KUPFFER CELL INACTIVATION ALLEVIATES ETHANOL-INDUCED STEATOSIS AND CYP2E1 INDUCTION BUT NOT INFLAMMATORY RESPONSES IN RAT LIVER

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Gadolinium chloride inactivates Kupffer cells and alleviates alcohol-induced liver lesions. We investigated the mechanism of gadolinium chloride protection in rats maintained ethanol-intoxicated for 6 weeks by feeding low-carbohydrate/high-fat ethanol liquid diet. At termination, liver samples and cell lysates obtained from the peripontal and perivenous region were analyzed for histopathology, mRNA expression of endotoxin-associated parameters and cytokines and for enzymes involved in oxidative stress.

Ethanol treatment alone caused marked microvesicular/macrovacuolar steatosis and focal inflammation. Macrophage inactivation by intravenous administrations of gadolinium chloride significantly alleviated pathology, by reducing steatosis but not inflammation. Ethanol also significantly increased mRNA expression of the LPS receptor CD14 and the LPS binding protein LBP, but not that of TNF-α and IL-1β. CD14 mRNA was found to be expressed preferentially in the perivenous region, and was not significantly affected by gadolinium treatment. However, gadolinium significantly moderated the ethanol induction of CYP2E1 and this effect correlated to the degree of steatosis. Ethanol increased glutathione transferase and reduced glutathione peroxidase activity, but these changes persisted after gadolinium treatment.

Our results suggest that gadolinium chloride reduces symptoms of ALD mainly by counteracting steatosis, and that CD-4 positive Kupffer cell populations are not involved in gadolinium protection. The strong correlation between pathology and CYP2E1 induction might suggest a steatopathogenic role for this enzyme.

IgG is more important than IgA in producing alcohol induced liver disease.

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A number of immunological changes are seen in alcohol induced liver disease (ALD). One of the most consistent observations is the raised semm level of IgA and this can also be demonstrated deposited along the liver sinusoids. IgA deposition is, however, also seen in other forms of liver disease including that associated with non-alcoholic hepatitis (NASH) and is therefore not specific. We therefore examined the deposition of IgG in ALD to see if this was more specific. Twenty biopsies from patients with ALD (10 fatty change and 10 alcoholic hepatitis) and 10 biopsies from patients with fatty change not due to alcohol and 10 from patients with NASH were stained for IgA and IgG using an indirect immunoperoxidase technique. As before there were no significant differences between the alcoholic and non-alcoholic groups with regards to IgA deposition. However IgG deposition was seen in 12/20 cases of ALD and only 2/20 cases on non ALD (which is statistically significant). Furthermore it was seen in 9/10 cases of alcoholic hepatitis and only 3/10 cases of alcohol induced fatty change. This shows that IgG deposition is most closely associated with alcoholic hepatitis. The further examine this specificity, we cultured Hep G2 cells, a liver cell line known to metabolise alcohol, in alcohol and then examined their ability to bind IgG antibodies derived from the serum of patients with ALD. Binding was seen with serum from patients with alcoholic hepatitis in 15/20 cases and in 3/12 cases of fatty change alone. We therefore conclude that not only are IgG antibodies more specific for ALD than for non-ALD, they are most closely
associated with alcoholic hepatitis. Further studies are in progress to examine whether these IgG antibodies play a role in producing the hepatocyte damage seen in alcoholic hepatitis.

A STUDY OF FATTY LIVER DISEASE AND PLASMA LIPOPROTEINS IN A KINDRED WITH FAMILIAL HYPOBETALIPOPROTEINEMIA DUE TO A NOVEL TRUNCATED FORM OF APOLIPOPROTEIN B (APO B-54.5)

