dismutase (SOD) catalase (CAT), glutathione...
peroxidase (GPx)$^{18}$, glutathione S-transferase (GST)$^{19}$, and glutathione reductase (GR)$^{20}$, and the levels of reduced glutathione$^{21}$, ascorbic acid$^{22}$ and $\alpha$-tocopherol$^{23}$ were measured.

**Statistical analysis:** Data of the groups were analysed using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test and $P<0.05$ was considered statistically significant.

**Results**

Body weight gain was similar for all groups and the final body weights were not significantly different from each other (174.0 ± 5.62g, 178.0 ± 5.43g, 185.0 ± 3.76g, 185.0 ± 3.16g, 185.0 ± 4.47g, and 172.5 ± 2.74g) for groups 1-6 respectively.

Fructose fed rats showed significantly higher ($P<0.05$) peroxidation compared to control rats. In LA (high dose) treated fructose fed rats, the levels of the peroxidation markers were significantly ($P<0.05$) lower as compared to untreated fructose fed rats and were near normal. However, in rats fed with fructose and treated with LA (low dose) lipid hydroperoxide levels in liver did not reach the level of control rats. In rats fed with control diet, administration of LA did not produce significant changes as compared to control groups (Table I).

The activities of SOD, CAT, GPX, GST and GR in liver and kidney were significantly ($P<0.05$) lower in fructose fed rats than normal control rats (Tables II, III). In fructose rats treated with LA (groups 3 and 4), activities of all these enzymes were significantly ($P<0.05$) higher as compared to fructose fed rats (group 2). The levels were near normal except for GR in kidney of group 3 rats (Table III).

The concentrations of non-enzymatic antioxidants GSH, vitamin C and vitamin E were found to be significantly ($P<0.05$) decreased in fructose fed rats (Table IV). Simultaneous treatment of fructose with LA brought the levels to near control values. However glutathione levels in liver was significantly ($P<0.05$) low in LA (low dose) treated fructose fed rats compared to LA (high dose) treated fructose fed, LA treated control and olive oil treated rats (Table IV).

No significant differences were observed in the effects of LA when administered at two different doses in fructose fed rats. Further, LA or olive oil when given to control rats did not produce significant alterations in the antioxidant parameters studied.

<table>
<thead>
<tr>
<th>Table I. Levels of lipid hydroperoxides, TBARS and conjugated dienes in liver and kidney of control and experimental animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Lipid hydroperoxides (µmol/mg protein)</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Kidney</td>
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<tr>
<td>TBARS (µmol/mg protein)</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Conjugated dienes (A 233)</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6). Group 1 - control, Group 2 - fructose-fed, Group 3 - fructose+lipoic acid (low dose), Group 4 - fructose+lipoic acid (high dose), Group 5 - control+lipoic acid, Group 6 - control+olive oil. TBARS, thiobarbituric acid reactive substances $^*$ $P<0.05$ compared to control (Group 1); $^† P<0.05$ compared to group 2
### Table II. Activities of enzymatic antioxidants in liver of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Units)</td>
<td>3.92±0.36</td>
<td>2.76±0.31*</td>
<td>3.81±0.82†</td>
<td>3.78±0.13†</td>
<td>3.86±0.34†</td>
<td>3.96±0.26†</td>
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<tr>
<td>CAT (µmol/min/mg protein)</td>
<td>53.24±3.22</td>
<td>35.88±0.74*</td>
<td>50.15±5.14†</td>
<td>52.82±1.24†</td>
<td>53.45±1.03†</td>
<td>52.74±0.55†</td>
</tr>
<tr>
<td>GPx (µmol/min/mg protein)</td>
<td>6.10±0.18</td>
<td>4.61±0.06*</td>
<td>5.82±0.09†</td>
<td>6.08±0.23†</td>
<td>6.05±0.16†</td>
<td>6.09±0.48†</td>
</tr>
<tr>
<td>GST (µmoles/min/mg protein)</td>
<td>5.79±0.11</td>
<td>4.67±0.27†</td>
<td>5.46±0.29†</td>
<td>5.55±0.25†</td>
<td>5.57±0.33†</td>
<td>5.62±0.24†</td>
</tr>
<tr>
<td>GR (µmoles/h/mg protein)</td>
<td>21.63±2.99</td>
<td>15.03±1.18*</td>
<td>19.87±1.98†</td>
<td>21.50±1.84†</td>
<td>22.47±1.82†</td>
<td>22.21±1.88†</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6)
Groups 1-6 as given in the foot note of Table I
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; GR, glutathione reductase
SOD unit-amount of enzyme, which gave 50% inhibition of NBT reduction/mg protein

