network and investigating the possibility of regenerative therapy for patients with neurobehavioral deficits of FASD. We have shown the potential migration of transplanted NSCs into the brain by visualizing a fluorescent cell marker and radioisotope, as well as the possible recovery of behavioral abnormalities observed in FASD model rats including the impairment of anxiety-like behavior, memory/cognitive function and social interaction.

In the present study, for the purpose of investigating the neurobiological mechanism of behavioral change by NSC transplantation, we assessed the characteristics of transplanted cells and interneurogenesis function in some brain field. Using immunohistochemical analysis, it was revealed that the GABAergic and/or cholinergic interneurons were increased in amygdala, DG, cingulated cortex and putamen areas in the model rat. In addition, in the amygdala and cingulated cortex, intravenous NSC transplantation appeared to reorganize the expression of post-synaptic density protein 95 (PSD95) which was decreased in FASD model rats. These results indicate that intravenous NSC transplantation might have the potential to become a new therapeutic approach for treatment of FASD patients, by their effect on neural network repair activity, through the increase in the interneurogenesis and synapse formation.

**P32**

**BINGE DRINKING AND/OR CHRONIC NICOTINE ADMINISTRATION ALTERS EXTRACELLULAR GLUTAMATE AND ARGININE LEVELS IN THE NUCLEUS ACCUMBENS OF ADULT MALE AND FEMALE WISTAR RATS**

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The effect of binge drinking coupled or not with chronic nicotine administration on the release of glutamate, arginine, taurine and hydroxyl radical from nucleus accumbens (NAc) has been studied in male and female adult rats administered a binge-type regime of ethanol, 2 or 3 g/kg for 3 weeks, ± nicotine.

The basal concentration of NAC glutamate increased 8-fold in the female adult rats but did not change significantly after further doses of ethanol. In contrast, the male adult rats showed no changes in basal glutamate content but exhibited a dose-dependent increase in NAC glutamate after further doses of ethanol. NAC arginine basal levels decreased significantly in both male and female adult rats after further doses of ethanol. Co-administration of nicotine modified the toxicity of ethanol as exemplified by diminishment of both the basal NAC glutamate release and modifying the release of this excitatory amino acid after further ethanol doses, particularly in female rats. In addition, the dramatic changes in arginine release after further ethanol doses were less evident. There was no evidence for increased hydroxyl radical production in the NAC after binge drinking ± nicotine. There appeared to be a greater vulnerability to ethanol toxicity in female adult rats after binge drinking. It remains unclear whether the increased release of glutamate during the microdialysis evokes activation of iNOS which would utilize arginine in the formation of nitric oxide.

**P33**

**BRAIN NGF AND BDNF ALTERATIONS IN AN FASD MOUSE MODEL**

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Ethanol exposure during pregnancy is one of the major causes of mental retardation in western countries by inducing fetal alcohol spectrum disorders (FASD). It has also been shown that neurotrophic factors as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are severely affected by ethanol during pre- and post-natal life and they may have a major role in FASD onset. The aim of the current study in an FASD mouse model was to investigate brain alterations in NGF and BDNF due to chronic early exposure to ethanol solution (11% vol) or to red wine at the same alcohol concentration starting from 60 days before pregnancy up to pups weaning. Data revealed no differences between groups of dams in pregnancy duration, neither in pups delivery, pups mortality and sex ratio. Data also showed that stress due to early ethanol exposure in adult animals disrupted the levels of both NGF and BDNF in the hippocampus and other brain areas. This profile was associated with impaired ChAT immunopositivity in the septum and nuclei basalis and with altered cognition and emotional behavior. Quite interestingly, mice exposed to red wine had no change in the behavior or in ChAT immunopositivity but a decrease in hippocampal BDNF and a mild NGF decrease in the cortex. Also NGF-induced neuritic outgrowth in PC-12 cells was still present when exposed to red wine but not when exposed to ethanol solution only. Data suggest differences in ethanol-induced neurototoxicity between red wine and ethanol solution only.

