ALDEHYDE DEHIDROGENASE LEVEL AND FATTY ACID ETHYL ESTER AS BIOCHEMICAL MARKERS PERSIST LONGER THAN ETHANOL IN WISTAR RATS AFTER CHRONIC ALCOHOL CONSUMPTION

Ni Made Suaniti[1)], A.A.Gede Sudewa Djelantik[2)],
Ketut Suastika[2)], Nyoman Mantik Astawa[3)]

[1)] Postgraduate Program, Udayana University
[2)] Faculty of Medicine, Udayana University
[3)] Faculty of Veterinary Madicine, Udayana University
Email: suanitisr@yahoo.com

ABSTRACT

Alcohol consumption in human has increased from year to year in Indonesia and more recently, an increasing number of cases of alcohol intoxication, alcoholic liver disease, and death were observed. The purpose of this experimental study was to examine the significance of two known biochemical markers of alcohol given by mouth in the Wistar rats. The study design used was the "True randomized experimental post test only control group design". The rats were randomly distributed according to the experimental design and were treated daily for six weeks (chronic intake) with 5% and 20% alcohol. This study used 15 rats with 5 rats for treatment group treated with 5% alcohol, 5 rats for treatment group treated with 20% alcohol, and 5 rats as control group treated with distilled water. The biochemical markers were aldehyde dehydrogenase (ALDH) and Fatty Acid Ethyl Esters (FAEE). ALDH and FAEE were two biochemical markers of ethanol which are sensitive and specific for alcohol consumption. The study was conducted in two phases. Initially, rats were treated orally everyday for six weeks with 5% and 20% alcohol, and then the blood level of ethanol, ALDH and FAEE were measured. Blood samples were collected at 6 and 24 hours after the last oral intake of chronic alcohol administration. Qualitative analysis was carried out to detect the presence of ethanol, ALDH, and FAEE in the treatment groups and quantitative analysis to determine their levels in the blood of Wistar rats. Statistical analysis of ALDH was done by using parametric test and the presence of FAEE persisting longer than ethanol by non-parametric test. The results showed that ALDH persisted and increased significantly following chronic consumption of alcohol in the rats. Similarly, FAEEs persisted longer than ethanol after alcohol intake. After six hours, the ALDH level increased by 108.14% in the rat treated chronically with 5% alcohol and by 85.07% in rat treated with 20% alcohol. After 24 hours, FAEE also persisted longer in the blood than ethanol following treatment with alcohol 5%. ALDH levels increased by 83.11% after chronic treatment with 5% alcohol and by 112.05% in the rats treated with 20% alcohol. In the blood collected 24 hours after the last treatment with 5% alcohol, ALDH increased by 95.11% and by 86.79% in the rats treated with 20% alcohol. FAEE persisted longer than ethanol in the blood following administration of 5 % and 20% alcohol both at 24 hours following chronic treatment. The longer persisting ALDH and FAEE were new and good biochemical blood markers for chronic alcohol consumption in the Wistar rats.

Key words: Aldehyde Dehydrogenase, Fatty Acid Ethyl Ester, ethanol consumption, Wistar rats

Introduction

Continued and persistent alcohol use and abuse may lead to alcoholism and a variety of chronic disorders. Persistent and prolonged consumption of alcohol drinks may result in saturation of digestive enzymes and rapid increase of blood alcohol concentration (BAC). The use of BAC level to determine the level of alcohol consumption has a time limitation since alcohol will be quickly eliminated from the blood circulation. It is therefore necessary to find other biomarkers which appear to persist longer in the body of an organism.

In the human body, alcohol was metabolized in the liver to produce fatty acid ethyl ester (FAEE), ethyl glucuronide (EtG), and ethyl sulfat (EtS) (Pawan, 1972; Wurst et al., 2003; Skipper et al., 2004; Weinmann et al., 2004; Costantino et al., 2006; Dahl, 2006). However, FAEE is considered as one of specific and sensitive marker as metabolite ethanol use because it can be detected longer in the blood (Wurst et al., 2004). Besides, FAEE is also a more stable marker and it is therefore regarded as a potential new biomarker for ethanol chronic consumption (Laposato, 1997; Bisaga et al., 2005). FAEE is non-oxidative ethanol metabolites which appears to be toxic to cells, both *in vitro* and *in vivo*. Large accumulation of FAEE following repeated exposure to ethanol in the muscles may cause myopathy. FAEE can still be detected in the serum for more than 24 hours after consumption of ethanol (Salem et al., 2006; Kulaga et al., 2006; SOASAS, 2006).