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BACKGROUND/AIMS - Familial hypobetalipoproteinemia (FHBL) is a co-dominant disorder characterized by reduced plasma levels of low-density lipoproteins. It can be caused by mutations in the gene encoding apolipoprotein B-100 (apo B), leading to the formation of truncated apo Bs which have a reduced capacity to export lipids from the hepatocytes as lipoprotein constituents. Case reports suggest the occurrence of liver disease in FHBL, but there are no studies of liver involvement in FHBL with defined apo B gene mutations. The presence of fatty liver disease was investigated in a large FHBL kindred. METHODS - Plasma lipoprotein and apolipoprotein analysis, liver function tests, and apo B gene sequence were performed in 16 members of a FHBL kindred. The presence of fatty liver was assessed by ultrasound and computed tomography scanning. RESULTS - The proband, a non-obese heavy drinker male with hypobetalipoproteinemia, had steatohepatitis with fibrosis. He was heterozygote for a novel nonsense mutation of apo B gene producing a truncated apo B of 2745 amino acids (designated apo B-54.5, having half the size of normal apo B-100). Seven other members of his kindred carried apo B-54.5. Although all of them were hypolipidemic, their lipid levels showed a large inter-individual variability not accounted for by polymorphisms of genes involved in apo B metabolism. Four carriers (two heavy drinkers and two teetotalers), irrespective of their plasma lipid levels, had ultrasonographic evidence of fatty liver. In the other four carriers, no evidence of fatty liver was found. CONCLUSIONS - In this kindred apo B-54.5 predisposes to fatty liver, which however may require some additional factors to become clinically relevant.

ARE THERE ANY SEX DIFFERENCES IN FATTY LIVER?

A STUDY OF GLUCOSE METABOLISM AND BODY FAT DISTRIBUTION

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ABSTRACT

Background and aims Fatty liver is a common condition found more often in males. Whether sex differences affect its development is presently unknown. The hypothesis that glucose metabolism alterations or central body fat distributions are gender-related in fatty liver was investigated.

Methods - Overall 199 consecutive subjects seen in the Division of Internal Medicine and Gastroenterology, Modena City Hospital, were enrolled. In the main arm of the present study, 44 men with sonographic fatty liver and 47 controls without and 18 women with and 19 without fatty liver had their body mass index (an index of overall adiposity), hepatobiliary serum enzymes, serum cholesterol and triglycerides determined. All underwent oral glucose tolerance test (estimated through the glucose area under the curve with the trapezoidal method). In the ancillary study, 17 other men with and 14 without, and 11 other women with and 29 without fatty liver had anthropometric measurements of body fat distribution (waist/hip, waist/height and skinfold thickness).

Results - Following statistical evaluation including univariate and multivariate analyses (main study), elevated body mass index was found to be an independent predictor of fatty liver in ether sex. Glucose area under the curve and central-type body fat distribution (ancillary study) predicted fatty liver) only in women.

Conclusions - Fatty liver could be gender-related in the present sense.

Hepatic ultrasonography versus liver biopsy in steatohepatitis diagnosis.

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Aim. The study aims to evaluate the diagnostic value of two methods of investigation:

one invasive liver biopsy -, and the other noninvasive - hepatic ultrasonography - in liver steatosis.

Material and methods. 300 patients with liver steatosis diagnosed by ultrasonography were compared with 51 patients, in whom the diagnosis was confirmed by percutaneous liver biopsy. The clinical, biochemical and morphological features of patients with steatohepatitis were analyzed. All patients were negative to hepatitis B and C virus.

Results. Steatohepatitis was usually discovered because of abnormal liver tests or hepatomegaly detected during evaluation for other medical problems. Ultrasonography was the first choice examination. The hepatomegaly was present in all patients and showed bright echoes. Accurate quantitation of the fat was not possible because of the known variation in echo pattern among healthy individuals. The biopsy specimens were characterized by the presence of striking fatty changes with evidence of lobular hepatitis, focal necrosis with mixed inflammatory infiltrates and, in most instances, Mallory bodies. Evidence of fibrosis was found in most specimens. Excess alcohol intake was the probable etiological association in 45%, obesity in 28% and diabetes mellitus in 8.5%. There were no significant differences in clinical presentation, biochemistry, ultrasonography or hepatic histopathology between alcoholic and non alcoholic steatosis. The severity or type of hepatic change did not correlate with the ultrasonographic characteristics. Ultrasound is not able to detect minimal changes, in fat content or fibrosis.

