Low Dose-Ethanol Modulates Toxic Effect of Iron-Overloading in the Liver

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Summary The oxidant properties of iron-overload and simultaneous ethanol consumption have received much interest, due to evidence reporting from hereditary hemochromatosis (HC). The full form of this disease is often associated with chronic alcoholism. An additive effect of toxicity of iron and ethanol was assumed. In this study, we examined nutritively iron-loaded Wistar rats (n=59) (TMH-Ferrocene) additionally fed with ethanol up to 8% in drinking water for 36wk. Methods: By reverse-phase HPLC we measured the concentration of ascorbic acid, tocopherole and retinol in serum and liver homogenates as well as transaminases in the serum. Lipid peroxidation was assessed utilizing the ethane-exhalation method. Iron concentration in the liver was measured with the Bathophenanthrolin method. Liver histology was performed to investigate the iron deposits and the organ damage (H.E., Azan and Berlin-blue-stainings). Results: 1. Vitamin C: A linear decrease of the concentration of vitamin C in serum and liver was found independent of alcohol and iron uptake. 2. Vitamin E: Animals fed iron and alcohol showed elevated vitamin E concentrations in the serum but not in the liver. 3. Vitamin A: Elevated levels in serum but strongly decreasing levels in liver could be measured. 4. Histology: All iron-fed animals showed massive deposits of iron in the liver. Iron diet caused liver cirrhosis, while an additional administration of ethanol could prevent this. 5. Lipid peroxidation increased in animals fed ethanol and iron, but was significantly lower in animals only receiving an iron diet. Conclusion: Evidence indicates that the additional exposition to ethanol in iron-loaded animals could modulate the organ damage and oxidative stress. The biochemical findings are positively correlated to the histology.

Key Words iron-overload, vitamin A, vitamin E, vitamin C, ethanol

Whether the chronic consumption of ethanol leads to enhanced intestinal iron-uptake has been a subject of much controversy over the last few years. Earlier studies show that almost 30% of the patients suffering from hereditary hemochromatosis (HC) also have an alcohol addiction. At that time, general opinion was that for the complete form of this disease to develop, an excessive intestinal uptake of ethanol played a vital role. In the meantime, it has been found that HC is autosomal recessively inherited and for the full form of the disease, homozygocity is essential. Heterozygote carriers of the HIC gene, whether or not alcoholic at the same time, only develop a light siderosis of the liver, with tissue iron being stored mainly in the Kupffer cells.

The only known case of an alcohol-induced, nutritively triggered iron-overload is the so-called Bantu-siderosis. The Bantu, an indigenous African tribe, traditionally brewed a form of iron-containing beer, which was heavily consumed by tribe members. The high levels of iron present in this beverage were caused by the preparation of the beer in iron kettles. Recently, a genetic defect relating to this condition has also been discussed (1–6).

Vitamin C has been linked to pathological processes by several studies. For example, diseases associated with a mild lack of vitamin C are generally cardiovascular diseases, cancer, and diseases of connective tissue. Ascorbic acid (vitamin C) is a powerful antioxidant in cells, a property mostly due to its high reactivity with free radicals (7). It even reacts with glutathione radicals in the aqueous phase and with vitamin E at the aqueous-lipid interface. The relative concentration of ascorbate has been suggested to be an indication of systemic oxidative stress. For example, plasma and lymphocyte
ascorbate concentrations decline ~20% throughout the aging process (7). Plasma ascorbate is also significantly lower in smokers, alcoholics (8), and as well as cancer sufferers (9), diabetes (10), hepatitis (11) or β-thalassemia major patients (12). Peroxidative effects of vitamin C have also been discussed.

