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Evaluation of Apolipoprotein E (ApoE) and Lipoprotein Profile in Severe Alcohol-dependent Individuals

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Abstract. *Background: Chronic alcohol consumption down-regulates the expression of sialytransferase genes resulting in impaired sialylation of apolipoprotein E (apoE) and decreased association with HDL. There are a limited number of studies with contradictory data on the effect of alcohol dependence on human plasma apoE. The aim of the present work is to determine and compare the levels of apoE in relation to the other lipoproteins in alcohol-dependent individuals in order to evaluate the possible role of apoE in lipoprotein metabolism in conditions of severe alcohol dependence. Patients and Methods: The sample of our study comprised 43 DSM-IV diagnosed alcohol-dependent/abusing subjects (33 males and 10 females), treated on an inpatient basis according to a standard detoxification protocol, and 27 healthy people (9 males and 18 females, as a control group). Serum concentration of hepatic enzymes (AST, ALT, γGT), as well as measures of cholesterol and lipoproteins were obtained at baseline and at discharge after a detoxification period of 4-5 weeks. Results: Upon admission, all alcohol-dependent individuals had significantly higher hepatic enzyme levels, apoE and HDL values compared to controls. After completion of alcohol detoxification, all the above parameters returned to normal levels. Additionally, a significant correlation was observed between alcohol consumption during the previous year of alcohol abuse and the apoE values both upon admission to and on discharge from the detoxification program. Conclusion: The statistical correlation between apoE on admission and discharge with alcohol consumption during the previous year suggests that apoE is dependent on alcohol consumption and can serve as a sensitive marker of severe alcohol abuse.*

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Apolipoprotein E (apoE) is a 299-amino-acid protein produced mainly by the liver and secreted in an O-glycosylated form. ApoE appears in three different forms, namely E_2, E_3 and E_4, coded by the three alleles ε2, ε3 and ε4 at a single gene locus (1). ApoE polymorphism is one of the common genetic factors responsible for inter-individual variations in lipid and lipoprotein levels. There are a few studies which have shown that the ε4 allele was associated with an increased risk of coronary atherosclerosis, and not the ε2 allele, whose role in the development of coronary atherosclerosis remains controversial (2, 3). ApoE synthesis has also been demonstrated in human brain, kidney and in steroid producing tissues such as the adrenal glands (2), as well as in ovarian granulosa cells (4, 5). It is found in very-low-density lipoproteins (VLDL), remnant lipoprotein, chylomicron, low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and serves as a ligand for several cell receptors. Thus, apoE determines the homeostasis of cholesterol and triglycerides (6).

It has been documented that chronic ethanol consumption leads to desialylated apoE and this results in a decreased affinity of apoE for HDL, leading to an impaired reverse cholesterol transport function of HDL (7, 8). There are very few studies regarding the effect of alcohol consumption on human plasma apoE (9-12) where it has been demonstrated that VLDL turnover was accelerated after alcohol abuse (13).

The aim of the present work was to determine the levels of apoE in relation to the other lipoproteins in alcohol-dependent individuals, in order to evaluate the possible role of apoE in lipoprotein metabolism in severe alcohol dependence.

Patients and Methods

Patients. The sample of our study comprised 27 healthy participants (9 males and 18 females, as a control group) and 43 alcohol dependent/abusing individuals (33 males, 10 females), enrolled over a 1-year period, who had consecutively contacted the Drug and Alcohol Addiction Clinic of the Athens University Psychiatric

Table I. *Lipoprotein and hepatic profile of alcohol-dependent individuals and control participants.*

