Dietary Modulation of Oxidative Stress in Alcoholic Liver Disease in Rats

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ABSTRACT Numerous studies dealing with prolonged feeding of rats with ethanol liquid regimens high in fat and low in carbohydrate showed that the resulting hepatic pathologic changes, including increased lipid peroxidation, are due to dietary aberrations rather than to ethanol toxicity. The amount and particularly the type of dietary fat significantly modulate the hepatic oxidative stress and morphofunctional reactivities. Although dietary vitamin E modulated oxidative stress or lipid peroxidation, it did not influence the development of hepatic pathologic changes in different animal models of chronic alcoholism. The old observation that lipotropes modulate the hepatic alterations associated with prolonged excessive ingestion of ethanol has been amply confirmed by even those who for years did not accept the importance of lipotropes. Our recent studies in rats indicated that prolonged feeding of large amounts of ethanol and diets with variable amounts of lipotropes, vitamin E and minerals did not significantly modulate a large series of hepatic prooxidants, but decreased several antioxidants (vitamin E, ubiquinols and glutathione peroxidase). Ethanol regimens relatively low in vitamin E increased the hepatic thiobarbituric acid–reactive substances and chemiluminescence and reduced some of the antioxidant factors. However, the hepatic prooxidant factors were unaffected, and no liver damage was detected. These and other findings indicated that the eventual detection of oxidative stress in experimental alcoholic liver disease primarily depends on the type of diet and that oxidative stress may play a significant pathogenic role in this condition. J. Nutr. 127: 912S–915S, 1997.

KEY WORDS: • ethanol • free radicals • lipid peroxidation • pro- and antioxidants • rats

The notion that hepatic oxidative stress or the consequent lipid peroxidation may play a pathogenic role in alcoholic liver disease (ALD) originated when Di Luzio (1964) reported that the acute ethanol-induced increase in hepatic triglycerides in rats was prevented by the administration of antioxidants. The detection of lipid peroxidation in hepatic homogenates and mitochondria seemed to support this interpretation (Di Luzio and Hartman 1969). While these findings were confirmed by some investigators, but not by others, data from the acute ethanol model seemed irrelevant in chronic alcoholism. On the other hand, the fatty liver that developed in rats fed for 3 wk a 35% ethanol liquid diet relatively low in fat (13%) was partially prevented by diphenyl-p-phenylenediamine, but not when the ethanol diet was high in fat (23%) (Di Luzio and Hartman 1969).

MODULATING EFFECTS OF DIETARY VITAMIN E AND THE TYPE AND AMOUNT OF FAT

After the introduction of the totally liquid ethanol regimen by Lieber et al. (1963), we repeated their experiment, but instead of feeding the rats for only 3 wk, as they did, we fed our rats for 3 mo (Porta et al. 1965). Because their original diet had high levels of unsaturated fat and did not contain vitamin E, we supplemented one of the two ethanol regimens with 16 mg a-tocopherol acetate/100 mL. Although vitamin E significantly reduced the thiobarbituric acid–reactive substances (TBARS) in hepatic homogenates of ethanol-treated rats, it did not significantly reduce the triglyceride level in relation to the unsupplemented ethanol group. On the other hand, because the level of TBARS in the unsupplemented ethanol group was significantly higher than in the non-ethanol control group, it was evident that under these conditions chronic ethanol consumption increased lipid peroxidation, even if this increase was unrelated to the development of fatty liver. These findings were later confirmed by others (Di Luzio and Hartman 1969, Lieber and DeCarli 1966, Odeleye et al. 1993, Sadrzadeh et al. 1995). Our suspicion that the high fat content of ethanol liquid regimens was responsible for the fatty changes and lipid peroxidation was reinforced when Bloom and Westerfeld (1971) showed that when rats were chronically fed a 36% ethanol liquid diet low in fat (5%) and relatively high in carbohydrate (35%), neither fatty liver nor lipid peroxidation occurred. These authors concluded that lipid peroxidation was not the cause of fatty liver but its consequence. Further studies with liquid ethanol regimens showed in addition that the high fat and low carbohydrate dietary contents, rather than the dietary levels of ethanol, were also responsible for the increases in the hepatic levels of triglycerides and cytochrome...
P450, microsomal ethanol oxidizing system activity, and blood alcohol level (Nakajima et al. 1992, Porta et al. 1967, Rao et al. 1992, Yonekura et al. 1993). Although the amount of dietary fat plays an important modulating role in some experimental models of chronic alcoholism, the type of fat seems to be even more important in relation to oxidative stress and pathophysiologic hepatic changes. For example, ethanol regimens high in saturated fat did not produce any significant liver changes (Lieber and DeCarli 1966, Lieber et al. 1967, Nanji et al. 1994, Theuer et al. 1972). Although the effects of unsaturated fat on hepatic histopathologic changes have been attributed mainly to the action of linoleate (Nanji and French 1989), other studies did not support this contention (Sankaran et al. 1994).