Vitamin A is essential for erythropoesis; vitamin A deficient people are unable to integrate haemoglobin into erythrocytes in a normal way. An association between vitamin A and iron metabolism was first reported in 1978 by Hodges et al. (13). These investigators fed vitamin A deficient diets to adult volunteers for ≥450 d, and observed that as serum retinol concentrations of the volunteers progressively decreased, so too did their haemoglobin concentrations, despite their diet containing adequate amounts of iron. The study subjects did not respond well to supplemental iron until their vitamin A deficiency was corrected. Several cross-sectional studies have since confirmed the interaction between iron and vitamin A. A positive correlation between serum retinol and haemoglobin values was reported in children in different countries from Southeast Asia and Africa (14). Several possibilities were discussed concerning this interaction. It was suggested that vitamin A deficiency decreases transferrin synthesis and thus reduces iron transport to the bone marrow (15); reduces the uptake of iron by the bone marrow; impairs the differentiation of blood cells due to lack of retinoic acid; results in ineffective erythropoesis; and impairs mobilization of iron from ferritin stores. Alternatively, it was suggested that the high prevalence of infections, which are frequently reported during vitamin A deficiency, is indirectly responsible for decreasing haemoglobin concentrations because the body sequesters iron during infections (13, 14).

Hepatic iron overload is commonly observed in alcoholics (16). The pathophysiological effects and the mechanism of hepatic iron overload in alcoholic liver disease are not yet fully understood. Iron within the liver is found in several biochemical forms, such as heme iron and non-heme iron, including ferritin, hemosiderin, and free iron. Free iron induces formation of free radicals and especially stimulates lipid peroxidation, with the resulting oxidative products causing direct cellular injury. Higher concentrations of liver malondialdehyde and 4-hydroxy-2-nonenal have been found in ethanol-fed rats with additional iron supplementation, than in those that were ethanol-fed alone.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver iron concentration (mg Fe/g Fgw.)</th>
<th>Total liver iron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (TMH-F, 2 vol% ethanol)</td>
<td>22.0±1.5</td>
<td>473±53</td>
</tr>
<tr>
<td>B (TMH-F, 5 vol% ethanol)</td>
<td>25.0±4.0</td>
<td>483±79</td>
</tr>
<tr>
<td>C (TMH-F, 8 vol% ethanol)</td>
<td>26.0±1.0</td>
<td>561±38</td>
</tr>
<tr>
<td>D (TMH-F)</td>
<td>28.0±5.0</td>
<td>631±174</td>
</tr>
<tr>
<td>E (Normal diet, 8 vol% ethanol)</td>
<td>0.9±0.1</td>
<td>6±1.2</td>
</tr>
<tr>
<td>F (Normal diet)</td>
<td>0.6±0.1</td>
<td>4±1</td>
</tr>
</tbody>
</table>

The liver iron was measured at regular intervals. These measurements are summarised in Table 2.

Table 2. The constituents of the normal animal diet.

<table>
<thead>
<tr>
<th>Energy</th>
<th>3,500 kcal/kg</th>
<th>14.6 MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals, % in diet (mean)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0.95</td>
<td>0.75</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.07</td>
<td>0.24</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Elements, mg in 1 kg diet (mean)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>Copper</td>
<td>5</td>
<td>816</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.4</td>
<td>30</td>
</tr>
<tr>
<td>Aminosacids, % in diet (mean)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysin</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>Cystin</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Tyrosin</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Histidin</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Threonin</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Leucin</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamins in 1 kg diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>15,000 IU</td>
<td>Vitamin D₃ 500.00 IU</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150.0 mg</td>
<td>Vitamin K₁ 10.00 mg</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>20.0 mg</td>
<td>Vitamin B₂ 20.00 mg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>15.0 mg</td>
<td>Vitamin B₁₂ 0.03 µg</td>
</tr>
<tr>
<td>Nicotin acid</td>
<td>50.0 mg</td>
<td>Panthenate acid 50.00 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>10.0 mg</td>
<td>Biotin 0.20 µg</td>
</tr>
<tr>
<td>Cholin</td>
<td>1,000.0 mg</td>
<td>Vitamin C 20.00 mg</td>
</tr>
</tbody>
</table>
their blood collected. Liver tissue was also harvested under carbon dioxide anaesthesia and two rats from each group were sacrificed after 4wk by exsanguination. Group F: n=9). Table 2 shows the content of the rat liver in drinking water at different concentrations, provided in load-diet (ID) (from Altromin, Germany, C1000 and Wiga, Hannover, Germany) at 8wk of age were randomly assigned to five experimental groups of ten animals and one control group of nine animals. The animals were individually housed in stainless-steel cages. The experimental groups were fed an iron-overload, comparable to the iron deposits in HC in these diseases.