Healthy controls		ApoE	CHOL	HDL	LDL	AST	ALT	γGT
	Male							
	n=9	3.2±1.2	218.7±37.3	48.7±7.1	143.9±34.5	27.0±14.7	23.0±9.5	21.67±11.3
	Female							
	n=18	5.0±2.3	277.8±58.2	60.7±11.5	185.9±53.6	22.0±5.9	22.6±14.4	18.7±9.3
	Total							
	n=27	4.4±2.2	258.0±58.8	56.7±11.7	171.9±51.5	23.8±9.9	22.8±12.8	19.7±9.9
Alcohol-dependent individuals	Male							
	n=33	6.7±4.4	263.0±55.7	58.27±2.0	167.2±5.0	49.2±45.4	43.3±30.5	175.6±304.0
	Female							
	n=10	7.8±3.5	273.0±32.6	78.4±26.6	169.0±27.3	36.8±22.3	32.0±22.3	81.2±91.8
	Total							
	n=43	6.9±4.1**	265.4±51.0	63.0±26.4**	167.6±41.0	46,2±41.3**	40.7±28.9*	153.6±272.0***

Values given are means ± sd; ***$p<0.001$, **$p<0.01$, *$p<0.05$.

Clinic at the Eginition Hospital in Athens, Greece. Informed consent was obtained from each participant; participation in the project was on a voluntary basis. Detailed information on the objectives of the study and the research-therapeutic protocol was provided to all participants. Ethical permission for the study was obtained from the special scientific committee of the Eginition Hospital and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

All patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, (DSM-IV) diagnostic criteria for alcohol abuse and dependence (14) presenting for detoxification on an inpatient basis. They were abstinent from alcohol for an average of 24.0±12.2 hs prior to admission to the clinic. Serious withdrawal symptoms, as monitored according to the CIWA-Ar Scale, were not present in our sample because all patients were treated with benzodiazepines.

Participants included in the study had to fulfill the following criteria: a) age between 25 and 65 years, b) absence of serious physical illness (as assessed through physical examination and routine laboratory screening), c) absence of other pre- or co-existing major psychiatric disorder on the DSM-IV axis I, and d) absence of other drug abuse. The mere presence of affective symptoms was not considered to be an exclusion criterion. Alcohol abusers who fulfilled the DSM-IV diagnosis of depressive disorder were excluded from the study if a major depressive episode had been recorded prior to the onset of alcoholism; participants were not excluded whenever a depressive episode was present concurrently with an alcohol-abusing period.

The mean age of the subjects was 48.1±9.9 years and mean alcohol consumption during the previous month before admission was 277.2±149.7 g/day, and the previous year was 86.9±20.9 g/day. The control group consisted of relatives of the alcohol dependent individuals, who were infrequent social alcohol consumers. The mean age of the controls was 47.1±8.7 years.

Study design. Upon admission, the detoxification protocol was initiated and completed over one week (7-10 days). Detoxification comprised vitamin replacement (vitamins C, E, and B complex) and oral administration of diazepam (30-60 mg in divided doses), with gradual taper off over a week. Thereafter, a standard treatment program with a short-term psychotherapy of cognitive-behavioural orientation was implemented that lasted for 4-5 weeks. It consisted both of individual sessions (twice a week) and family interventions (at least once every two weeks). Alcohol consumption was prohibited during hospitalization.

Assessments. Blood samples were drawn on the morning of their arrival at the hospital and following 30 days of abstinence. The blood was centrifuged at 1000xg for 15 min and serum samples were analyzed immediately for aspartate transaminase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γGT), cholesterol and lipoproteins. One aliquot of serum was kept at –70˚C until apoE measurement. Laboratory testing showed that 28% of patients had elevated serum AST and 32% of them had elevated serum ALT, although none showed clinical or laboratory signs of severe liver disease such as low serum albumin (<4 mg/dl), jaundice, ascites or hepatic encephalopathy.

Serum concentration of total cholesterol (total-CH), HDL cholesterol (HDL-CH), LDL cholesterol (LDL-CH), albumin, and activities of AST, ALT and γGT were measured with commercially available kits (Medicon Hellas, Athens, Greece) on an Olympus 550 analyzer (Medicon Hellas).

Total apoE was determined by electroimmunodiffusion in agarose gel with the use of Sebia Hydragel ApoE particle kits (Issy-les-Moulineaux, Rue, France). The gels contained human anti-apoE monospecific antibody. After migration, the resulting "rockets" electrophoresis were stained with acid violet and the excess stain was removed with an acidic solution. A calibration plot was constructed from assayed values obtained on a calibrated apoE standard serum.

