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Alcohol-Induced Hyperlipidemia and Beta Lipoproteins

Abstract. *Alcohol addicts with a primary type IV hyperlipoproteinemia show a striking elevation of triglycerides in the serum during long periods of alcohol consumption as compared with controls, without an accompanying significant increase in free fatty acids in the serum. These data suggest that this genetically related lipid abnormality may be a significant factor in the pathogenesis of alcohol hyperlipemia and the alcohol-induced fatty liver.*

Although there is a high correlation between chronic alcohol abuse and cirrhosis of the liver, not all alcoholics develop this form of hepatic disease. The specific factors that predispose certain individuals to the development of the alcohol-induced fatty liver, acute hepatic necrosis, or Lannec's cirrhosis, associated with chronic drinking, are as yet undetermined and have puzzled biomedical scientists for more than a century (*1, 2*). This report describes for the first time the role of a genetically related derangement in lipid metabolism which may be a significant factor in the pathogenesis of hyperlipemia in alcohol addicts. The impetus for our studies was provided by the comprehensive investigations of fat transport and lipoproteins carried out by Frederickson and his associates (*3*). A possible relationship between the expression of a type IV hyperlipoproteinemia and excessive alcohol intake, as well as emotional stress and carbohydrate metabolism alterations, was also suggested by the studies of Frederickson and his associates (*3*).

Although the exact incidence of type IV primary hyperlipoproteinemia is unknown, the presence of this disorder in the general population is quite common. Genetically determined primary type IV hyperlipoproteinemia is expressed as a dominant trait and is often observed in 50 percent of adult relatives of those who have the genetic abnormality.

Primary type IV hyperlipoproteinemia has also been implicated in the pathophysiology of pancreatitis and premature atheromatosis.

The mechanisms by which alterations in lipid transport and metabolism produce derangements of hepatic function are unknown. However, chronic alcohol intake in alcoholics has been associated with reciprocal elevations in serum triglycerides and free fatty acids (*4*). During experimentally induced intoxication, there was an initial fall in free fatty acids and an elevation in serum triglycerides. As drinking continued and blood alcohol concentrations increased, serum triglycerides fell with a concomitant elevation in free fatty acids (*4*). However, two limitations affect the interpretation of these several studies: (i) lipoprotein profiles of subjects were not determined and (ii) alcohol was administered on a programmed dosage basis along with an adequate diet. Although programmed alcohol administration represents a good clinical research design, it does not simulate the patterns of real life drinking accompanied by the marginal dietary intake observed in chronic alcohol addicts and therefore may limit the generality of associated biological changes (*5*).

The major purpose of our study was to determine whether there is any relation between primary lipoprotein abnormalities and ethanol-induced alterations in serum lipids. Concentrations of lipids and lipoprotein profiles in serum were determined in alcoholic males prior to, during, and after free-choice alcohol consumption (a pattern of drinking analogous to the manner in which such individuals drink in real life). Although these subjects had access to an adequate diet, on the basis of previous clinical findings, it was anticipated that they would tend to decrease caloric intake during prolonged drinking periods (*5*).

Thirteen male alcohol addicts between the ages of 28 and 51 were admitted to the clinical research ward of the National Institute of Alcohol Abuse and Alcoholism and informed consent was obtained from each subject. All subjects were in good health and showed no evidence of any medical (including hepatic and pancreatic) or major psychiatric disorder as determined by appropriate clinical and laboratory examinations. Subjects had a 3- to 33-year history of alcoholism (as defined by tolerance and physical dependence). The history of recent alcohol intake and the duration of abstinence was comparable for all subjects.

After the subjects completed a period of acclimation to the research ward, a minimum of three consecutive daily blood samples were obtained and used for assay of serum lipids and lipoproteins. All samples in our study were collected from fasted (8 hours) subjects. During the remainder of the study, blood samples were obtained each morning for serum lipid and lipoprotein determinations (*6–8*). Triglycerides were determined by the method of Noble and Campbell (*6*). Cholesterol was determined by the procedure of Zondag and Van Boelzeler (*7*). Free fatty acids were determined by a method described by Dole (*8*). Profiles of pre-beta lipoprotein (these proteins migrate in front of beta lipoproteins on paper electrophoresis and lag behind beta lipoproteins on acrylamide gel electrophoreses) were determined with a disc-gel technique described by Frings and his associates (*9*). Reagent kits (Q.D.L., Canalco Diagnostic Products, Rockville, Maryland) were used for these determinations. In any instance where disc gel electrophoresis data were not clear, confirmation of serum lipoprotein phenotyping was carried out by analytical ultracentrifugation.