This study further explores the relationship between alcohol-uptake and iron-overload and its effects on lipid peroxidation. Groups of animals with and without iron-overload and up to 8% alcohol-ingestion were compared. Whether or not a nutritive iron overload contributes in an additional toxic effect on liver tissue was examined, along with the antioxidative status of the tissue. Female Wistar rats (n=59) that were nutritively iron-loaded and additionally fed with ethanol up to 8% in drinking water for a maximum of 36 wk were examined. Iron-overload of liver tissue was achieved by feeding a diet containing 0.5% trimethylhexanoyl-ferrocene. This diet leads to a rapidly progressive hepatocellular iron-overload, comparable to the iron deposits in HC in man (18, 19). Using HPLC, we measured the levels of the vitamins A, C and E in serum and liver homogenates of the animals. We also examined liver histology (Azan, and Berlin-blue-stainings).

**MATERIALS AND METHODS**

**Animals.** Fifty-nine female Wistar rats (130–140 g: Wiga, Hannover, Germany) at 8 wk of age were randomly assigned to five experimental groups of ten animals and one control group of nine animals. The animals were individually housed in stainless-steel cages. The experimental groups were fed an iron-overload-diet (ID) (from Altromin, Germany, C1000 and C1038 with 0.5% TMH-ferrocene) and/or ethanol (Eth) in drinking water at different concentrations, provided ad libitum for 36 wk: Group (A) ID and 2% Eth; (B) ID and 5% Eth; (C) ID and 8% Eth; (D) ID; (E) 8% Eth; (F) Control (experimental Groups A–E: n=10, control Group F: n=9). Table 2 shows the content of the rat diet.

Body weights were recorded once per week. One or two rats from each group were sacrificed after 4 wk by exsanguination under carbon dioxide anaesthesia and their blood collected. Liver tissue was also harvested and after being frozen in liquid nitrogen, specimens were stored at −80°C for subsequent analysis. All procedures involving animals were in accordance with guidelines of the local Animal Care and Use Committee.

**Plasma and liver sample extraction.** Extraction of plasma and liver samples was performed as described previously (20). Briefly, serum samples were prepared as follows: 400 µL of the serum sample was mixed with alpha-tocopherole-acetate (diluted 1:1,000) and 800 µL n-hexane, followed by centrifugation of these samples at 2,000 rpm for 2 min. A 400 µL aliquot was collected from the n-hexane phase and completely dried in saturated nitrogen atmosphere. The supernatant was then eluted in 400 µL of methanol-solution after which 100 µL of the eluate was injected into a HPLC-column.

Preparation of the liver samples: 0.5 g of liver tissue was homogenized in 3 mL of ice-cold NaCl-solution. One milliliter of 0.1 molar sodium-dodecyl-sulfate-solution was then added to the homogenate and a subsequent 2 mL of this suspension removed and mixed with 2 mL of 10% meta-phosphoric-acid. This mixture was centrifuged at 5,000 rpm, 0°C, for 5 min after which it was then diluted 1:10 and then ultra-filtrated using a Millipore 0.2 µm filter.

**HPLC analysis.** Vitamin C: The HPLC-pump 2248 was used for analysis (Pharmacia Biotech, UK), along with the following equipment specifications: low pressure mixer (Pharmacia Biotech), electrochemical detector (Pharmacia LKB Biotech), HPLC-column SuperPac Sephasil C18 5 µm (4×250 mm), Chromato-integrator D-2500 (Merck), Mobile phase 2.5 mM di-potassium hydrogen phosphat-trihydrat-solution (pH 2.95). For HPLC analysis, the system was calibration using a standard vitamin C solution.