Until recently, determination of level of alcohol consumption in a suspected individual is generally carried out by detection of ethanol level in the blood. In practice, detection of liver disorder induced by chronic alcohol consumption is done by measuring the serum levels of transaminases: glutamic piruvic transaminase (SGPT) and glutamic oxaloacetic transaminase (SGOT), which have been used as biochemical markers (Wallach, 2004 and POA, 2006). In spite of it, ALDH and FAEE both appear to have a higher level of specificity and stability, and so we decided to study the practical significance of ALDH and FAEE as Biochemical markers because they simply persist longer in the blood stream of an organism (Zakhari, 2006).

Materials and Methods

The study method was a true experimental design using randomized post test only control group. All Wistar rats were adapted in cages in the Pharmacology Department of Udayana University Medical Faculty. This study used 15 rats with 5 rats for treatment group treated with 5% alcohol, 5 rats for treatment group treated with 20% alcohol for six weeks, and 5 rats treated with distilled water was used for the control group. Qualitative analysis was carried out to detect the presence of ethanol and FAEE by GC-MS with retention time. Quantitative analysis was carried out to detect ALDH level by Elisa. GC can be used to analyze qualitative and quantitative ethanol and FAEE automatically. Elisa method was used to know the level of ALDH. The study was sanctioned by the Ethics Commission for Research and Research Development Unit Udayana University Medical Faculty/ Central General Hospital of Denpasar.

Results

Aldehyde Dehydrogenase Levels in Blood of Wistar Rats

ALDH levels in the blood of Wistar rats following oral treatment with alcohol were as follows. In chronic alcohol consumption, the level of ALDH after 6 hours were 7.2878 ± 0.1465 U/L for alcohol 5% and 8.4399 ± 0.5844 U/L for alcohol 20%. Whereas after drinking 24 hours, they were 7.7228 ± 0.6406 U/L for alcohol 5% and 7.3933 ± 0.2739 U/L for alcohol 20%. Kolmogorov-Smirnov test showed that all the data were normal ($p > 0.05$). Furthermore, the homogeneity test data indicated that the data content in the blood of rats ALDH was homogeneous, as the p value was > 0.05. Thus, all data can be analyzed by parametric tests using one-way ANOVA. The levels of ALDH in the blood of Wistar rats taken 6 and 24 hours after alcohol administration 5 % are significantly different ($p < 0.05$) (Table 1). This indicated that ALDH levels after 6 hours and 24 hours in six weeks after alcohol administration was significantly different.

Table 1. The levels of aldehyde dehydrogenase in the blood of Wistar rats after chronic alcohol administration

Treatment	6 hours	p value	24 hours	p value
Control	3,9801	< 0.05*	3,9581	< 0.05*
Alcohol 5%	7,2878		7,7228	
Alcohol 20%	8,4399	< 0.05*	7,3933	< 0.05*

Description:* different significant statistically between control and treatment rat (alcohol 5% and 20%) in blood after 6 and 24 hours alcohol consumption.

One-way Anova test showed that ALDH level in rat's blood were significantly different between control and 5% alcohol treatment for six weeks ($p < 0.05$). In chronic of alcohol treatment 6 hours, the ALDH level for alcohol 5% (7.2878 U/L) and for alcohol 20% (8.4399 U/L) were significantly higher than the control rats (3.9801 U/L). Similarly, in chronic alcohol treatment in 24 hours, the ALDH level for rat with 5% alcohol treatment (7.3933 U/L) and for rat with 20% alcohol treatment were also significantly higher than control rats (3.9581 U/L).

Fatty Acid Ethyl Ester persist longer than Ethanol in blood of Wistar Rats

After repeated chronic alcohol administration in 6 hours after the last treatment were 0.0000 ppm for control rats, 5.9357 ppm for 5% alcohol treatment and 1.3423 ppm for 20% alcohol treatment. And in 24 hours after the last treatment showed that concentration of ethanol were 0.0000 ppm for control rats, 1.0283 ppm for 5% alcohol treatment and 1.5269 ppm for 20% alcohol treatment. However, data on FAEE were not quantitative but as detectablity category 1 if there was no peak, category 2 if there was only 1 or 2 peaks and category 3 if there were 3 peaks or more occurring at the same retention time for FAEE standards.