After a baseline phase of 7 to 9 days during which the subjects did not consume any alcohol, an 11- or 12-day period of spontaneous alcohol intake was instituted; this period was followed by a 7- to 10-day withdrawal period. Subjects could consume up to 32 ounces of 100-proof beverage alcohol daily. Each morning subjects were given 32 tokens to buy alcohol from an automatic dispensing apparatus; 1 ounce of alcohol was dispensed for each token spent. Blood alcohol concentrations were determined every 4 hours with a breathalyzer device. Subjects could drink at any time, but were encouraged to sleep during the hours of 12:30 and 7:30 a.m. to permit electroencephalogram recording of sleep patterns. Although all subjects reduced caloric intake from food when drinking, there

were no significant differences in amount of caloric intake between subjects during the period of spontaneous alcohol consumption.

Because it has been shown that hyperlipemia may be induced by carbohydrate in healthy individuals (*10*), it was necessary to differentiate between subjects with primary and carbohydrate-induced abnormal pre-beta lipoprotein profiles according to the following criteria. Subjects with a carbohydrate-induced type IV disorder had an abnormal pre-beta lipoprotein profile and elevated triglyceride only when they were overweight and consuming excessive carbohydrate. On reduction of carbohydrate intake and weight loss, triglyceride concentrations and pre-beta lipoprotein profiles returned to normal. In contrast, those subjects in the primary type IV category had persistent elevations of triglyceride in the serum and abnormal pre-beta lipoprotein profiles after reduction of carbohydrate intake and maintenance of ideal body weight. In the primary type IV subjects triglycerides were significantly higher ($P < .05$) than in the carbohydrate-induced type IV subjects prior to alcohol ingestion.

Three subjects showed no evidence of serum lipoprotein abnormalities or derangement in serum lipid as determined by at least three consecutive fasting blood samples prior to initiation of drinking. Five subjects showed some evidence of occasional type IV carbohydrate-induced increase in lipoproteins with elevation of serum triglyceride prior to drinking. Five subjects showed evidence of a primary type IV hyperlipoproteinemia with elevated serum triglycerides which were not associated with obesity or with excessive caloric intake prior to initiation of drinking.

A comparison of concentrations of triglyceride, cholesterol, and free fatty acid in the serum, as a function of the amount of alcohol detectable in the blood, for each of the three groups—normal, type IV primary, and type IV carbohydrate-induced subjects—is shown in Fig. 1. Within each group, the significance of changes in serum lipid concentration at each blood alcohol concentration (in comparison to baseline) was evaluated by a nonparametric Mann-Whitney U test (one-tailed) (*11*). Between-group comparisons of concentrations of lipids in the serum at each concentration of blood alcohol were evaluated by the same technique (*11*).