**INTERNAL MEDICINE**

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**P34**

**THIAMINE DOSE FOR SUSPECTED WERNICKE ENCEPHALOPATHY?**

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There appears no clear consensus about what dose of thiamine is best indicated for the treatment of acute Wernicke encephalopathy. Classical textbooks and some literature refer to doses of 50–100 mg being required even though these doses exceed the recommended daily nutritional requirement. Recently, in Australia, a trend toward giving even higher thiamine doses has been observed, e.g. 200 mg three times daily, often parenterally administered. Data are presented from a survey of hospital physicians indicating that Wernicke encephalopathy is still considered under-detected, often an uncertain diagnosis and that thiamine dosing selection is quite variable. This presentation briefly reviews the evidence concerning thiamine dose selection and then discusses the risk/benefit merits of either high or low-dose selection. The case for high-dose thiamine usage is strongly advocated.

**P35**

**EFFECT OF N-ACETYL-L-CYSTEINE AND GLUTAMINE-BASED COMPOSITION ON ALCOHOL-INDUCED METABOLIC AND MORPHOLOGIC LIVER INJURIES**


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Since activation of free radical processes plays a crucial role in the damaging effects of ethanol on the liver, we studied the effects of glutamine in combination with N-acetyl-cysteine that influence ethanol and acetaldehyde metabolism and possess antioxidant properties, on the hepatotoxicity induced by ethanol. Wistar male rats consumed a liquid alcohol diet in which ethanol produced 36% of calories during 6 weeks. Following that we studied the effect of 2-week intragastric administration of N-acetyl-cysteine (100 mg/kg) in combination with glutamine (340 mg/kg) on the metabolic and morphologic alterations induced by alcohol. One of the control groups received a standard laboratory diet, while the second-group rats consumed a liquid alcohol-free diet. It was shown that N-acetyl-cysteine in combination with glutamine normalized blood serum ASAT and GOT activities, decreasing them to the control levels. The administration of N-acetyl-cysteine and glutamine combination decreased blood serum concentrations of total lipids, triglycerides, cholesterol and total bilirubin in comparison with alcoholized placebo-treated animals. The content of liver triglycerides was significantly elevated after chronic alcohol intoxication as opposed to the control groups receiving either the liquid alcohol-free diet or a standard laboratory diet. After the 2-week administration of the composition studied, the liver triglyceride level was significantly lower than that found immediately after chronic alcohol intoxication and did not differ from the controls on the liquid alcohol-free diet. The majority of the animals consuming the liquid alcohol diet (6 rats of 8–75%) showed pronounced centrilobular microvesicular hepatocyte steatosis, and signs of hepatitis and focal necroses were sometimes noticed. The N-acetyl-cysteine and glutamine combination normalized the liver morphologic picture in the majority of alcoholized animals (in seven rats of 8–87.5%). Therefore, a hepatoprotective effect of the N-acetyl-cysteine and glutamine-based composition was demonstrated using the model of chronic alcohol intoxication. These data support the pharmacological potential of the above substances in the management of alcoholic liver injury and as agents for metabolic therapy during a post-intoxication period.
P36

**EFFECT OF GLUTAMINE AND N-ACETYL-CYSTEINE ON THE SYSTEMS OF ALCOHOL AND ALDEHYDE METABOLISMS IN THE LIVER OF RATS FOLLOWING ALCOHOL INTOXICATION**

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Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the key enzymes in alcohol and aldehyde metabolisms. Under alcohol intoxication, these enzymes are involved in metabolisms of ethanol and its toxic metabolite, acetaldehyde. This causes impairments in metabolisms of other alcohols and aldehydes. Chronic ethanol intake induces accumulation of malondialdehyde and oxynonenal, which, in turn, can inactivate ALDH. The aim of this work was to study the effect of glutamine and the antioxidant N-acetylcysteine on rat liver ADH and ALDH in chronically intoxicated rats. Male Wistar rats with the initial body weight of 180–250 g were used in the experiment. Alcohol intoxication induced according to Lieber C.S. by a liquid ethanol diet (36% of the total caloric value from ethanol). Control animals received a similar diet in which ethanol was substituted by sucrose. The average ethanol consumption with the diet was 18 g/kg body weight per day. The animals were intragastrically administered with N-acetylcysteine (100 mg/kg) + glutamine (140 mg/kg) during 14 days after 40 days of intoxication. From the day of the substance administration, the liquid alcohol diet was substituted by the liquid control diet. ADH and ALDH activities were determined spectrophotometrically using ethanol and acetaldehyde as substrates. Alcohol intoxication induced activation of ALDH with high Km for acetaldehyde and did not affect the intramitochondrial enzyme. Ethanol withdrawal significantly decreased the activity of high Km ALDH. The glutamine and N-acetylcysteine treatment during withdrawal dramatically activated ADH and normalized the activity of ALDH with high Km. Since it is known that N-acetylcysteine prevents formation of SH-group cross-links, it can be suggested that the activation was induced by protection of SH-groups in the ADH and ALDH active centres from toxic aldehydes which accumulate during the ethanol withdrawal period. Thus, glutamine and N-acetylcysteine protect the system of ethanol and aldehyde metabolisms from degradation after chronic alcohol intoxication.

