Biochemical evaluation of lipid and oxidative stress status in relation to high fat-high antioxidant diets

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Free radicals are produced through biological processes and environmental interactions. They are metabolised by the enzymatic and non-enzymatic antioxidants present in the tissues. In this study, a 90 days long feeding of high fat diet to rats, resulted in significantly elevating the lipid and oxidative stress levels of the rat liver and blood as became evident from the changes in the levels of lipids, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and three hepatic antioxidant enzymes; glutathione peroxidase (EC 1.11.1.9), catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1). However, a concurrent feeding of high antioxidant combination, as high fat high antioxidant diets, reduced the lipid levels and diminished the oxidative stress. The results suggest that apart from reducing lipid levels, dietary antioxidants also support endogenous antioxidants in their oxidative stress reducing endeavours.

Free radicals are known to be generated through biological processes and environmental interactions\(^1\). Their detrimental effects are minimised by the enzymatic and non-enzymatic antioxidants present in body tissues\(^2\). Oxidative stress, caused by free radicals, is suggested to be involved in the initiation and progression of several chronic and degenerative diseases like coronary heart disease and cancer as well as in the process of ageing\(^3\). There is growing interest in the role of dietary constituents, including fat as risk factors in the aetiology of chronic diseases\(^4\). Since dietary antioxidants have been shown to possess protective properties against chronic diseases\(^5\),\(^6\), the present studies were carried out to probe the interrelationship among lipid nutrition, oxidative stress and dietary antioxidant factors.

Thirty two male Wistar albino rats (75-85g) were randomly divided into four equal groups, consisting of 8 animals each. The groups A,B,C and D received the diets, A (isoenergetic normal fat), B (high fat), C (Height fat high all antioxidants) and D (High fat high lipophilic antioxidants) respectively. Diets C and D were prepared by mixing additional amounts of three antioxidants (β-carotene, L-ascorbate and α tocopherol acetate) and two lipophilic antioxidants (β-carotene and α-tocopherol acetate) respectively to the diet B. Composition of each diet has been given in Table 1. The animals were housed individually in polypropylene cages in a room with a 12hr light dark cycle, 20°C-25°C temperature, 40-50% humidity level, and had free access to food and water. Diet consumed by each animal and animal weights were recorded daily.

Rearing of animals in the pre-experimental period and their upkeep during the entire experimental span conformed to ethical guidelines laid down by Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh. At the end of 90 days experimental period, food was withheld from the rats the previous night and the following morning blood sample collected from orbital plexus of each animal using mild ether anaesthesia. Thereafter, the animal was sacrificed by cervical dislocation. The liver was quickly removed, washed with ice cold saline, freed of adhering connective tissue, weighed and a small part used for enzyme activity assay or other instant biochemical investigations. The rest of the hepatic tissue was stored at -25°C for further biochemical analysis.

Enzyme assay and other biochemical analysis carried out included serum triglycerides\(^12\), phospholipids\(^13\), serum and liver cholesterol\(^14,15\), total lipids\(^16\), lipid peroxidation\(^17\) red cell and liver reduced glutathione\(^18\) and liver antioxidant enzymes; glutathione peroxidase (EC 1.11.1.9)\(^19\), catalase (EC 1.11.1.6)\(^20\) and superoxide dismutase (EC 1.15.1.1)\(^21\). Statistical analysis was carried out by using Student’s ‘t’ test. Values with P<0.01 and beyond were considered statistically significant.

High fat diet raised lipid levels significantly except for serum HDL-cholesterol. However, the effect was
found to be reduced significantly in animal groups fed high fat high all or lipophilic (C or D) antioxidant diets, with the former registering a greater reduction than the latter as becomes evident from Table 2.

Table 3 depicts the effect of antioxidant nutrition on the high dietary fat induced oxidative stress in rat blood and liver tissues. Whereas high fat diet had significantly increased the levels of TBARS in both serum and liver, it caused a decrease in red cell and liver GSH, and hepatic antioxidant enzyme activity levels. Again, high fat high antioxidant diets tended to reverse these trends in significant terms in almost all of these cases.

Increased levels of lipids due to high dietary fat (Table 2) seemed to be related to their more effective utilization in tissue lipid synthesis than that of carbohydrates, for all the alteration in the dietary fat component of high fat diet was made at the expense of carbohydrates (Table 1). It is speculated that the reduction in the lipid raising effect of fat by high dietary antioxidant combination seemed to be due to their capacity to increase the thermic effect of fat, thereby weaning them away from the process of tissue lipid synthesis.