**MODULATING EFFECTS OF DIETARY PROTEIN AND LIPOTRIC FACTORS**

It has been known for a long time that the hepatic changes associated with chronic consumption of ethanol by rats were essentially due to the increased requirements of lipotropic factors and were completely prevented by dietary supplementation with choline, methionine or casein (Best et al. 1949). Lipotrope factors include not only these nutrients but also several constituents of the transmethylation pathway (i.e., betaine, S-adenosylmethionine, vitamin B-12 and folate). The modulating importance of protein and lipotropes on the hepatic fatty changes associated with prolonged feeding of rats with liquid ethanol regimens (Lieber's model) or with solid diets and sweetened ethanol solution offered separately (Porta's model) was repeatedly shown (Barak et al. 1994, Hartroft et al. 1969, Koch et al. 1969, Porta et al. 1967 and 1971). Lipotropes also modulate hepatic lesions in monkey and baboon models of chronic alcoholism (Lieber et al. 1990, Rogers et al. 1981).

Mechanisms involved in the modulating effects of protein and lipotropes on hepatic oxidative stress or lipid peroxidation are not clear. If, as suggested by Bloom and Westerfeld (1971), lipid peroxidation is a consequence of increased hepatic triglyceride, then the lipotropes probably prevent lipid peroxidation by preventing triglyceride accumulation. Furthermore, because dietary methionine modulates hepatic levels of reduced glutathione (GSH) (Leaf and Neuberger 1947), this amino acid may modulate oxidative stress by elevation or preservation of the hepatic levels of GSH. However, this possible protective mechanism in experimental ALD is problematic because, as observed and reviewed in one of our studies (Azzalis et al. 1992), the commonly found increase in hepatic GSH in different animal models seems to be a specific effect of ethanol metabolism apparently unrelated to the dietary levels of methionine, cystine or choline.

**RECENT FINDINGS ON DIETARY MODULATION OF HEPATIC PROOXIDANT AND ANTIOXIDANT FACTORS IN ALCOHOLIC LIVER DISEASE**

In collaboration with Virginia Junqueira and co-workers from the University of Sao Paulo, Brazil, we used our method of chronic alcoholism in male Wistar rats (Porta and Gomez-Dumm 1968) to study the modulating effects of several dietary micronutrients on a series of prooxidant and antioxidant hepatic factors. Control rats had free access to tap water and a basal purified diet; the experimental animals had free access to the same basal diet and an aqueous solution containing 25% sucrose and 32% pure ethanol (wt/v). Details regarding the composition of the basal diets and analytical methods were described previously (Azzalis et al. 1992, 1994 and 1995, Junqueira et al. 1993). The amounts of the diverse essential nutrients used in these studies are presented in Table 1 and compared with the amounts recommended for growing rats by the National Research Council (1995) and the American Institute of Nutrition (Reeves et al. 1993). In these studies, the ethanol consumption of the rats was 14–20 g/(kg d). The ranges in the proportions of energy in the regimens of the ethanol-fed rats were as follows: ethanol 33–37%, carbohydrate 40–41%, fat 16–21%, protein 7–9%. It is important to realize that these ethanol regimens were not low in carbohydrate or high in fat. At the end of the experiment, the hepatic activities of alcohol dehydrogenase, catalase and microsomal ethanol oxidizing system were not significantly different between the ethanol-fed and control groups. None of the proxi-