Vitamin E and A: Pump 2248 (Pharmacia Biotech), low pressure mixer (Pharmacia Biotech), HPLC-column ChromSa×ID=250×4.0 [mm], Gel: Inertsil ODS-2 5 µm, spectral photometer “Knauer.” A fluorescence HPLC monitor RF-535 T (Shimadzu, Japan) was used for the detection of vitamin A at 325 nm, vitamin E and vitamin E-acetate at 275 nm. Chromato-integrator D-2500 (Pharmacia Biotech) was used as previously described (21–24) (Fig. 1).

**Synthesis of trimethylhexanoyl-ferrocene.** The first step is synthesis of 3,5,5-trimethylhexanoylchlorid, 3,5,5-Trimethylhexanoylchlorid (2.73 mol) was incubated for 36 h in a saturated oxygen atmosphere. The resulting carbon acid was then added 1.27 mol phosphotrichloride. This stock was kept for 5 d free of humidity. The liquid phase could be fractioned by distillation in a vacuum. Out of this 356 g 3,5,5-trimethyl-
hexanoylchlorid was synthesized. The second step was the synthesis of trimethylhexanoyl-ferrocene (TMH-F). Therefore 1.34 mol ferrocene (dicyclopentadienyl-iron) was solved in 1.4 L methylenchloride and added 1.06 mol aluminium-chloride (as catalyst). Then, it was added over a 6 h period very slowly at 0.87 mol 3,5,5-trimethylhexanoylchloride under constant stirring and cooling at 4°C. This stock was then put onto ice and the methylenchloride-phase was separated using a funnel. The methylenchloride was evaporated. The leftover was solved in toluole and separated using gel-chromatography-column. Next, the toluole was evaporated and the left over oil lyophilized. Output 211 g 3,5,5-trimethylhexanoylferrocene.

**Determination of liver-iron concentration.** For the determination of the liver iron concentration we used the bathophananthrolin-method as previously described (25-27). All chemicals were purchased from Merck.

**Ethane exhalation.** As ethane-exhalation positively correlated to lipid-peroxidative processes in-vivo, we used this method to assess the time course in the different groups. This method was previously described by Dresow et al. in 1995 (28). Before measurement of the ethane, food and ethanol was withdrawn 12 h in advance. We used the gas-chromatograph Hewlett Packard 5840A with F1 80–100 mesh-column (Supelco, SA, Switzerland). The carrier gas was nitrogen while the burner gas was hydrogen and synthetic air (air liquide, France).

**Settings of the gas-chromatograph.** Start-temperature 60°C for 3 min, ramp speed 30°C/min, final temperature 180°C for 7 min, injection temperature 250°C, detector temperature 300°C, nitrogen flow 25 mL/min, hydrogen flow 14 mL/min, synthetic air flow 16 mL/min.

**RESULTS**

**Vitamin C**

In serum and liver, vitamin C levels were determined using HPLC electrochemical detection. The reference level of vitamin C in serum was observed at 0.29±0.07 μg vitamin C/mL of serum, and in liver 30.5±1.9 μg vitamin C/g of tissue, as detected in control animal groups (no ID, no E). In comparison, in ID-, E- and ID/E treated groups, both serum- and liver-levels of vitamin C were below the reference, with all groups showing a linear decrease in vitamin C concentrations. After 36 wk, the ID-alone group of animals (Group D) showed statistically significant lower levels of vitamin C (p<0.05) compared to all the other groups, with no significant variations in vitamin C levels being observed between all the other groups (p>0.05). All experimental groups exhibited a severe and linear decrease of vita-
Fig. 3. Increase in serum vitamin A in iron-loaded rats following ethanol administration. Using a reversed phase HPLC, the serum (a) and liver (b) were determined over 35 wk of monitoring in animals feeding with different % (2% as Group A, 5% as Group B, 8% as Group C) together with TMH. In addition, TMF only or ethanol only fed rats were represented in Group D and E respectively. The samples of serum and liver tissues were obtained at regular 4–6 wk intervals. The results are represented as mean in the line forms (--, Group A, ---, Group B, --•--•; Group C, --•--•; Group D, --•--•, reference; ----). The symbols are representative of animals (Ÿ: Group A, £: Group B, ¡: Group C, ž: Group D, ¢: Group E).