* P<0.05 compared to control (Group 1)
† P<0.05 compared to Group 2

### Table III. Activities of enzymatic antioxidants in kidney of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Units)</td>
<td>4.02±0.48</td>
<td>3.17±0.06*</td>
<td>3.89±0.26†</td>
<td>4.04±0.11†</td>
<td>4.12±0.07†</td>
<td>4.15±0.05†</td>
</tr>
<tr>
<td>CAT (µmol/min/mg protein)</td>
<td>57.42±3.51</td>
<td>43.68±1.36*</td>
<td>54.10±2.65†</td>
<td>55.72±0.97†</td>
<td>56.30±1.17†</td>
<td>55.75±4.27†</td>
</tr>
<tr>
<td>GPx (µmol/min/mg protein)</td>
<td>5.29±0.59</td>
<td>4.15±0.48*</td>
<td>5.05±0.46†</td>
<td>5.2±0.29†</td>
<td>5.27±0.29†</td>
<td>5.3±0.25†</td>
</tr>
<tr>
<td>GST (µmoles/min/mg protein)</td>
<td>5.41±0.27</td>
<td>4.24±0.18*</td>
<td>5.15±0.13†</td>
<td>5.31±0.29†</td>
<td>5.37±0.30†</td>
<td>5.42±0.18†</td>
</tr>
<tr>
<td>GR (µmoles/h/mg protein)</td>
<td>23.91±2.32*</td>
<td>17.94±1.52*</td>
<td>20.02±1.45†</td>
<td>22.20±2.17††</td>
<td>24.17±0.60††</td>
<td>23.08±1.27††</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6)
Groups 1-6 as given in the foot note of Table I
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; GR, glutathione reductase
SOD unit-amount of enzyme, which gave 50% inhibition of NBT reduction/mg protein

* P<0.05 compared to control (Group 1)
† P<0.05 compared to Group 2
‡ P<0.05 compared to Group 3
Administration of LA to fructose fed rats resulted in a significant decrease in peroxidation indices namely lipid hydroperoxides, TBARS and conjugated dienes in liver and kidney. The activities of both enzymic and non-enzymic antioxidants were markedly elevated. LA is both lipid and water soluble and is highly effective in scavenging free radicals, lipid peroxides in cellular membranes and free radicals in the cytosol. LA can function as unique and effective antioxidant recycling vitamins C and E and elevating glutathione levels. LA possibly reacts with free radicals that are prooxidants for ascorbic acid and tocopherol. The redox potential $E^\circ$ of LA is $-290$ mV while that of vitamin E is $+370$ mV. Lipoic acid could maintain the intracellular redox balance, thereby offering a protective effect against free radical attack.

Our results show that fructose feeding to the rats results in development of oxidative stress in both liver and kidney. This oxidative stress may play a role in pathology associated with fructose feeding such as insulin resistance. In uncontrolled diabetes, oxidative stress results from increased free radical production.
and depletion of antioxidants like SOD, catalase, vitamin E and α-lipoic acid. Being a potent quencher of reactive oxygen species, LA inhibited the free radical-mediated lipid peroxidation, preserved the antioxidant enzymes and maintained the non-enzymic antioxidant concentrations. This ameliorative effect of LA on tissue lipid peroxidation might also be attributed to its ability to increase glucose disposal thereby abolishing the consequences of hyperglycaemia. The findings of the present study corroborates the utility of LA as a therapeutic tool in the management of diabetic complications in which induction of oxidative stress is the major contributing mechanism.

References