**TABLE 1**

<table>
<thead>
<tr>
<th>Amounts of diverse nutrients in the basal diets of our studies, and the currently recommended amounts for growing rats</th>
<th>Our diets</th>
<th>NAS-95</th>
<th>AIN-93G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E, mg</td>
<td>0.53–2.13</td>
<td>0.67</td>
<td>1.87</td>
</tr>
<tr>
<td>Choline (free base), mg</td>
<td>28–330</td>
<td>19–25</td>
<td>25</td>
</tr>
<tr>
<td>Methionine, mg</td>
<td>558</td>
<td>—</td>
<td>115</td>
</tr>
<tr>
<td>Methionine + cystine, mg</td>
<td>—</td>
<td>245</td>
<td>—</td>
</tr>
<tr>
<td>L-Cystine, mg</td>
<td>122</td>
<td>—</td>
<td>92</td>
</tr>
<tr>
<td>Folate, µg</td>
<td>38</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin B-12, µg</td>
<td>0.574</td>
<td>1.250</td>
<td>0.625</td>
</tr>
<tr>
<td>Se, µg</td>
<td>1.5–3.7</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Zn, µg</td>
<td>31–371</td>
<td>300</td>
<td>750</td>
</tr>
<tr>
<td>Cu, µg</td>
<td>30–306</td>
<td>125</td>
<td>150</td>
</tr>
<tr>
<td>Mn, µg</td>
<td>108–770</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

1 Expressed in units/418 kJ (100 kcal).
2 All-rac-α-tocopheryl acetate.

**TABLE 2**

<table>
<thead>
<tr>
<th>Hepatic prooxidant-related variables in ethanol-fed and control rats</th>
<th>Ethanol-fed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P-450 nmol/mg protein</td>
<td>0.81 ± 0.09 (22)</td>
<td>0.73 ± 0.06 (21)</td>
</tr>
<tr>
<td>Cytochrome b₅₅₅ nmol/mg protein</td>
<td>0.61 ± 0.06 (12)</td>
<td>0.60 ± 0.04 (12)</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase U/mg protein</td>
<td>0.034 ± 0.005 (20)</td>
<td>0.031 ± 0.005 (21)</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase U/mg protein</td>
<td>0.64 ± 0.08 (12)</td>
<td>0.72 ± 0.05 (12)</td>
</tr>
<tr>
<td>NADPH oxidase, U/mg protein</td>
<td>2.83 ± 0.41 (19)</td>
<td>2.87 ± 0.20 (21)</td>
</tr>
<tr>
<td>O₂⁻ production, nmol/mg protein-min</td>
<td>15.10 ± 1.94 (21)</td>
<td>16.74 ± 1.35 (21)</td>
</tr>
</tbody>
</table>

1 Pooled data from two or three experiments. Values are means ± SEM, with the numbers of rats in parentheses. Differences between ethanol-fed rats and their controls were not statistically significant (P > 0.05).
Table 3