Vitamin A

In serum, reference levels of vitamin A were seen at 0.31±0.09 µg vitamin A/mL of serum, and in liver 33.0±4.31 µg vitamin A/g of tissue. In all experimental groups, serum levels followed a linear increase above the reference value, with no significant difference between the animals. In the liver, all animals followed an exponential decrease in levels of vitamin A, with detected levels falling below the reference (typically towards 0). Again, no significant difference was observed when comparing the different experimental groups (p>0.05) (Fig. 3, a and b).

Vitamin E

In both serum and liver tissue, the reference levels of vitamin E (0.33±0.09 µg vitamin E/mL of serum; 15.63±3.2 µg vitamin E/g of liver tissue) were exceeded by all experimental groups. The 8%-ethanol treated group showed statistically significant lower levels of vitamin E in serum (p<0.05) for up to week 20 of the study, after which no difference from the other groups was observed. In comparison, no difference in liver-vitamin E levels was seen among the different groups, however all animals showed a general linear decrease in liver-vitamin E (Fig. 4, a and b).

Ethane-exhalation

Animals receiving ethanol, iron or a combination of the two showed a significant increase of lipid-peroxidative activity (p=0.01). There was no difference between the animals receiving 2, 5 or 8% of ethanol and ID. A significant difference was found between Group E and the other experimental groups (Fig. 5).

Serum-transaminases

Hepatotoxicity was evaluated by measuring the activity of serum enzymes (alkaline phosphatase [ALP], alanine aminotransferase [ALT], and aspartate aminotransferase [AST]). The enzyme levels were determined as previously described (29, 30). The administration of TMH-ferrocene alone significantly increased the activity of ALP (62.4 to 517.3 U/L), ALT (from 102.3 to 932.7 U/L) and AST 121.5 to 1,232.3 (U/L). Whereas the administration of ethanol up to 8% and iron led to a significantly increased level of the enzyme activities.
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Fig. 5. Reduced ethane exhalation in ethanol fed and iron-loaded rats. As in Figs. 1–3, the experiments were set up with Groups A to C as being the animals fed with percentages (2% as Group A, 5% as Group B, 8% as Group C) of ethanol in combination with TMH. Similar controls were carried out in animals fed with ethanol only (Group D), TMH only (Group E) or normal diet (reference). The results are represented as mean in the line forms (--; Group A, ------; Group B, "" Group C, -"" Group D, "" reference; ----). The symbols are representative of animals ("": Group A, "": Group B, "": Group C, "": Group D, "": Group E).

over the normal animals, but showed significantly lower activities compared to the animals receiving soli
tarily the iron diet ALP (62.4 to 211.3 U/L), ALT (from 102.3 to 234.4 U/L) and AST 121.5 to 244.3 (U/L) (p=0.01) (Fig. 6).

Liver iron
Liver iron was determined using the bathophenanthrolin-method as previously described. The four exper
cimental groups showed no significant differences compared to each other, but were showing increased
levels of iron compared to the non-iron overloaded, ethanol-exposed group. It shows significantly higher liver
iron concentrations (p=0.01) and the total liver iron
(p=0.02) compared to the non iron-overloaded, non
ethanol-exposed group. The feeding of 8% ethanol
alone did not cause iron storage (Table 1).

Histology
Histological analysis of liver samples from all animals was performed after 36 wk using H&E- Azan- and Berlin-Blue staining. All animals in groups which were fed ID, showed massive iron-deposits in hepatocytes, Kupffer-cells, and also in macrophages localised in the periportal areas. However, a difference was detected within these groups as to the degree of fibrosis present in these iron-loaded animals. Animals in Group D, which were fed ID without ethanol, showed severe liver cirrhosis after 36 wk. Animals that additionally received 2% ethanol (Group A) only showed a mild increase in connective-tissue, without destruction of the liver structure. An increase in the concentration of ethanol applied, up to 8% (Groups B and C), did not pre
vent cirrhosis. In comparison, the solitary 8% ethanol
diet (Group E) did not induce fibrotic conversion (Fig.
7).