FAEE persisted longer than ethanol in the blood of rats after alcohol treatment. After chronic alcohol treatment in 6 hours after 5% alcohol treatment, FAEE was significantly less datectable (1.8) than ethanol (3) ($p < 0.05$). However, in 24 hours, FAEE was more detectable (2.4) than ethanol (1) ($p < 0.05$). In 6 hours after 20% alcohol treatment, FAEE was more detectable (1.4) than ethanol (1) and at 24 hours, FAEE was more detectable (2.4) than ethanol (1) (Figure 1).

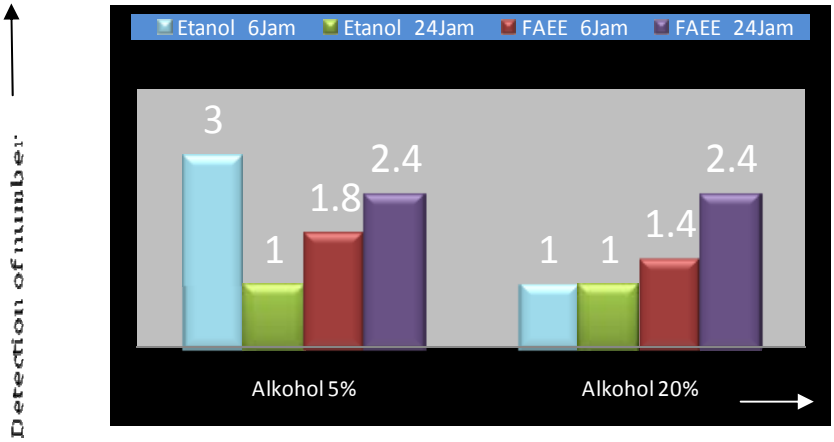

Figure 1. Mean of detection number FAEE and etanol after chronic alcohol consumption (5 dan 20%)

Discussion

Aldehyde dehydrogenase level in the blood of Wistar rats

The results showed that ALDH level persisted longer (more higher) after peroral alcohol 5% consumption in the blood of Wistar rats both chronic compared to control rats.

In chronic alcohol consumption, the different of mean ALDH levels were 3.3077 and 4,4598 after 6 hours significant different between control and alcohol treatment (5 % and 20%) respectively. The data indicated that the increase of ALDH levels were 83.11% and 112.05% at 5% and 20% after last alcohol administration.

A similar result was also obtained in chronic alcohol administration, in 24 hours after alcohol consumption, it was also found significant different between control and alcohol treatment (5% and 20%). ALDH levels in blood rats were 3.7647 and 3.4352 and it seemed there was the increase of 95.11% and 86.79%. This result was not different from the result obtained in 6-hours examination. In general, the results obtained from repeated peroral administration of alcohol in rats can cause ALDH levels as a biochemical marker, in chronic. Marchitti et al. (2008) stated that the aldehyde is a reactive molecule that can be oxidized by ALDH into acetate. In people who consume alcohol will be increased acetaldehyde levels and become toxic to various organs/tissues. To prevent of poisoning by acetaldehyde, the body produces ALDH to oxidize acetic acid (Hoek et al., 2004; Lieber, 2005; Moon et al., 2007). Thus, it could be understood that consuming alcohol continuously can make ALDH produce more enzymes. The consumption of chronic alcohol that can be cause someone to be accustomed (tolerance to alcohol) was closely related to ALDH level in the body. The role of ALDH oxidize acetaldehyde into acetate was also proved in embryonic development, neurotransmision, oxidative stress, and cancer as well as functioning hydrolyze esters, antioxidants, bioaktivasi toxins, and ultraviolet light absorption (Marchitti et al., 2008). In this case acetaldehyde converted to acetate for subsequent metabolism.