Statistical analysis. Groups were compared using Student's *t*-test. The correlation analysis was performed using the Pearson correlation coefficient (r). Level of significance was set at $p=0.05$.

Results

The two groups under study did not differ significantly in age (controls *versus* alcohol-dependent individuals: 47.1 years ±8.7 years *versus* 48.1 years ±9.9 years, ns), marital status

Table II. *Effect of abstinence from alcohol on various hepatic measures.*

Serum level (U/L)	ApoE1	ApoE 2	AST 1	AST 2	ALT1	ALT2	γGT1	γGT2
Alcohol-dependent individuals (n=43)	6.9±4.2	2,6±2.1***	46.3±41.3	28.1±7.5**	40.7±28.9	27.3±10.7**	153.6±272.0	38.7±20.5***

Values given are means ± sd. ApoE1, AST1, ALT1, γ-GT1: Admission; ApoE2, AST2, ALT2, γ-GT2: Discharge. ***$p<0.001$, **$p<0.01$.

(chi-square=1.14, ns), level of education (chi-square=6.73, ns) or their socioeconomic status (chi-square=7.73, ns). Mean values of the laboratory measurements in the studied groups are shown in Table I.

Upon admission all alcohol-dependent individuals presented elevated serum levels of AST (46.3±41.3 U/L), ALT (40.7±28.9 U/L) and a marked elevation of γGT (153.7±272.0 U/L). The normal range, given by the laboratory, is 7.00-40.00 U/L for AST, 7.00-40.00 U/L for ALT and 7.00-49.00 U/L for γGT. After the detoxification period, values were significantly reduced. Specifically, AST was 28.1±7.5 U/L ($p<0.01$), ALT 27.3±10.7 U/L ($p<0.01$) and γGT 38.7±20.5 U/L ($p<0.001$).

Total cholesterol and LDL concentrations had no statistically significant difference; between the two groups ($p>0.10$). However, apoE and HDL were significantly higher in alcohol-dependent individuals compared to healthy controls.

The distribution for the serum apoE concentrations in the studied groups is shown in Figure 1.

The values of the studied laboratory parameters on admission and on discharge after the completion of the detoxification period are shown in Table II. The abnormal serum levels of the studied laboratory markers returned to normal after completion of detoxification (after 4-5 weeks).

There was a statistical correlation between the apoE value on admission and the quantity of alcohol which had been consumed during the previous year. Moreover, a significant correlation was observed between apoE levels at the end of treatment and alcohol consumption during the previous year (apoE admission-quantity per year r=0.474, $p<0.001$; apoE discharge – quantity per year r=0.379, $p<0.01$).

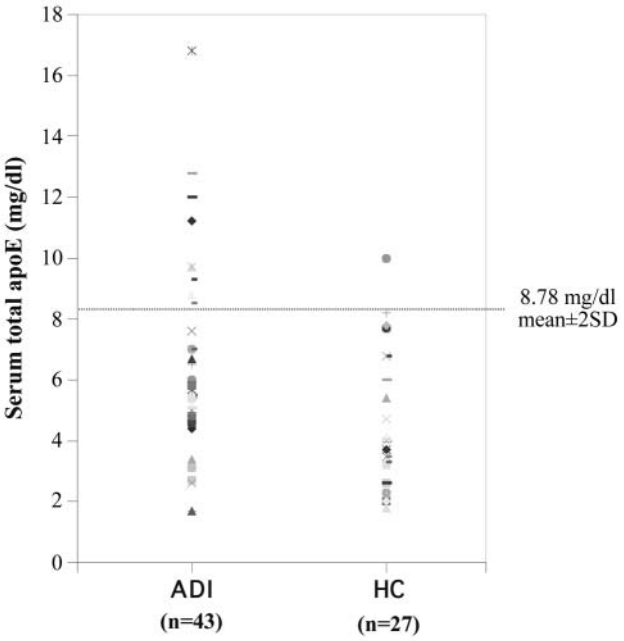

Figure 1. *Distribution of serum total apoE in the studied groups. The horizontal line indicates the cut-off level. Student's t-test, T=3.05, p<0.01.*

Discussion

The objective of the present study was to evaluate the effect of chronic alcohol consumption on circulating lipids, lipoproteins and apoE in alcohol-dependent individuals without severe liver disease in comparison with a control group.