Conclusions. Percutaneous liver biopsy is essential for the diagnosis of steatohepatitis. It is a safe, effective and acceptable procedure in selected patients. Liver biopsy is also important for establishing the prognosis: perivenular sclerosis is indicative for a more severe disease than the fat deposition alone.

THE ETIOLOGY AND EVOLUTION OF NONALCOHOLIC STEATOSIS

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Aim of the study. The aim of this clinical trial was to study nonalcoholic steatohepatitis (NASH), taking into account the fact that its prevalence is lower compared to alcoholic steatohepatitis and the fact that we have insufficient knowledge regarding the etiological factors and evolution of NASH.

Material and methods. This clinical trial was conducted on a group of 62 patients. Diagnosis of NASH was based on hepatomegaly associated with high levels of aminotransferases, of \( \gamma \)GT, of alkaline phosphatase, of triglycerides and of cholesterol. Liver biopsy showed the presence of steatosis, necrosis and inflammatory infiltrate.

Results. The study of the etiological factors revealed the implication of diabetes mellitus in 59.67% of cases, followed by obesity in 40.32%, intake of amiodarone in 1 case and of thyroid hormones in 2 cases. We remarked the predominance of female patients (80.64%, p<0.001). Mean age at the moment of diagnosis was 49±20 years for females and 53±4 years for males. Hepatomegaly was present in all cases. The incidence of jaundice was similar in males and in females (27%). Polynevritis appeared more frequently in patients with diabetes (50% vs. 8%).
Cirrhogenic evolution was present only in patients with diabetes (13.8%). Ascites was present in only 1 case—patient with diabetes. The cholestatic and hepatocytolitic syndromes appeared in similar proportions in both groups of patients.

**Conclusions.** 1. The authors differentiate nonalcoholic steatohepatitis from alcoholic steatohepatitis on the basis of absence of alcohol intake, on the predominance of female gender (80.64%) and on a younger age at the moment of diagnosis (approximately 49 years). 2. The clinical signs are dominated by hepatomegaly. 3. The most important biological data are represented by high levels of aminotransferases (especially ALAT), of yGT, of cholesterol and of triglycerides. 4. Cirrhogenic evolution is not significant.

**Ursodeoxycholic acid or lipostabil in the treatment of NASH**

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Nonalcoholic steatohepatitis (NASH) is described as inflammation of the liver, associated with the accumulation of fat in the liver. It is not connected with other causes of chronic liver disease, including hepatitis B. No established treatment exists for this potentially serious disease. Our goal was to study the efficacy of Ursodeoxycholic acid (UDCA) and lipostabil in the treatment of NASH.

20 patients were chosen with NASH, who did not consume substantial quantities of alcohol, who had negative results on serologic examinations for viral hepatitis, who had elevated plasma liver enzyme levels aspartate aminotransferase (AST) and alanine aminotransferase (ALT) who had all features of fatty liver on ultrasound examination (hyperechoic texture or a bright liver), who had hyperlipidemia (hypertriglyceridemia or hypercholesterolemia, or both). The diagnosis of NASH was confirmed with liver biopsy. The histologic features of the condition included macrovesicular fatty infiltration, lobular (acinar) inflammation, apoptosis, Mallory hyaline bodies (in two cases), and fibrosis (in 5 cases).

Ten patients received 13 to 15 mg/kg/d of UDCA, during 6 months. Ten patients with hypertriglyceridemia received lipostabil 1,8g/day during 6 months. 14 women and 6 men entered the study. One of 20 patients (5%) withdrew because of side effects. Despite its lipid-lowering properties, lipostabil was not found to be beneficial in the treatment of NASH. In contrast, Ursodeoxycholic acid therapy significantly reduced plasma levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GTT) triglycerides and cholesterol. Histological grade of steatosis, and inflammation decreased significantly in UDCA group. UDCA has both lipid-altering properties and direct cytoprotective effects. Randomized, controlled trials are required to validate the effect of UDCA therapy in patients with NASH.