Repeated alcohol administration which becomes a social problem is called alcohol abuse or alcoholism. Lately, the experimental studies on the oxidation of ethanol have been carried out (Xin-sheng Deng and Deitrich, 2008). It is said that ethanol is a complex problem and is not only seen from the ethanol itself, but also the metabolite acetaldehyde into acetic acid. ALDH plays an important role in tolerance and dependence of someone to alcohol. In the brain, the enzyme catalase which functions similar to Alcohol dehydrogenase (ADH), ethanol into acetaldehyde can alter the work of several mediators that interfere with central nervous system. Nakamura et al. (2004) also mentioned that the accumulation of acetaldehyde causes the release of Kupffer cells, TNF-α, and acute liver inflammation in rat.

Chronic alcohol consumption as much as 90 g alcohol per day for five years or more for humans can cause pathological changes and disease on multiple organ systems such as liver, heart, and pancreas, especially those associated with fibrosis settled and cardiopathy. Research in animal models by Jones (2005) showed that the fibrosis caused by alcohol poisoning can be cured by zinc supplementation in the diet. Zinc is an antioxidant compound that can inhibit cell apoptosis and liver damage by blocking the formation of FAEE that trigger apoptosis and inflammation of liver cells (HepG2) (Ciesielska et al. (2007). The role of ALDH in the metabolism of alcohol is also reviewed by Zakhari, 2006. Variations of ALDH-gene influenced the levels of alcohol consumption, tissue damage, and alcohol dependence. Lachenmeier, 2008 says that the use of alcohol can cause skin irritation, especially in human with ALDH deficiency. In the meantime, Seitz et al. (2001) states that alcohol consumption can increase the risk of cancer in the digestive system, especially in individual who suffer from lack of ALDH.

Presence of Ethanol and FAEE in the blood of Wistar Rats

Calibration is performed with GC obtained correlation coefficients of ethanol was 0.997 and the correlation coefficient of FAEE palmitic acid ethyl ester was 0.993. Results showed that the GC can be used to measure the levels of both compounds in the samples. The correlation coefficient and a resolution of the separation of many compounds are two important things in the validate method of analysis of certain compounds was done by GC. The study using a patient's hair samples showed that the correlation coefficient of palmitic acid ethyl ester was 0.945 with a cut-off value between heavy drinkers and non drinkers at 0.40 ng/mg (Wurst, et al., 2004). Furthermore Yegles et al. (2004) compared EtG and FAEE levels among heavy drinkers (EtG 0.03 to 0.42 ng/mg; FAEE 0.65 to 20.50 ng/mg) and lightweight (EtG <0.002 ng/mg; FAEE from 0.26 to 0.50 ng / mg). Strong positive relationship with R^2 value of 0.0914 was obtained between EtG and FAEE as determined by GC-MS. EtG in the study were purified by SPE method using aminopropil column, whereas FAEE

microextraction purified by solid phase method (solid phase microextraction/SPME). From these results one could prove with heavy drinkers to see FAEE cut-off value of 1 ng/mg, the presence of EtG has been enough to prove that someone is a heavy alcohol drinker. FAEE measurement validation in rat blood was done by standard palmitic acid ethyl ester, myristic, linoleic, oleic, and stearic. With some of the standard equation of each line in succession FAEE was y = 0.056 x with R^2 = 0.995, y = 0.059 x with R^2 = 0.993, y = 0.013 x with R^2 = 0.889, y = 0.070 x with R^2 = 0.989; y = 0.064 x with R^2 = 0.992. However, with these standards using the original blood sample of rat, the results showed the qualitative data in the form of chromatogram peak FAEE that can not be converted to FAEE concentration using standard equations above. In fact, the chromatogram using some of the above standard has been in line with research Wurst et al., 2004 which found that the cut-off value of ethyl palmitate amounted to 0.40 ng/mg with R^2 = 0.945 in hair and has been used as biological markers of consumption 30-60 g alcohol per week. Some factors that cause the chromatogram peak not to be converted into FAEE levels were the low levels of FAEE in the blood sample. This situation is caused by sample preparation by SPE which were not maximal and small FAEE levels in the rat's blood.