P37

**CHRONIC ETHANOL CONSUMPTION STIMULATES DIETHYLNITROSAMINE-INITIATED HEPATIC CARCINOGENESIS**

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Background. Obese insulin-deficient, Leptin-deficient Zucker rats (fa/fa) develop spontaneously metabolic syndrome and fatty liver. It has been shown that obese Zucker rats exhibited increased hepatic oxidative stress and showed a significant increase in carcinogenic exocyclic etheno-DNA adducts when compared with their lean littermates (Wang et al., *Hepatology* 50,453:2009). Therefore, we wondered whether such an effect could also be observed in an animal model in which steatosis is induced by diets and which resembles better the human situation.

Methods. In the present study, male Wistar rats received a semi-synthetic high-fat, choline, methionine-deficient (HFCMD) diet. After 10 weeks of feeding, the animals were killed. Rats with the HFCMD diet developed fatty liver with inflammation. In the serum ALT, AST, cholesterol, tumor necrosis factor-α (TNF-α), adiponectin and leptin were determined. In the liver, cholesterol and triglyceride were measured and in addition exocyclic etheno-DNA adducts were immunohistologically determined.

Results. The HFCMD diet results in a significant increase in ALT- and AST-activity and in TNF-α, while serum adiponectin and leptin were found to be significantly decreased. In addition, significant increased levels of carcinogenic exocyclic etheno-DNA adducts were also detected in the livers of the rats with the HFCMD diet when compared with their controls.

Conclusions. Non-alcoholic fatty liver induced by HFCMD diets is associated with an increased level of carcinogenic DNA adducts deriving from oxidative stress. This may explain at least in part the carcinogenic potential of this liver disease.

P38

**ENHANCED GENERATION OF CARCINOGENIC DNA LESIONS IN RATS FED A HIGH FAT CHOLINE, METHIONINE-DEFICIENT DIET**

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Conclusions. Non-alcoholic fatty liver induced by HFCMD diets is associated with an increased level of carcinogenic DNA adducts deriving from oxidative stress. This may explain at least in part the carcinogenic potential of this liver disease.

P39

**HISTOLOGICAL DETERMINANTS OF INCREASED LIVER STIFFNESS IN ALCOHOLIC LIVER DISEASE**

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Background. Transient elastography (Fibroscan) is a rapidly expanding non-invasive technique to assess liver fibrosis. Besides matrix deposition, several other factors such as inflammation or hepatic congestion are known to affect liver stiffness (LS). Thus far, histological determinants of LS are poorly understood.

Methods. LS was measured in 103 patients with histologically confirmed alcoholic liver disease. Liver fibrosis was assessed by two histological scores (Kleiner versus semiquantitative Chevallier score). In addition, collagen stain was quantified using a morphometric computer-assisted analysis of collagen stain (Sirius red). LS was further correlated with histological subscores for steatosis, inflammation and other ALD features.

Results. The semiquantitative Chevallier (0.800, all P < 0.001) correlated higher with LS than the Kleiner score (0.719). Both histological scores correlated better when compared with morphometry. Interestingly, within the Chevallier subscoring system, number and width of septa alone were highly correlated with LS (0.772 and 0.706). Classical features such as Mallory’s hyaline (0.367), ballooning (0.347) and lobular inflammation (0.248) also correlated well with LS. In contrast, steatosis, portal inflammation, number of megamitochondria and glycogenated nuclei were not related to LS.