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**ASSESSMENT OF INSULIN RESISTANCE AND INSULIN SECRETION IN NASH**

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Hyperinsulinemia and insulin-resistance are increasingly recognized as important associations of NASH. Few studies have compared insulin resistance and secretion in NASH and its common clinical associations - obesity and type 2 diabetes mellitus (DM). **Subjects and Methods: We evaluated insulin resistance and insulin secretion in 20 subjects with NASH—one, 24 subjects with NASH and type 2 DM, 15 subjects with type 2 DM, 15 obese subjects, and 10 lean subjects. Sixteen subjects with NASH were studied before and after a ten-day course of anabolic steroid.**

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>NASH</th>
<th>NASH+DM</th>
<th>TYPE 2 DM</th>
<th>OBESE</th>
<th>LEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA INSULIN</td>
<td>43.19±1.33**</td>
<td>40.14±1.98*</td>
<td>8.92±0.74</td>
<td>11.25±1.3</td>
<td>7.83±1.06</td>
</tr>
<tr>
<td>HOMA - INSULIN</td>
<td>9.88±1.64*</td>
<td>15.87±3.05*</td>
<td>10.61±3.14*</td>
<td>2.61±0.33</td>
<td>1.75±0.28</td>
</tr>
<tr>
<td>RESISTANCE INDEX</td>
<td>544±106**</td>
<td>312±58**</td>
<td>13.18±0.63***</td>
<td>144.7±18.105±16.49</td>
<td></td>
</tr>
<tr>
<td>INDEX INSULIN PULSE</td>
<td>15.1±3.33**</td>
<td>5.98±1.4</td>
<td>3.7±0.7</td>
<td>3.65±0.79</td>
<td></td>
</tr>
<tr>
<td>AMPITUDE uIU/ml</td>
<td>35.22±8.8</td>
<td>20.9±4.2</td>
<td>24.5±5.3</td>
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</tbody>
</table>

=p<0.05 vs Obese and lean; **p<0.05 vs non-NASH subjects, ***p<0.05 vs all other; HOMA = Homeostasis Assessment Model
Treatment with anabolic steroids improved liver enzymes, decreased hepatic fat, and increased hepatic insulin extraction but did not affect plasma insulin levels. **Conclusions:** Insulin resistance in NASH and type 2 DM were similar, but NASH subjects had better islet beta-cell function. Hyperinsulinemia in NASH was mainly due to insulin resistance since enhanced insulin extraction does not result in lower insulin levels. Insulin secretion in NASH remained pulsatile with increased amplitude and area under the insulin pulse. Changes in insulin secretion and action may play a significant role in the pathogenesis and clinical manifestations of NASH.

The immunologic aspects of alcoholic genesis in the development of chronic liver pathology

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The experimental and clinic researches testify to the significant role of alcohol in development of chronic diseases of the liver. The direct toxic influence of ethanol on hepatocytes and the violation of systemic immunity lie in the basis of the organ's affection.

42 men (aged 39-63) having chronic alcohol steatohepatitis with the disease duration of 1-7 years have been observed. The role of the alcoholic affection based on the grounds of a thoroughly analyzed anamneses, clinical features of the liver pathology, and on the evidence of non-liver features of alcoholic intoxication, laboratory data - the absence of infectious hepatitis virus markers in blood, revealed with the help of immunofefermental analysis and polymerase chain reaction. Within the patients examined, the chronic alcoholic steatohepatitis was characterized by minimum (29 persons), average (12) and maximum (~) degrees of activity.

The violation of immune indices showed up with the reduction of the total quantity of T-lymphocytes 60,813.2% (donors 72 0+3 0%, P < 0.05), T-helpers (CD4+) down to 27 2+1 4% (46 0+2 5% - P < 0.05), insignificant of T-suppressors, immunoregulatory coefficient was 1,1+0,06 (2,3+0,14- P < 0.05). The number of B-lymphocytes has not been significantly changed. While examining the humoral link of immunity the rise of IgA 4,8+0,35 g/l (1,8+0,4 - P < 0.05) presence has been found out. The change of Ig classes M and G presence has not been so significant. The presence of circulating immunocomplexes (3,72+0,2 g/l and 1,85+0,1lg/l - P<0,05), because of the more pathogenic of average- and smallmolecular fractions in blood has been increased.