Sample preparation is an important factor in determining the levels of FAEE in biological samples. In this study, FAEE were isolated and purified from rat's blood using SPE and then injected into a Varian GC-MS equipment DB-5% phenyl methyl Polysiloxane calibrated with internal standard ethyl caproate. This method has been applied by previous researchers (Chaterine et al., 2003; Caprara et al., 2006) for biological samples such as blood, tissues, meconium, and human hair with an extraction efficiency rate reaches 40 to 73% and detection limit (limit of detection /LOD) reached 0.008 to 0.084 pmol/mg. In addition FAEE levels by SPE method can also be determined by binding of albumin carrying fatty acids (Best et al., 2006). FAEE levels were obtained between 0.1 and 2.0 mol per mol protein by using hair samples. The use of hair samples to detect FAEEs have been held by Pragst and Yegles, 2008 and it is one technique to detect alcohol abuse during pregnancy retrospectively. Tracking FAEE in hair samples is better than in blood sample as performed in this study but it needs further research.

FAEE and EtG are metabolite of ethanol specific markers and sensitive and can be obtained with a mixture of solid phase microextraction n-heptana/dimetil sulfoxide tetra-hydrofuran and GC-MS. The detection limit of FAEE and EtG was 10-10 pg/mg (Pragst and Yegles, 2008). However, Wurst et al., 2006 mention that FAEE is a new marker to identify sensitive and specific alcohol abuse compared with other alcohol metabolites. The presence of FAEE in hair has been used as biological markers in the prenatal period for diagnosis of fetal abnormality rate due to alcohol (Fetal Alcohol Spectrum Disorder/FASD) in pregnant women who suffered from alcoholism (Kulaga et al., 2009). Moore et al., 2003 state that the total FAEE concentration > 10 000 ng/g in meconium specimens was strong enough to show that new births have been exposed to some alcohol during pregnancy.

FAEE was detected by Infrared (IR) spectroscopy to prove the functional group of FAEE compound. The specific functional group FAEEs were C-H (3600-2500 cm^{-1}); C = O of aldehydes, ketones, and carboxylic ester (1870-1540 cm^{-1}); C = C (1455 and 1390 cm^{-1}); and C-O (1190-1140 cm^{-1}) (Silverstein, 1991).

In this study it was also observed that in chronic alcohol administration, ethanol is fast disappearing from the blood when compared with the acute alcohol administration. The higher ALDH level after chronic alcohol consumption (Table 1) due to loss of alcohol from the blood. It is known that ALDH oxidizes acetaldehyde (a toxic compound) to acetic acid (is not toxic) (Moon et al., 2007; Nakamura et al., 2004; Giebultowicz et al., 2008; Marchitti et al., 2008). In chronic alcohol consumption, ethanol is rapidly disappearing from the blood and can cause tolerance of alcohol consumption. Tolerances were not easily visible, intoxicated when they became accustomed to consuming alcohol and ethanol could be as enzyme inducer (Katzung, 2002;Yoon et al., 2006).

Conclutions

Aldehyde dehydrogenase level persisted longer in the blood of Wistar rats after peroral alcohol consumption for six weeks. In the blood taken after 6 hours, at 5% alcohol ALDH level was higher or increased by 83.11% and 20% alcohol by 112.05%. But, after 24 hours, 5% alcohol content of ALDH was higher or increased by 95.11% and 20% alcohol by 86.79%.

Fatty Acid Ethyl Esters persisted longer than peroral ethanol after peroral alcohol consumption for six weeks. It looked at providing alcohol 5% and 20% in blood collected after 24 hours.

Suggestions

ALDH and FAEE biochemical markers can be used as a diagnostic test after 24 hours of peroral alcohol consumption and the test can provide a longer time.

Searching on FAEE detection by ELISA to obtain quantitative results simultaneously and in cases of alcohol abuse.

REFERENCES

Best CA, Laposato M, Proios VG, Szczepiorkowski ZM. 2006. Method To Assses Fatty Acid Ethyl Ester Binding To Albumin. *J Alcohol and Alcoholism*. 41: 240-246.

Bisaga A, Laposato M, Shan Xie, Evans SM. 2005. Comparison of Serum Fatty Acid Ethyl Esters and Urinary 5-Hydroxytryptophol as Biochemical Markers of Recent Ethanol Consumption. *J. Alcohol and Alcoholism*. 40: 214-218.

Caprara DL, Klein J, Koren G. 2006. Diagnosis of Fetal Alcohol Spectrum Disorder (FASD): Fatty Acid Ethyl Esters and Neonatal Hair Analysis. J Ann Ist Super Sanita. 42: 39-45.