Conclusion. Histological subanalysis indicates that, besides collagen deposition, morphological indications for volume increase as lobular inflammation and ballooning are important determinants of LS.

P40

**CORRECTION OF METABOLIC DISTURBANCES BY COMBINATION OF URSODEOXYCHOLIC AND OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RATS WITH ALCOHOLIC STEATOHEPATITIS**

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Background. Chronic excessive alcohol consumption is a risk factor for hepatocellular cancer (HCC). However, studies in animal models have yet to conclusively determine whether ethanol acts as a tumor promoter in HCC. We examined whether prolonged alcohol consumption alone could act as a hepatic tumor promoter after tumor initiation by low-dose diethylnitrosamine (DEN) in a rat model.

Methods. DEN/kg body weight 1 week before introduction of either an ethanol liquid diet or an isocaloric non-ethanol liquid diet. Hepatic histology was assessed after 10 months. Hepatic carcinogenic exocyclic etheno-DNA adducts were shown that obese Zucker rats exhibited increased hepatic oxidative stress and showed a significant increase in carcinogenic exocyclic etheno-DNA adducts when compared with their lean littermates (Wang et al., *Hepatology* 50,453:2009). Therefore, we wondered whether such an effect could also be observed in an animal model in which steatosis is induced by diets and which resembles better the human situation.

Results. The HFCMD diet results in a significant increase in ALT- and AST-activity and in TNF-α, while serum adiponectin and leptin were found to be significantly decreased. In addition, significant increased levels of carcinogenic exocyclic etheno-DNA adducts were also detected in the livers of the rats with the HFCMD diet when compared with their controls.

Conclusions. Non-alcoholic fatty liver induced by HFCMD diets is associated with an increased level of carcinogenic DNA adducts deriving from oxidative stress. This may explain at least in part the carcinogenic potential of this liver disease.
Ursodeoxycholic acid (UDCA) is widely used for treatment of cholestatic liver diseases. Recent studies have shown positive results of UDCA in patients with alcoholic liver disease. The basic mechanism of hepatoprotection by UDCA is prevention of liver membrane structure damage. Polyunsaturated fatty acids also improve liver membrane structural and functional properties. We tried to study the effects of UDCA, ω-3 polyunsaturated fatty acids (PUFA) and their combination on the development of alcoholic steatohepatitis in rats fed ethanol-containing high-fat Lieber–DeCarli diet (LCD). Male Wistar rats (180–200 g) were divided into six groups: first, intact control; second, ethanol-free LCD—8 weeks; third, LCD—8 weeks; fourth, LCD + PUFA (300 mg/kg)—8 weeks; fifth, LCD + UDCA (40 mg/kg)—8 weeks; sixth, LCD + UDCA (40 mg/kg) + PUFA (300 mg/kg)—8 weeks. After 8 weeks of the experiment, animals were decapitated. Liver histology improved in rats treated with UDCA and their combination. The results showed that the treatment with either PUFA, UDCA, or the combination significantly reduced liver sudanophilic area and triglycerides content. The treatment with both PUFA and UDCA + PUFA decreased the serum activity of ALT and triglyceride content as well as the levels of TNF-α in liver lymphocytes. UDCA, PUFA and UDCA + PUFA significantly reduced the number of circulating immune complexes and γ-globulin fraction in serum. Thus, PUFA and UDCA developed an anti-inflammatory effect in liver tissue and prevented alcoholic steatosis. The anti-inflammatory effect of the combination was more pronounced when compared with both monopreparations. The demonstrated immunomodulatory and hepatoprotective properties of this combination may be proposed for introduction in the clinic as a promising tool for a treatment and prevention of alcoholic liver disease.