The data obtained testify to the violation of the cell and humoral link of immunity within the chronic affection of the liver of alcoholic genesis, and all these changes depended on the activity and the heaviness of the pathological process.

Changes in the expression of apoptosis related genes in rat liver fibrosis


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Liver fibrosis is characterized by excessive deposition of extracellular matrix and loss of liver function. Necrotic cell death has been described in fibrotic liver, but less is known about apoptotic cell death of hepatocytes. Apoptosis is regulated by cytokine-receptor interactions, such as pro-apoptotic Fas/Fas Ligand and TNF/TNF-α and anti-apoptotic IL40/IL-lORo and by the balance of pro- and anti-apoptotic Bcl-2 family proteins. **Aim:** To investigate the regulation of Bcl-2 family proteins and apoptosis modulating cytokine–receptor couples in liver fibrosis. Methods: Liver fibrosis was induced by bile duct ligation (BDL). Bcl-2 family members and cytokine-receptor couples were determined by RT-PCR and Western blotting. Apoptosis in liver homogenates was determined by caspase-3 assay. Results: after BDL collagen type I ~A expression was strongly increased.

<table>
<thead>
<tr>
<th></th>
<th>mRNA</th>
<th>Control</th>
<th>BDL day8 mRNA</th>
<th>Control</th>
<th>BDL day8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 (anti-apoptotic)</td>
<td>+</td>
<td>++</td>
<td>Fas</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Bcl-xl (anti-apoptotic)</td>
<td>+</td>
<td>+</td>
<td>Fas ligand</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bax (pro-apoptotic)</td>
<td>+</td>
<td>+++</td>
<td>TNF</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bad (pro-apoptotic)</td>
<td>+</td>
<td>++</td>
<td>TNF-R1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Bak (pro-apoptotic)</td>
<td>+1-</td>
<td>++</td>
<td>IL-1O</td>
<td>+/</td>
<td></td>
</tr>
<tr>
<td>Bid (pro-apoptotic)</td>
<td>+1-</td>
<td>+1-</td>
<td>IL-1ORa</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

Expression of Bcl-2 (mRNA and protein), Bax, Bak, TNF, Fas and IL-10Ra were strongly increased. Expression of Bcl-XL and Bid mRNA and protein did not change after BDL. No significant cleavage of Bid was observed. FasL expression was barely detectable. Caspase-3 activity showed a clear induction upon BDL, peaking at day 4 and almost returned to normal levels at day 8. **Conclusion:** Liver fibrosis induced by BDL is accompanied by increased expression of Bcl-2 family members and increased expression of cytokine-receptor couples. These changes resulted in a transient rise in caspase-3 activity, indicating that apoptosis in this model is self-limited.
Ultrastructure of hepatocytes, especially of mitochondrial abnormalities, in non-alcoholic steatohepatitis in children

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The aim of the study was to evaluate the ultrastructure of hepatocytes in liver biopsies obtained from four children (2, 3, 4 and 9-year-old boys) with non-alcoholic steatohepatitis-NASH (idiopathic steatohepatitis) revealed in histologic findings steatosis, inflammation and fibrosis in the absence of clinical and serologic markers of viral hepatitis, autoimmune hepatitis, other identifiable etiologies or excessive alcohol intake.

Material and methods: Fresh small tissue blocks (1mm³ in size) from the liver biopsies were fixed in glutaraldehyde and in the routine way processed for ultrastructural studies and examined using Opton 900 PC microscope.

Results: The most prominent abnormalities in hepatocytes consisted in macrovesicular steatosis (in two patients with associated microvesicular component), polymorphism of mitochondria with defects of mitochondrial cristae, broadening of rough endoplasmic reticulum. Megamitochondria (without special localization within the cell) containing linear crystalline inclusions were evident in three of four patients. These intramitochondrial crystal-like structures of unknown composition had an average diameter of 10 run. Moreover dilatation of the perisinusoidal spaces of Disse and presence of numerous bundles of collagen fibres within them and intercellular spaces, sometimes within basal cytoplasm of steatosed hepatocytes were observed.