All the alcohol-dependent individuals who participated in this study had elevated AST and ALT levels on admission, concomitantly with highly elevated γGT levels. This abnormal profile indicates that considerable liver dysfunction is present with co-existing liver tissue injury. Previous studies corroborate our findings regarding the elevation of γ-GT concentrations in alcohol-dependent individuals; this enzyme is known to be elevated in about 65% of those who chronically abuse alcohol, with a rough correlation between the amount of alcohol intake and γGT activity (15).

The serum transaminases AST and ALT are routine tests of liver function and excessive alcohol consumption can lead to raised levels due to increased cell membrane permeability and cell necrosis. AST and ALT are elevated minimally in alcohol liver injury; AST usually less than 8-fold and ALT less than 4-fold the upper reference limit (16).

The relationships found between the levels of the three hepatic enzymes reflect the significance of their combined usage for the determination of alcohol-induced liver dysfunction (AST/ALT: r=0.747, $p=0.001$, AST/γ-GT: r=0.805, $p=0.001$, ALT/γ-GT: r=0.557, $p=0.001$).

No significant differences were found for total cholesterol or LDL cholesterol between the two groups, a finding which is in agreement with previous studies (11, 12).

In the present study, alcohol-dependent individuals had higher HDL cholesterol levels, a finding which is in agreement

with the literature (8, 17-20). The mechanism through which chronic alcohol consumption increases HDL-cholesterol is by affecting the function of HDL in reverse cholesterol transport (RCT) (21, 22). The removal of cholesterol from peripheral tissues to the liver (RCT) is mediated by HDL through association with apoE *via* B/E receptor and HDL-apoE receptor (7, 23). It has been demonstrated that chronic alcohol exposure destabilizes sialyltransferase (ST) mRNA resulting in a concomitant reduced steady-state level of (ST)mRNA (7) and the formation of asialo-apoE, which has a lesser binding affinity to HDL than sialylated apoE (22). In our alcohol-dependent sample without severe liver disease, the concentrations of the hepatic enzymes (AST, ALT, γGT) and HDL normalized after the completion of detoxification. The observation that apoE elevation was independent of the concentrations of AST and ALT and that the concentrations of AST, ALT, γGT and HDL returned to normal following one month of abstinence from alcohol, strongly suggests that alcohol abuse was the cause of their elevation. Furthermore, the significant correlation between apoE on admission and discharge, and the quantity of alcohol consumption during the previous year, suggests that apoE is positively related with alcohol consumption.

Conclusion

The present study provides preliminary evidence in favor of the use of apolipoprotein E levels as a sensitive marker of prolonged alcohol consumption. However, further investigation of the possible interrelationships between apoE and alcohol consumption, in studies with a larger sample size, is needed.

References

1 Bednarska-Makaruk M, Rodo M, Markuszewski C, Rozenfeld A, Swiderska M, Habrat B and Wehr H: Polymorphisms of apolipoprotein E and agiotensin-converting enzyme genes and carotid atherosclerosis in heavy drinkers. Alcohol Alcoholism *40*: 274-282, 2005.

2 Davignon J, Gregg RE and Sing CE: Apolipoprotein E polymorphism and atherosclerosis. Atherosclerosis *8*: 1-21, 1988.

3 Wilson PW, Schafer EJ and Larson MG: Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol *16*: 1250-1255, 1996.

4 Siest G, Pillot T, Regis-Bailly A, Leininger-Muller B, Steinmetz J, Galteau MM and Visvikis S: Apolipoprotein E: An important gene and protein to follow in laboratory medicine. Clin Chem *41*: 1068-1086, 1995.