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EFFECT OF MERCASOLYL-INDUCED HYPOTHYROIDISM ON ETHANOL OXIDATION RATE AND ANTIOXIDANT STATUS IN THE LIVER OF RATS WITH CHRONIC ALCOHOL INTOXICATION

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The administration of mercasolyl (15 mg/kg) to rats caused a decrease in blood serum T3 and T4 concentrations, which confirms development of hypothyroidism in the animals. Chronic alcohol intoxication was induced by administration of a 25% ethanol solution at a dose of 4 g/kg (i.e., 8 weeks). The state of hypothyroidism was maintained by daily administration of mercasolyl at a dose of 10 mg/kg till the end of the experiment. A significant elevation of alcohol dehydrogenase (ADH) activity was found in the liver of ethanol-treated hypothyroid rats. The level of ethanol in the blood of hypothyroid rats was decreased by 52% after the ethanol load. The data obtained can indicate elimination of the inhibitory effect of thyroid hormones on ADH activity after mercasolyl administration. The intensity of hydroxyl radical formation by isolated liver microsomes of alcoholized animals was higher by 15% in comparison with euthyroid rats. After the mercasolyl and ethanol treatments, the formation of hydroxyl radicals was reduced by 18% when compared with animals treated only with ethanol. The mercasolyl administration elevated the concentration of reduced rat liver glutathione. The chronic alcohol intoxication in hypothyroid animals was accompanied by a significant increase in reduced glutathione level, whereas the MDA liver concentration remained unchanged, and it was significantly lower as opposed to the group of euthyroid ethanol-treated animals. Glutathione reductase activation might contribute to the increase in the reduced glutathione level. The mercasolyl administration caused a significant increase in rat liver glutathione transferase activity, and ethanol contributed to further enzyme activation. Under chronic alcohol intoxication, the experimental animal groups did not significantly differ in catalase, glutathione peroxidase and superoxide dismutase activities. It is suggested that the mercasolyl-induced decrease in thyroid hormone levels prevents development of oxidative stress in the liver of rats with chronic alcohol intoxication.

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ALCOHOLIC PATIENTS VALUED FOR LIVER TRANSPLANT: NEW PREDICTIVE FACTORS OF RELAPSE

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Background and aims. The alcoholic cirrhosis is a consolidated indication to the liver transplant (OLT) and is the first indication in Europe. Ninety-five percent of patients with end-stage alcoholic liver disease has never been formally valued for liver transplant. A documented alcoholic abstinence of almost 6 months is strictly necessary in order to be included in a waiting list. It is important to underline that some factors such as age, socio-economic stability, absence of consumption of other substances have turned out to be prognosis positive factors for the maintaining of post-transplant abstinence. The object of our research is to identify new relevant predictive factors of relapse in these patients.

Methods. Since 2004 to date, we have been valuing 231 men and 40 women (total 271 patients) aged 23–68, affected by liver cirrhosis, in order to set alcoholism diagnosis according DSM-IV criteria and to monitor, as well as sustain abstinence in pre- and post-OLT. Data analysis was performed by using SPSSW 18.

Results. 96.3% of patients presented alcoholic dependence diagnosis: 12.9% abuse; 2.6% active polyabuse, while just 1% of patients turned out non-drinker. The average age of first contact with alcoholic beverages was around 15; the risk consumption period was within those aged 25–27, with average consumption of 9 UA/die and maximum of 15 UA/die. Fifty-five percent of patients presented positive familiarity for alcoholism. 53.9% of the sample were smokers, 29.9% of patients consumed illicit drugs in the past; among them 9.2% came out positive to the toxicological exam. A high number of patients (78.9%) presented a stable family support, fundamental for the compliance pre- and post-OLT. The percentage of patients without scholastic failures was 48.3%.

Conclusions. The relapse percentage of our sample in pre- (18.5%) and post (13.5%) OLT is lower than the data present in literature; this can be due to the identification of new predictive factors of relapse (positive familiarity for alcoholism, premature first contact, risk consumption years, scholastic failures) as well as to a strict monitoring with specific medical management in a specialist alcohol service. Hence, the importance of the figure of the specialist in alcoholism in transplant team.
HISTONE DEACETYLASE INHIBITOR IN A MODEL OF ALCOHOL DEPENDENCE

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The development of effective treatments for alcoholism is a high public health priority. Alcoholism is dependent upon both genetic and environmental factors and occurs over time, requiring neuronal adaptations. One area of intensive research aimed at identifying the long-term neuro-adaptations in the regulation of changes in gene expression induced by alcohol consumption. The molecular mechanisms which control expression of genes, known as epigenetic mechanisms, have been underappreciated in the nervous system. Recent studies provided evidence suggesting the importance of chromatin remodeling in controlling gene transcription and revealed its possible implication in cocaine addiction and in alcohol withdrawal-induced anxiety. Epigenetic therapeutic strategies for the treatment of neuropsychiatric diseases such as schizophrenia, Huntington’s disease, anxiety, depression and addiction have been suggested.