Further, higher oxidative stress status of the high fat fed group pointed towards a possibly higher free radical activity generation resulting in peroxidative damage, lowered GSH level and activities of the antioxidant enzymes than those of normal fat fed groups (Table 3). However, feeding either high all-antioxidant or lipophilic antioxidant combination, along with high fat diet to the two groups (C or D) effectively diminished the oxidative stress build up recovering all the indices close to those of normal fat fed control group. The restoration trends noticed in antioxidant enzyme activity levels on feeding high antioxidant appear to be due to the sparing action of dietary or non-enzymatic antioxidants bestowed on

Table 1 — Composition of normal, high fat and high fat high antioxidants diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Isoenergetic normal fat</th>
<th>High fat</th>
<th>High fat high all antioxidants</th>
<th>High fat high lipophilic antioxidants</th>
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<tbody>
<tr>
<td>Casein</td>
<td>220.0</td>
<td>220.0</td>
<td>220.0</td>
<td>220.0</td>
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<tr>
<td>DL-Methionine</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>Mineral Mix</td>
<td>45.0</td>
<td>45.0</td>
<td>45.0</td>
<td>45.0</td>
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<tr>
<td>Vitamin Mix</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20.0</td>
<td>145.0</td>
<td>145.0</td>
<td>145.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>75.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>378.0</td>
<td>378.0</td>
<td>378.0</td>
<td>378.0</td>
</tr>
<tr>
<td>Oil</td>
<td>50.0</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Mineral mixture provided the following g/kg of all diets: CaHPO4 2H2O 2H2O, 1.17; K2HPO4, 10.8; CeCO3, 8.1; NaCl 3.1; Mg 0.98; MnSO4.7H2O 4.0; FeSO4.7H2O 0.39; ZnSO4.7H2O 0.22; MnSO4.7H2O 0.046; NaF 0.046; Al2(SO4)3 0.009; KI 0.003; K2S2O8 0.0036; FeSO4.7H2O 0.0004.

2 Vitamin mixture provided the following (per kg of diet): oxotocopherol, 50 mg; L-ascorbic acid 0.05 g; choline chloride, 0.75g; D-calcium pantothenate, 30mg; inositol, 50 mg; niacinamide, 22 mg; thiamine, 45 mg; p-aminobenzoic acid, 50 mg; pyridoxine, HCl, 10 mg; riboflavin, 10 mg; tocopherol acetate, 9 mg; ergocalciferol, 0.0025 mg; biotin, 0.2 mg; folic acid, 0.9 mg; vitamin B12, 0.0135 mg.

3 All the dietary formulations contained blends of corn, groundnut, rapeseed and sesame oils in equal proportion by weight.

4 Antioxidants: a-tocopherol acetate, 1.1 g/kg; B-carotene equivalent to retinol, 0.18 g/kg and L-ascorbic acid, 10 g/kg of diet.

Table 2 — Effect of high fat fed rat lipid status [Value are means(SE of 8 rats in each group]

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver total cholesterol (mg/100g of tissue)</th>
<th>Serum total cholesterol (mg/100ml)</th>
<th>Serum HDL-cholesterol (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>360.2±5.34</td>
<td>77.2±1.96</td>
<td>34.3±1.12</td>
</tr>
<tr>
<td>II</td>
<td>443±5.39</td>
<td>95.2±2.94</td>
<td>33.5±0.99</td>
</tr>
<tr>
<td>III</td>
<td>373±5.20</td>
<td>79.8±6.07</td>
<td>31.5±1.03</td>
</tr>
<tr>
<td>IV</td>
<td>397±4.40</td>
<td>83.0±5.34</td>
<td>28.2±1.27</td>
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</table>
their enzymatic counterparts. The results are in agreement with those of Khan et al., where diet encompassing natural antioxidants have been reported to reclaim the rat liver antioxidant enzyme activity levels that have been rendered lower by high fat feeding. In present findings also pointed towards the L-ascorbate containing high all antioxidant combination to be more effective as compared to high lipopholic combination, implying the potential positive role of L-ascorbate in antioxidant nutrition.

In conclusion, the study seemed to reveal that high fat and high antioxidant nutrition play the aggravative and alleviative roles respectively in the context of lipid and oxidative stress status as high fat feeding for 90 days resulted in raising both lipid and oxidative stress status in blood and hepatic tissues. However, these effects were considerably lowered by high all antioxidants and lipopholic antioxidant diets each when fed in combination with high fat diet.

Reference