Conclusion: Ultrastructural abnormalities of hepatic mitochondria including swelling and development of crystalline inclusions may be morphologic marker for NASH and could indicate either an adaptive process within these organdies or their injury.

Evidence of intestinal bacterial overgrowth in patients with non-alcoholic steatohepatitis

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Introduction: Non-alcoholic steato-hepatitis (NASH) is a well-known risk factor for liver cirrhosis. Although there are accepted metabolic factors leading to development of liver steatosis, factors triggering and maintaining inflammation are not known. Intestinal bacterial overgrowth (IBO) has been suggested by several authors to play a pathogenetic role in patients with NASH. Therefore the aim of this work was to assess the presence of IBO and the effect of non-absorbable antibiotics on liver function tests in patients with NASH. Methods: 10 patients with NASH were recruited. They fulfilled the following criteria: ALT elevation, absence of alcohol intake, abdominal ultrasound with diffuse increase in liver echogenicity and absence of other causes of chronic hepatitis. Biopsy was performed in 5 patients, all of which had steato-hepatitis, with diverse stages of fibrosis, but no cirrhosis. We recruited also a group of 10 controls, matched by sex, age and body mass index. Lactulose breath test, measurement of fasting transaminases, glucose, cholesterol and triglycerides was performed in all patients. The group with NASH received norfloxacin 400 mg daily for 14 days, after which it was repeated lactulose breath test and transaminases. Results: Basal values of breathed H₂ in NASH patients was 10.8 ± 3.3 parts per million (ppm), versus 6.3 ±3.3 ppm (p<0.0084). There were statistically significant differences between patients and controls in breathed H₂ at 15 and 105 minutes after lactulose administration. Orocecal transit time was greater in patients with NASH (127 ± 61 versus 57 ± 23 minutes; p=0.0037). After
norfloxacin administration; there was no significant variation in lactulose breath test or ALT in patients with NASH. **Discussion:** Present data support the hypothesis that NASH can be linked to endotoxin-induced liver damage from intestinal origin. The administration of norfloxacin in this group of patients did not modify IBO demonstrated in lactulose test.

**OXIDATIVE STRESS IN ALCOHOLIC AND VIRAL CHRONIC LIVER DISEASES**

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In liver cells, oxidative stress and oxy-free radicals are formed especially during the alcohol metabolism by the microsomal ethanol oxidizing system, or the reaction of acetaldehyde or parines with xanthine oxidase. Membrane lipid peroxidation represents the most important mechanism among those inducing cellular oxidative aggression and acts through a chain mechanism. Hepatic lipid peroxidation has been shown to be increased after both acute and chronic ethanol consumption and has been implicated in the development of hepatic steatosis and cirrhosis. More recently, lipid peroxidation seems to be intensified also in other inflammatory - for example viral - liver diseases.

We studied the lipoperoxide serum level using the thiobarbituric acid method (Satoh) in 101 patients with chronic liver diseases: 32 with decompensated liver cirrhosis (22 alcoholic and 10 viral), 34 with chronic active hepatitis (17 alcoholic and 17 viral) and 35 patients with compensated cirrhosis or chronic persistent hepatitis. In decompensated liver cirrhosis high levels of serum lipoperoxides were noted especially in alcoholic forms (4.59+/−1.81 nM/ml, p=0.001), as well as in chronic active alcoholic hepatitis (5.63+/−0.89 nM/ml, p=0.001) as compared to 20 controls (1.65+/−0.88 nM/ml) and nonactive liver diseases (1.40+/−0.13 nM/ml). In the viral decompensated liver cirrhosis (2.65+/−0.68 nM/ml, p=0.01) and active hepatitis (3.09+/−2.12, p=0.01) the lipoperoxides levels were also higher than compensated cirrhosis or chronic persistent hepatitis.