We investigated the effect of a histone deacetylase inhibitor (HDACi) on different components of ethanol addiction. This dependence has been induced in rats by chronic and intermittent exposure to ethanol vapors. To further investigate the role of brain chromatin remodeling caused by histone modifications, we studied the expression of histone acetylation in this model of alcohol dependence. Preliminary results show changes in histone acetylation in the central amygdala (CeA) and the basolateral amygdala (BLA) in dependent rats compared with non-dependent. Treatment with an HDACi might balance these changes and correlates with behavioral data that suggest that the pharmacological modulation of epigenetics attenuated different components of alcohol addiction. Thus, HDACi might be an interesting target in the development of new treatments in alcoholism.

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ETHANOL CONCENTRATIONS OF PROBIOTIC AND BASIC DIETARY PRODUCTS: A POTENTIAL RISK FOR UPPER DIGESTIVE TRACT CANCER

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Background. Acetaldehyde (ACH) formed endogenously from ethanol was recently classified as a Group I carcinogen. Many microbes representing normal oral flora are able to oxidize ethanol to ACH locally in the oral cavity. This pathway is activated already at very low ethanol concentrations. Fermented dairy products, especially yoghurts, may contain ethanol levels that are more than enough for local microbial ACH production. Moreover, lactic acid bacteria added to probiotic dairy products can also metabolize ethanol to ACH. Thus, these ethanol-containing products may expose the upper digestive tract to endogenously formed ACH. Therefore, the aim of this study was to measure ethanol concentrations of different fermented dairy products either with or without probiotics.

Methods. Ethanol concentrations were determined from 21 different fermented dairy products: 13 yoghurts, 4 drinkable yoghurts and 4 sour milk products. Five yoghurt products contained added probiotic lactic acid bacteria (isolates of lactobacillus, bifidobacterium and propionibacterium). Ethanol was analyzed from the samples by headspace gas chromatography. Results. Ethanol was found in every product. The mean ethanol concentration in yoghurts was 0.19 ± 0.06 mg/ml, in drinkable yoghurts 0.19 ± 0.04 mg/ml and in sour milk products 0.56 ± 0.14 mg/ml. There was no significant difference in the ethanol concentrations of dairy products with and without added probiotic bacteria (0.47 ± 0.39 vs. 0.46 ± 0.25 mg/ml, with vs. without probiotics, P = 0.092).

Conclusions. Considering that there was no difference in the ethanol concentration in probiotic and normal dairy products, these probiotic products do not seem to cause more risk for local ACH exposure in the upper digestive tract than normal dairy products. However, in every product analyzed, we did find some levels of ethanol which can potentially be metabolized to ACH by oral microbes. Furthermore, probiotics are able to colonize the gut for a long period of time and thus produce ACH locally in the digestive tract both from ingested ethanol and glucose and thereby increase the total exposure to ACH. The classification of endogenously formed ACH as a Group 1 carcinogen to humans warrants for further studies on ethanol and probiotic containing dairy products.