DISCUSSION
The in vivo biochemical evidence presented by this study indicates that chronic ethanol intake, when com
bined with iron-overload, interferes with tissue metabo
lism of ascorbic acid, tocopherole and retinol. More
specifically, we have demonstrated that chronic iron
overload leads to (1) a significant decrease in serum and liver ascorbic acid concentrations and that (2) animals fed with iron and alcohol showed a significantly higher concentration of ascorbic acid in serum and liver tissue compared to animals in other experimental groups. (3) No specific results were obtained from the analysis of alpha-tocopherole concentrations in serum and liver. (4) The combined feeding of ethanol and iron diet also led to a significant decrease in retinol concentrations in the liver, whereas serum concentrations were signifi
cantly elevated.

The relative concentration of ascorbate has been sug
gested to be an indication of systemic oxidative stress.
For example, plasma and lymphocyte ascorbate con
centrations decline by ~20% throughout aging (31).
Plasma ascorbate is also significantly lower in smokers,
alcoholics, and sufferers of cancer, diabetes, hepatitis, HIV, cystic fibrosis, or β-thalassemia major (7). Yama
moto et al. has previously described how a significant
decrease in plasma ascorbate levels in groups of
patients with hepatitis, cirrhosis or liver cancer, is indic
ative of oxidative stress that is evident in these diseases
(11). Our results could confirm these findings. In ani
mals fed with an iron diet and additionally with etha
nol, the reduction of ascorbate levels seen was signifi
cantly less than in the group fed on iron alone, which
suggests an antioxidative influence of ethanol under iron-overload. This was also confirmed by our histological findings.
Similar to other reports (32), we have shown that chronic ethanol feeding results in a significant decrease in retinol concentrations in the liver of rats. Elevated serum-retinol concentrations in terms of oxidative stress have not previously been recorded. Retinoic acid, the most active form of vitamin A, plays an important role in the control of cellular proliferation and differentiation. Therefore, a decreased cellular retinoic acid level may lead to enhanced cell proliferation and potentially to tumour formation. Decreased liver and elevated plasma retinoic acid concentrations as a result of alcohol feeding and iron overload in our in vivo study could be caused by several mechanisms. First, ethanol acts as a competitive inhibitor of retinol oxidation in the liver and also other tissues (33). Ethanol metabolism undergoes a two-step process: Initially, it is oxidized to acetaldehyde, and then acetaldehyde is subsequently oxidized to acetic acid. Similarly, retinol is converted into retinaldehyde, and then retinaldehyde can be oxidized to retinoic acid. The reversible oxidation of retinol to retinal by class I alcohol dehydrogenase (ADH) is the limiting step in retinoic acid biosynthesis (34, 35). Human class I ADH, which also functions as a retinol dehydrogenase, consists of ADH1, ADH2, and ADH3 and is mainly localized to the liver (36). High doses of ethanol may competitively inhibit these ADH enzymes (37) and thus biosynthesis of retinol. The elevated levels of retinol in serum observed could thus be explained as a temporary event, where retinol is used extra-hepatically to compensate oxidative stress and therefore the hepatic deposits are released.

As previously described by Kawase et al., the effects of chronic ethanol feeding on hepatic lipid peroxidation showed that dietary vitamin E is an important determinant of hepatic lipid peroxidation. Hepatic lipid peroxidation significantly increased after chronic ethanol feeding in rats receiving a low-vitamin E diet. Here plasma alpha-tocopherol was also elevated after ethanol feeding (38). This could be explained by the associated hyperlipemia. Nevertheless, decreasing concentrations of alpha-tocopherol in serum and liver indicate consumption of this antioxidant. These data suggest that ethanol and iron feeding causes a remarkable alteration of vitamin E metabolism in the liver and that the combination of ethanol with high iron intake results in a decrease in hepatic alpha-tocopherol content which renders the liver more susceptible to free radical attack.

Conclusion

Evidence exists that the additional exposition of iron-loaded rats to ethanol has an antioxidative effect as well as a positive influence on the development of liver-fibrosis, in spite of the reduction in anti-oxidative vitamins. Biochemical findings were confirmed by and correlated to the histology. Further investigations will follow.

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