The results of our study demonstrate a good correlation of the lipoperoxide level to disease activity and suggest the involvement of oxidative stress not only in the pathogenesis of alcoholic but also in the viral hepatic induced damage in humans.

**EFFECTS OF CLOFIBRIC ACID ON THE FUNCTIONS AND ULTRASTRUCTURE OF RAT MITOCHONDRIA AND PERFUSED LIVER**

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Fibrates are a class of aromatic compounds used as hypolipemic drugs in humans. In rat, their most conspicuous effect is peroxisomal proliferation. The significance of this phenomenon is not entirely clear, since it is not evident in all mammals, including guinea pigs and humans. For clarifying this aspect, we studied the effects of clofibrac acid (CA) at biochemical and ultrastructural level on hepatic mitochondria and perfused liver of the two laboratory species. The present abstract refers to the data obtained on rat.

Respiration rates, phosphorylation ability and mitochondrial membrane potential were estimated as previously described for the effects of other drugs (Tarba and Craciun: B.B.A. 1019, 1990, 19-28). Gluconeogenesis and ketogenesis in the perfused liver were also assayed as described (Petrescu and Tarba: B.B.A. 1318, 1997, 385-394). Electron microscopy was performed on samples of hepatic mitochondria and liver pieces according to standard procedures.

Upon addition of increasing concentrations of clofibrac acid (0.05-0.2 mM) to isolated hepatic mitochondria, one can observe a slight increase of state 2 (state 4) respiration with a consequent decrease of respiratory control ratio and phosphorylation ability, an instability of succinate-generated membrane potential, a rarefaction of mitochondrial matrix, swelling, inhibition of glucose synthesis and stimulation of lipid transit.

In subchronic treatments (20 mg CA/100 g body weight/day, for 7 days), one can observe no effects on oxidative phosphorylation, but a moderate depletion of lipids occurs, associated with ultrastructural changes, such as hypertrophy of the nucleolus and smooth endoplasmic reticulum and above all a very strong peroxisomal proliferation.

**Nonalcoholic steatohepatitis and insulin resistance metabolic syndrome.**

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**Background:** Nonalcoholic steatohepatitis (NASH) is a disease of emerging importance, and is now considered as one of the commonest liver diseases in Western Countries. This condition is frequently associated with Type II diabetes, hyperlipidemia and obesity. Recent studies suggest insulin resistance may be an important feature of NASH. **Aims:** The aim was to investigate the relationship between NASH, insulin resistance
and body mass index (BMI). Furthermore, to evaluate disturbances in iron studies in this cohort of patients. **Methods:** Consecutive patients with liver biopsy proven NASH were recruited from the Liver Clinic. Investigations included standard anthropometric measurements, fasting blood sugar levels, insulin and C-peptide levels as well as iron studies. **Results:** A total of 24 patients were evaluated of which 54% were male. The mean age was 43 years. The mean body mass index in this cohort was 32.8. Overall 48% of patients were obese BMI males > 31.1; Females > 32.3. C-peptide and insulin levels were elevated in 94% and 59% respectively (Reference values: c-peptide < 1.8 mcg/l, Insulin < 21 mlt/lml), while elevated blood glucose levels were observed in 21% (> 5.9mmo1/L). Elevated ferritin (> 250 mcg/L) was detected in 52% of patients. **Discussion/Conclusions:** While blood glucose levels did not fulfill the criteria for diabetes mellitus, there is a strong evidence of insulin resistance as indicated by high insulin/c-peptide levels. This may be related to the fact that the mean age of this cohort is younger than most of the published literature and many may subsequently present with overt hyperglycemia. There appears to be a significant association between NASH and features of the metabolic syndrome, particularly insulin resistance. Elevated levels of ferritin are also observed in patients with NASH, although none of the patients were homozygous for the FIFE gene. Iron may be important in the liver injury associated with NASH and this requires further investigation.