ALTERATIONS TO LIVER ENERGY METABOLISM AND CELL DEATH FOLLOWING ALCOHOL EXPOSURE

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The precise mechanisms of alcoholic liver disease (ALD) in relation to tissue injury/cell death at the initial fatty liver stage remains poorly understood. A key feature in the development of ALD is diminished capacity of the liver to generate and maintain mitochondrial adenosine 5’-triphosphate (ATP) levels. This decreased bioenergetic capacity leads to decreased hepatocyte viability; however, the proportion of cells dying by necrosis and apoptosis is not known, or changes to the mitochondrial membrane potential, a key indicator of cell death. Therefore, the focus of this study was to determine hepatic cell death in relation to bioenergetic capacity using HepG2 (VA-13) cells over-expressing alcohol dehydrogenase. VA-13 cells were cultured in Dulbecco’s modified eagle medium containing either high glucose or galactose and exposed to increasing concentrations of alcohol (50–200 mM) for 24, 48 or 72 h. The effect of alcohol on ATP levels was determined using a Luciferase assay and the mitochondrial membrane potential was measured by flow cytometry using rhodamine 123. Lactate dehydrogenase (LDH) release and trypan blue dye uptake were utilized as markers of necrosis, and externalization of phosphatidyl serine (PS) detected by annexin V-fluorescein isothiocyanate staining was used as a marker of apoptosis. Following alcohol exposure, glucose grown VA-13 cells demonstrated a significant decrease in cellular ATP levels with time and concentration (24 h: 7–10%; 48 h: 13–29%; 72 h: 22–33%). A similar pattern was also observed with the mitochondrial membrane potential following alcohol exposure (24 hr: 29–115%; 48 hr: 26–115%; 72 h: 26–111%). LDH leakage markedly increased with elevated alcohol concentration (24 h: 38–85%; 48 h: 99–141%; 72 h: 66–97%) mainly occurring at 48 h, and a similar trend was also evident with trypan blue dye uptake, providing evidence of significant necrosis. There was also a significant increase in apoptosis with...
increasing concentrations of alcohol (24 h: 21–162%; 48 h: 76–182%; 72 h: 36–111%). In conclusion, alcohol exposure caused maximum cell death at 48 h, with cells showing some recovery by 72 h. These findings highlight the importance of hepatic bioenergetic function and the involvement of the alcohol dehydrogenase pathway in the early stages ALD.

**P48**

**EFFECT OF GLUTAMINE-CONTAINING Dipeptides ON THE LEVELS OF BRAIN TISSUE AMINO ACIDS DURING ACUTE ALCOHOL INTOXICATION IN RATS**

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The effects of glutamine and the glutamine-containing dipeptides, t-alanlyl-t-glutamine and glycyl-t-glutamine on free amino acids were studied in the different regions of rat brain during acute alcohol intoxication. Adult male Wistar rats were used. The acute ethanol intoxication was induced by a single i.p. injection with 20% w/v ethanol solution at a dose of 3.5 g/kg body weight. Control animals received saline. t-glutamate (146 mg/kg), t-alanyl-t-glutamine (216 mg/kg), glycyl-t-glutamine (203 mg/kg) or an equimolar amount of saline was injected intraperitoneally 5 min before the ethanol injection. Rats were decapitated after 30 min following ethanol administration. Amino acids were measured by HPLC/FD in the large hemispheres, brain stem and cerebellum. During acute ethanol intoxication, the rat brain structures showed a considerable imbalance among neurotransmitter amino acids. The glutamate levels were significantly decreased in the large hemispheres, brain stem and cerebellum, whereas the aspartate concentrations were diminished only in the brain stem. On the contrary, the GABA level was elevated in the large hemispheres and cerebellum. The amino acid imbalance during acute ethanol intoxication was most marked in the brain stem. Glutamine, t-alanyl-t-glutamine and glycyl-t-glutamine significantly decreased the raised brain GABA levels and increased glutamate and aspartate concentrations to normal values. The results obtained show the ability of t-glutamine and its dipeptides to normalize the levels of both excitatory and inhibitory neurotransmitter amino acids in the hemispheres, brain stem and cerebellum of rats during acute alcohol intoxication. We showed previously that these compounds significantly decreased the severity of ethanol withdrawal in rats, normalized neurotransmitter amino acid levels in various brain regions during alcohol withdrawal, and diminished the voluntary ethanol consumption in rats. These findings suggest that glutamine and glutamine-based dipeptides might possess some therapeutic benefits in treatment for alcohol-use disorders.