Hepatic antioxidant variables in ethanol-fed and control rats

<table>
<thead>
<tr>
<th>Ethanol-fed</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-tocopherol, nmol/g liver</td>
<td>29.6 ± 1.9 (17)</td>
<td>55.0 ± 5.6 (17)</td>
</tr>
<tr>
<td>Ubiquinol 9, nmol/g liver</td>
<td>27.0 ± 7.0 (5)</td>
<td>54.6 ± 8.7 (5)</td>
</tr>
<tr>
<td>Ubiquinol 10, nmol/g liver</td>
<td>3.4 ± 0.7 (5)</td>
<td>5.9 ± 0.8 (5)</td>
</tr>
<tr>
<td>Reduced glutathione, μmol/g liver</td>
<td>8.4 ± 0.2 (22)</td>
<td>7.3 ± 0.2 (22)</td>
</tr>
<tr>
<td>Glutathione peroxidase, U/mg protein</td>
<td>0.70 ± 0.06 (12)</td>
<td>1.20 ± 0.09 (12)</td>
</tr>
<tr>
<td>Glutathione reductase, U/mg protein</td>
<td>0.086 ± 0.006 (22)</td>
<td>0.078 ± 0.005 (22)</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase, uM/mg protein</td>
<td>18.66 ± 1.63 (22)</td>
<td>16.25 ± 2.48 (21)</td>
</tr>
<tr>
<td>Catalase, U/mg protein</td>
<td>320 ± 20 (20)</td>
<td>264 ± 15 (21)</td>
</tr>
<tr>
<td>Superoxide dismutase, U/mg protein</td>
<td>124 ± 12 (19)</td>
<td>150 ± 11 (20)</td>
</tr>
</tbody>
</table>

1 Values for α-tocopherol and ubiquinols 9 and 10 are from one experiment, and the rest are from three experiments. Values are means ± SEM, with the numbers of rats in parentheses. NS = not significant.

Dant factors were significantly influenced by the different dietary levels of lipotropes, vitamin E or minerals, and no significant differences were found between ethanol-fed and control rats (Table 2). On the other hand, the ethanol regimen was associated with a significant increase in the hepatic concentration of GSH by significant decreases in the activity of hepatic glutathione peroxidase and in the concentrations of α-tocopherol and ubiquinols 9 and 10 in relation to the values in the control rats (Table 3). The activities of hepatic glutathione reductase, glucose-6-phosphate dehydrogenase, catalase and cytosolic superoxide dismutase were statistically similar in ethanol-fed and control rats (Table 3).

When the vitamin E level was relatively high, the levels of TBARS (nmol/MDA/mg protein/120 min) in hepatic microsomes of ethanol-fed rats (1.59 ± 0.29) were significantly different from those of control rats (1.50 ± 0.5), but when the vitamin E level was relatively low, both the microsomal levels of TBARS and chemiluminescence (cpm 10⁻⁸) in the ethanol-fed rats (1.32 ± 0.20 and 408.7 ± 36.5, respectively) were significantly higher than those in control animals (0.65 ± 0.09 and 72.4 ± 8.8, respectively). As expected, when the dietary Se was low, the activities of hepatic glutathione peroxidase (U/mg protein) in the ethanol-fed groups as well as in the controls were lower (0.09 ± 0.01 and 0.43 ± 0.03, respectively) than when Se was normal (0.70 ± 0.06 and 1.20 ± 0.08, respectively). Finally, when dietary levels of Cu and Zn were low, the hepatic activities of cytosolic superoxide dismutase (U/mg protein) in the ethanol-fed and control rats were lower (44 ± 5 and 57 ± 7, respectively) than when the dietary levels of these two minerals were normal (163 ± 15 and 197 ± 13, respectively). Despite the observed significant decreases in antioxidant factors observed in the ethanol-fed rats, and despite the significant differences in these antioxidants and indices of lipid peroxidation created by variations in the dietary levels of vitamin E, Se, Cu and Zn in the ethanol-fed rats and the controls, in none of our studies was there any evidence of hepatic damage by the ethanol regimen. The levels of ethanol-fed rats were histologically normal, and no significant differences were found in the serum concentrations of alanine aminotransferase between ethanol-fed rats and their controls. All these results clearly show that the mere presence of oxidative stress in experimental ALD in rats does not indicate ALD. Various indicators of oxidative stress are not necessarily associated with ALD, and hence oxidative stress does not have a significant pathogenic role.

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