**Steatohepatitis in patients undergoing laparoscopic cholecystectomy for cholelithiasis.**

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**Background:** Liver function tests are frequently found to be abnormal in patients requiring cholecystectomy. The clinical profiles of patients with cholelithiasis and NASH have strong similarities (female predominance, frequently obese, hyperlipidemia). **Aim:** The aim of this study was to assess the prevalence of underlying steatosis and steatohepatitis in patients undergoing laparoscopic cholecystectomy when the operative cholangiogram was normal, but the pre-operative liver function tests (LFTs) were abnormal. **Methods:** Consecutive patients undergoing laparoscopic cholecystectomy with normal operative cholangiogram and serial abnormal LFTs were enrolled in the study. All patients had a Trucut liver biopsy at the time of laparoscopic cholecystectomy. Patients with a preoperative diagnosis of an organic liver disease including positive serology for hepatitis B and C were excluded from analysis. **Results:** 68 patients were included in this study with 53% females and a mean ± SEM age of 47 ± 1 yrs. There were no post-operative complications associated with the biopsy. Histological assessment revealed that 25% had steatosis, 40% mild to moderate steatohepatitis, and 12% had steatohepatitis with advanced fibrosis/cirrhosis. Another hepatic disorder was identified in 4% of biopsies (haemochromatosis, lymphoma and autoimmune liver disease). Investigation for etiological causes identified a history of alcohol abuse in 13%. Furthermore, in those with histological evidence of steatosis, 81% either had an underlying metabolic disorders such as obesity, diabetes mellitus and/or hyperlipidemia. Another hepatic disorder was identified in 4% of biopsies (haemochromatosis, lymphoma and autoimmune liver disease). Investigation for etiological causes identified a history of alcohol abuse in 13%. Furthermore, in those with histological evidence of steatosis, 81% either had an underlying metabolic disorders such as obesity, diabetes mellitus and/or hyperlipidemia. **Conclusion:** The high prevalence of significant histological abnormalities in this select cohort of patients is an indication that liver biopsy in this group of patients should be a routine procedure to allow further management, appropriate follow up and referral where necessary.

**PROTECTION OF ETHANOL INDUCED MEMBRANE DAMAGE BY UR SODEOXYCHOLIC ACID (UDCA)**

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**Introduction:** Previous studies have shown that UDCA has protective effects on ethanol induced cell damage. Electron microscopic investigations have suggested a much less enlargement of smooth endoplasmatic reticulum and mitochondria on ethanol incubated Hep 02 cells (1). Furthermore increased amounts of cytokines on Hep G2 cells induced by ethanol, show a significant UDCA dependent reduction (2). In this study we investigated the effect of UDCA on membrane damage induced by ethanol.

**Methods:** Phosphatidylcholine (PC) liposomes with different UDCA contents { 10% (w/w), 20% (w/w)} in the vesicle membrane and entrapped 6-carboxyfluorescein (6-CF) were prepared by sonication. 1. Liposomes without UDCA containing membranes were incubated with
different concentrations of ethanol (1% (v/v), 5% (v/v), 7.5% (v/v), 10% (v/v), 20% (v/v)) and the release of entrapped 6-CF was measured by increase of fluorescence. The experiments were repeated with liposomes containing 10% (w/w) and 20% (w/w) of UDCA.

**Results:** Ethanol shows significant damage on membranes made of phosphatidylcholine. Membranes containing 10% (w/w) UDCA show less losses of entrapped 6-CF. The amount of 6-CF-release at 1004 (v/v) ethanol on PC-liposomes is 26.8% (p < 0.0001 vs control) versus a loss of 6-CF of 18.3% (p < 0.0001 vs control) at liposomes including 10% (w/w) UDCA in their membrane. The highest decrease of ethanol induced damage with a release of 14.2% 6-CF (p < 0.0001 vs control) was observed with 20% (w/w) UDCA in the bilayer. 100% loss of entrapped 6-CF is reached after incubation with 10% Triton X-100.

**Conclusions:** The incorporation of UDCA into PC-liposomes stabilizes the membrane structure and decreases 6-CF-liberation from the interior. The data are in accordance with previous investigations using electron paramagnetic resonance spectroscopy (3).

(1) Neuman Ct al., Gastroenterology 1995; 109: 555-563