Chaterine AB, Joanne E, Cluette-Brown, Teruya M, Teruya, A, Laposato M. 2003. Red Blood Cell fatty Acid Ethyl Esters: A significant Component of Fatty Acid Ethyl Esters in the Blood. J. of Lipid Research. JLR Papers in Press. Published on January 1, 2003 as Manuscrpt M200398-JLR200. Division of Laboratory Medicine, Departement of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts USA 02114.mlaposato @partners.org.

Ciesielska AS, Plewka K, Daniluk J, Szerszen MK. 2007. Zink inhibits HepG2 cell Apoptosis induced by Acetaldehyde and Fatty Acid Ethyl Esters. Journal of pre-Clinical and Clinical Research. 1:127-134.

Costantino A, Digregorio EJ, Korn W, Spayd S, dan Rieders F. 2006. The effect of the Use of Mouthwash on Ethylglucuronide Concentrations in Urine. *J. of Analytical Toxicology*. 30:659-662.

Dahl H. 2006. Ethyl glucuronide, a new biochemical marker for acute alcohol intake: Studies on possible causes for false-negative or false-positive results. *Departement of Clinical Neuroscience section of Alcohol and Drug Dependence Research*. Karolinska Institutet, Stockholm, Sweden.

Fernandez A, Koval M, Fan X, Guldot DM. 2007. Chronic alcohol ingestion alters calusin expression in the alveolar epithelium of rats. *J. Alcohol*. NIH Public Access. Agustus; 41:371-379.

Giebultowicz J, Wroczynskii P, Piekarczyk J, Wierzchowski j. 2008. Fluorometric Detection of Aldehyde Dehydrogenase activity in Human Tissues in Diagnostic of Cancers of Oral Cavity. Acta Poloniae Pharmaceutica-Drug Research. 65: 81-84.

Jones WK. 2005. A Murine Model of Alcoholic Cardiomyopathy: A Role for Zinc and Metallothionein in Fibrosis. *J. of American Phatology*. 167:301-304.

Katzung BG, 2002. Basic and clinical oh Pharmacology. McGraw-Hill, 8th Ed. New York.

Kulaga V, Caprara D, Iqbal U, Kapur B, Klein J, Reynolds J, Brien J, Koren G. 2006. Fatty Acid Ethyl Esters (FAEE): Comparative Accumulation in Human and Guinea Pig as a Biomarker for Prenatal Alcohol Exposure. *J Alcohol and Alcoholism*. 41:534-539.

Kulaga V, Pragst F, Koren G. 2009. The Fatty Acid Ethyl Esters (FAEE) Hair Test: Emerging Technology for the Diagnosis of Fetal Alcohol Spectrum Disorders (FASD). Annales of Toxicologie Analytique. 21: 61-65.

Lachenmeier DW. 2008. Safet evakuation of topical application of ethanol on the skin and inside the Oral Cavity. Review Journal of Occupational Medicine and Toxicology. 3:26. Lachenmeier@web.de.

Laposato M. 1997. Fatty acid ethyl esters, *J. Clinical Chemistry*. American. 43: 1527-1534.

Marchitti SA, Brocker C, Stagos D, Vasiliou V. 2008. Non-P450 Aldehyde Oxidizing Enzymes: The Aldehyde Dehydrogenase Superfamily. Expert Opin Drug Metab Toxicol. 4: 697-720.

Moon K, Abdelmegeed MA, Song B. 2007. Inactivation of Cytosolic Aldehid Dehydrogenase via Nitrosylation in Ethanol-Exposed Rat Liver. FEBS Lett.21:3967-3972.

Moore C, Jones J, Lewis D, Buchi K. 2003. Prevalence of Fatty Acid Ethyl Esters in Meconium Specimens. Clinical Chemistry. 49:133-136. Drug Monitoring and Toxicology.

Nakamura Y, Yokohama H, Higuchi S, Hara S, Kato S, Ishi H. 2004. Acetaldehyde Accumulation Suppresses Kupffer Cell release of TNF-α and modifies acute hepatic inflammation in rats. J Gastroenterology. 39: 140-147.