5 Blue ML, Williams DL, Zucker S, Khan SA and Blum CB: Apolipoprotein E synthesis in human kidney, adrenal gland and liver. Proc Natl Acad Sci USA *80*: 283-287, 1983.

6 Driscoll MD, Schreiber JR, Schmit VM and Getz GS: Regulation of apolipoprotein E synthesis in rat ovarian granulose cells. J Biol Chem *260*: 9031-9038, 1985.

7 Lakshman MR, Manjunath N Marmillot R and Marmillot P: Alcohol and molecular regulation of protein glycosylation and function. Alcohol *19*: 239-247, 1999.

8 Rao MN, Liu Q-H, Marmillot P, Seeff LB, Strader DB and Lakshman MR: High-density lipoproteins from human alcoholics exhibit impaired reverse cholesterol transport function. Metabolism *49*: 1406-1410, 2000.

9 Guegen S, Herbeth B, Pirollet P, Paille F, Siest G and Visvikis S: Changes in serum apolipoprotein and lipoprotein profile after alcohol withdrawal: effect of apolipoprotein E polymorphism. Alcohol Clin Exp Res *26*: 501-508, 2002.

10 Wilhelm J, von Ahsen N, Frieling H, Hillemacher T, Bayerlein K, Bonsch D, Ziegenbein M, Kornhuber J and Bleich S: Apolipoprotein E4 genotype is not associated with short-term cognition deficits during alcohol withdrawal. Alcohol *37*: 151-156, 2005.

11 Bleich S, Wilhelm J, Graesel E, Degner D, Sperling W, Rossner V, Javaheripour K and Kornhuber J: Apolipoprotein E4 is associated with hippocampal volume reduction in females with alcoholism. J Neural Transm *110*: 401-411, 2003.

12 Simopoulos AP: Genetic variation and nutrition. Biomed Environ Sci *9*: 124-129, 1996.

13 Sane T and Nikkila EA: Accelerated turnover of very low density lipoprotein triglycerides in chronic alcohol users. Atherosclerosis *5*: 185-193, 1984.

14 American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 4th ed. APA, Washington, DC, 2000.

15 Daeppen JB, Schoenfeld-Smith K, Smith TL and Schukitt MA: Characteristics of alcohol dependent subjects with very elevated levels of gamma-glutamyltransferase (GGT). J Stud Alcohol *60*: 589-594, 1999.

16 Henry JB (ed.): Clinical Diagnosis and Management by Laboratory Methods. 20th edition, Philadelphia, USA, W.B. Saunders Company, pp. 224-225, 2001.

17 Lecomte E, Herbeth B, Paile F, Steinmetz J, Artur Y and Siest G: Changes in serum apolipoprotein and lipoprotein profile induced by chronic alcohol consumption and withdrawal: determinant effect on heart disease? Clin Chem *42*: 1666-1675, 1996.

18 Andrade RJ, Escolar JL, Valdivielso P and Gonzalez-Santos P: Apolipoprotein distribution in plasma HDL subfractions in alcohol consumers. Drug Alcohol Depend *26*: 161-168, 1990.

19 Sharpe PC, McBride R and Archbold GPR: Biochemical markers of alcohol abuse. Q J Med *89*: 137-144, 1996.

20 Srivastava LM, Vasisht S, Agarwal DP, Agarwal DP and Goedde HW: Relation between alcohol intake, lipoproteins and coronary heart disease: the interest continues. Alcohol *29*: 11-24, 1994.

21 Whitehead TP, Clarke CA and Whitfield AGW: Biochemical and haematological markers of alcohol intake. Lancet *1*: 978-981, 1978.

22 Marmillot P, Rao MN, Liu QJ and Lakshman MR: Desialylation of human apolipoprotein E decreases in binding to human high density lipoprotein and its ability to deliver cholesterol to the liver. Metabolism *48*: 1184-1192, 1999.

23 Tall AR: Plasma high-density lipoproteins. Metabolism and relationship to atherogenesis. J Clin Invest *86*: 379-384, 1990.

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