**POSTER SESSION 2: MARKERS, PSYCHIATRY AND TREATMENT**

**MARKERS**

doi:10.1093/alcalc/agr119

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**BIOMARKERS DEMONSTRATE INCREASED CONSUMPTION BUT NOT ABUSE OF ETHANOL IN ESSENTIAL TREMOR**

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**Background.** Essential tremor (ET) is one of the most common neurological disorders. ET is characterized by postural and kinetic tremor in the upper extremities, head and less commonly other areas. A distinctive feature of ET is its responsiveness to ethyl-alcohol. The temporary amelioration of tremor following ingestion of a small amount of alcohol has been reported in 45–75% of ET patients. Increased incidence of alcoholism has been suspected in ET; however, no objective evaluation has been performed using laboratory markers to date. Only self-reported data obtained by questionnaires were assessed in all previous studies. We therefore elected to undertake an assessment of alcohol consumption in ET patients, using both questionnaires and laboratory markers of alcohol intake.

**Methods.** Data on alcohol intake in the last 30 days were acquired in 95 ET patients and 35 healthy controls. Blood and urine markers related to alcohol metabolism and liver function were evaluated.

**Results.** The intentional use of alcohol to suppress tremor was reported by 25.5% of patients. Mean alcohol intake in last 30 days was 17.8 ± 20 units in ET patients and 16.5 ± 16 units in controls. No signs of alcohol dependency were recorded in any of the patient or control subjects. The levels of blood-borne biomarkers tended to be higher in the ET than in controls, but the difference was only significant for carbohydrate-deficient-transferrin (CDT) (P < 0.05, corrected), with mean CDT in the ET group found to be 2.22 ± 0.7% and in controls 1.78 ± 0.8%. A significant correlation was found between CDT levels and self-reported alcohol intake in patients but not in controls (P = 0.366, P < 0.01). A relationship between urinary ethyl-glucuronide and self-reported alcohol intake was found in both the ET (P = 0.856, P = 0.000) and control (F = 4.422, P = 0.020) groups. No other significant correlations between clinical and laboratory parameters were found.

**Conclusion.** Our data do not reflect a higher incidence of alcoholism in patients with ET. Their alcohol intake is well controlled and does not exceed the limits of healthy social drinking.

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**DRD2 TAQIA POLYMORPHISMS ARE ASSOCIATED WITH PERSONALITY TRAITS THAT CORRELATE WITH ALCOHOL CRAVING IN ALCOHOL-DEPENDENT SUBJECTS**

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Dopaminergic neurotransmissions play indispensable roles in modulation of addictive behaviours through personality and stress response. This study aims at exploring the relationships among personality traits, salivary cortisol and alcohol craving in alcohol-dependent subjects, and the associations between these parameters and gene polymorphisms involved. Alcohol-dependent subjects (n = 156) were recruited during a 5-day detoxification treatment. Neuroticism (N), extraversion (E) and conscientiousness (C) were measured by TIDimensional Character Inventory. Alcohol craving level was measured by Alcohol Urge Questionnaire in the morning of day 3–day 5, and a saliva sample was collected subsequently for cortisol measurement. Genotyping was done on by PCR-RFLP. Results showed that alcohol craving correlated with salivary cortisol level in female subjects (Spearman’s ρ = 0.430, P = 0.022) but not in male subjects. Alcohol craving is significantly (P < 0.05) correlated with N, C, NS and RD scores for male subjects but with E, C, NS and P scores for female subjects. Significant correlation between alcohol craving and these personality traits was only observed in DRD2 TaqIA A1-allele carriers, whose craving level was negatively correlated with RD scores (adjusted r² = 0.191, P < 0.001). Structural equation modeling revealed the effect of DRD2 TaqIA on alcohol craving is mediated through RD trait. The current findings suggest that correlations between alcohol craving, salivary cortisol level and certain addiction-related personality traits are gender-specific. DRD2 TaqIA polymorphisms may be associated with alcohol craving level through mediation of RD trait: alcohol-dependent A1-carrying patients with more prominent RD trait may benefit from a detoxification treatment environment in which alcohol craving level is low.

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**AUDITORY EVENT RELATED POTENTIALS (P3) AND COGNITIVE CHANGES INDUCED BY FRONTAL DIRECT CURRENT STIMULATION IN ALCOHOLICS ACCORDING TO LESCH ALCOHOLISM TYPOLOGY**

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