Pawan GLS. 1972. Metabolism of alcohol (ethanol) in man. *Proc. Nutr*. Soc. 3X, 83. Metabolic Division, Department of Medicine, The Middlesex Hospital Medical School, London W_1P_7PN.

POA (Pathology of Alcohol). 2006. Pathology of Alcohol: The legs go before the Liver. *The Biomedical Sciencest*. January 2006. 48-49.

Pragst F, Yegles M. 2008. Determination of Fatty Acid Ethyl Esters (FAEE) and Ethyl Glucuronide (EtG) in Hair: A Promising Way for Retrospective Detection of Alcohol Abuse During pregnancy. *Therapeutic Drug Monitoring*. Proquest. April. 30: 255.

Salem RO, Laposato M, Rajendram R, Cluettle-Brown, JE, Preedy VR. 2006. The Total Body Mass of Fatty Acid Ethyl Esters in Skeletal Muscles Following Ethanol Esposure Greatly Exceeds that found in the Liver and the Heart. *J\ Alcohol & Alcoholism*. 41:598-603. Advance Access publication 16 September 2006.

Seitz HK, Matsuzaki S, Yokohama A, Hormann N, Vakevainen S, Wang Xian Dong. 2001. Alcohol and Cancer. Alcoholisme: Clinical and Experimental Research. May Suplement 2001. 25:137s-143s.

Silverstein RM, Bassler GC, Morrill TC. 1991. Spectrometric Identification of Organic Compounds. 5th Ed. John Wiley & Sons, INC

Skipper GE, Weinmann W, Thierauf A, Schaefer P, Wiesbeck G, Allen JP, Miller M, Wurst FM. 2004. Ethyl Glucuronide: A Biomarker To Identify Alcohol Use By Health Professionals Recovering From Substance Use Disorders, J *Alcohol and Alcoholism*. 39: 445-449.

SOASAS (State Office of Alcoholism and Substance Abuse Services). 2006. Addiction Medicine Unit knowledge Workbook IV: The Assessment of Alcohol use Utilizing Biomarkers. New York State Office of Alcoholism and Substance Abuse Services 1450 Western Avenue Albany, New York 12203. http://www.oasas.state.ny.us/AsMed/.

Wallach J. 2004. Interpretation of Diagnostic tests. 8th Ed. Lippincott Williams & Wilkins.

Weinmann W, Schaefer P, Thierauf A, Schreiber A, Wurst FM. 2004. Confirmatory Analysis of Ethylglucuronide in Urine by Liquid-Chromatography/ Electrospray ionization/Tandem Mass Spectrometry According to Forensic Guidelines. *J of The American Society for Mass Spectrometry. Science Direct. 15: 188-193.*

Wurst FM, Vogel R, Jachau K, Varga A, Alling C, Andreas A, Skipper GE. 2003. Ethyl Glucuronide Discloses Recent Covert Alcohol Use Not Detected by Standard Testing in Forensic Psychiatric Inpatients. J *Alcoholism: Clinical and Experimental Research. 27: 471-476.*

Wurst FM, Alexson S, Wolfersdorf M, Bechtel G, Forster S, Alling C, Aradóttir S, Jachau K, Huber P, Allen JP, Auwärter V, Pragst F. 2004. Concentration of Fatty Acid Ethyl Esters in Hair of Alcoholics: Comparison to other Biologycal State Markers and Self Reported Ethanol Intake. J Alcohol and Alcoholism. 39: 33-38.

Xin-sheng Deng dan Deitrich, R.A. 2008. Putative Role of Brain Acetaldehyde in Ethanol Addiction. Current Drug Abuse Reviews.1, 3-8. Alcohol Research Center USA.

Yegles M, Labarthe A, Auwarter V, Hartwig S, Vater H, Wennig R, Pragst F. 2004. Comparison of Ethyl glucuronide and fatty acid ethyl ester concentration in hair of alcoholics, social drinkers and teetotallers. *Abstract Forensic Sci Int*. 29 Oktober 2004. 145:167-173.

Yoon M, Madden MC, Barton HA. 2006. Developmental Expression of Aldehyde Dehydrogenase in Rat: a Comparison of Liver and Lung Development. Toxicological Sciences. 89: 386-398.

Zakhari S. 2006. Overview: How is Alcohol Metabolized by the Body. Alcohol Metabolism and The Body. 29:245-254.