Subjects with normal pre-beta lipo-

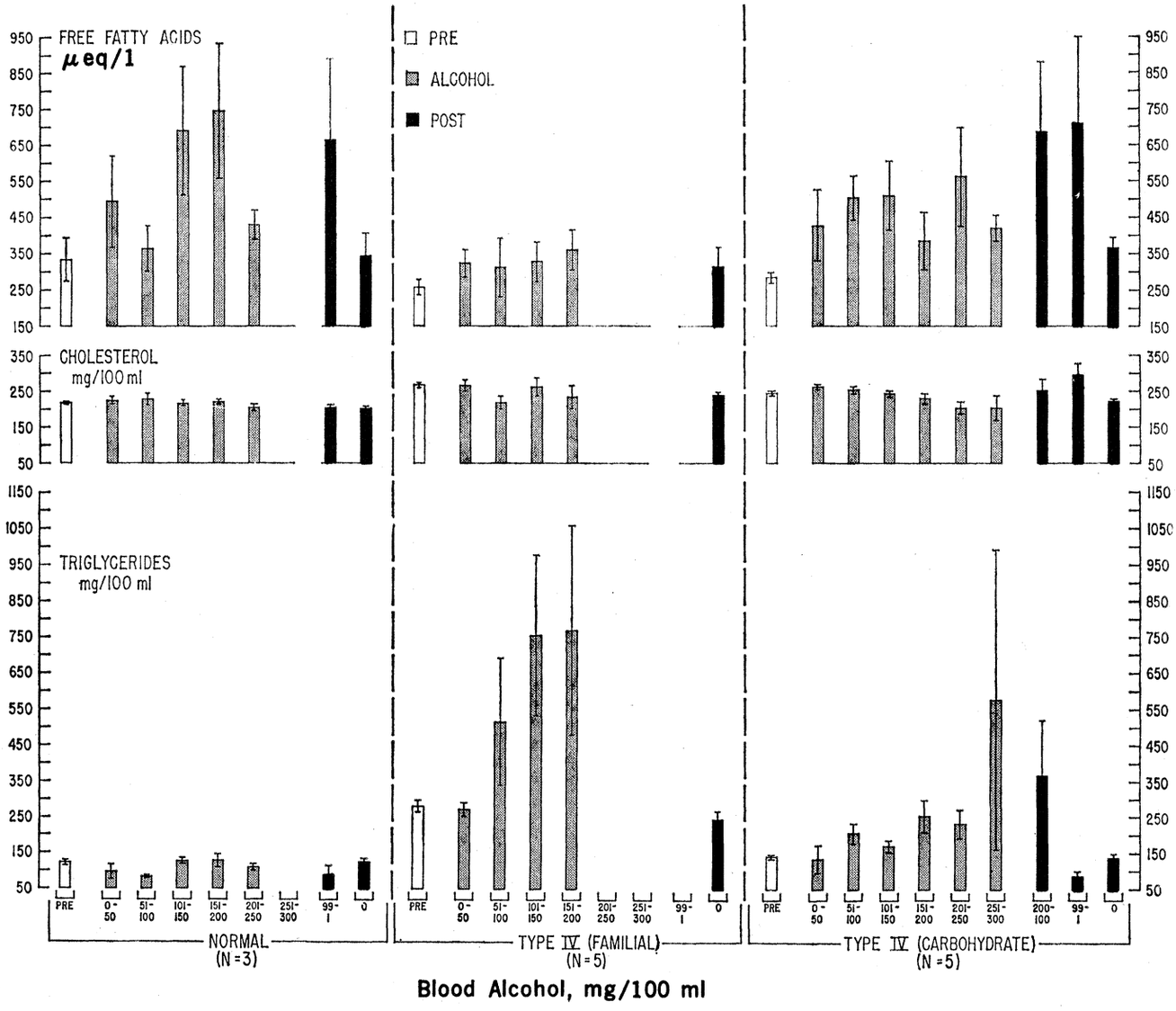

Fig. 1. Serum lipid levels (mean ± standard error) are presented for each of three groups of subjects: normal, type IV primary, and type IV carbohydrate induced.

protein profiles prior to drinking showed no significant increase in serum triglyceride or cholesterol, even when the blood alcohol was above 200 mg/100 ml (Fig. 1, column 1). Free fatty acid increases were statistically significant (except when the blood alcohol was 51 mg to 100 mg/100 ml), and the highest elevations occurred when the blood alcohol was between 101 and 200 mg/100 ml ($P < .01$).

In striking contrast to the normal group, subjects with evidence of a type IV primary pre-beta lipoprotein abnormality during baseline showed significant, alcohol-dosage related increases in triglyceride concentrations during drinking ($P < .01$) (Fig. 1, column 2). Although concentrations of free fatty acid also increased significantly during drinking ($P < .05$) in comparison to baseline values, the magnitude of this increase was considerably smaller than that shown by the normal group (Fig. 1, column 1). Cholesterol concentrations were unchanged or slightly decreased as a function of increasing alcohol consumption.

Those subjects who showed evidence of a type IV carbohydrate-induced lipoprotein abnormality prior to drinking also developed striking elevations of triglycerides when the concentration of alcohol in the blood was high (251 mg to 300 mg/100 ml)—concentrations never reached by the primary type IV group (Fig. 1, column 3). This group did show small but significant ($P < .01$, $.05$) elevations of serum triglycerides at lower concentrations of blood alcohol (51 mg to 250 mg/100 ml). Cholesterol tended to decrease during drinking and this trend achieved significance when the blood alcohol was 201 mg to 250 mg/100 ml ($P < .05$). Free fatty acid did increase significantly during prolonged periods of alcohol consumption ($P < .05$, $.01$), and the magnitude of this increase was most comparable to that shown for the normal subjects.

The dramatic increase in serum triglycerides in patients during drinking periods which is shown by the type IV primary subjects was further demonstrated by between-group statistical comparisons. The serum triglycerides of primary type IV subjects were significantly higher than those of carbohydrate-induced type IV subjects at blood alcohol concentrations between 50 and 150 mg/100 ml ($P < .05$, $.01$). In both type IV groups triglycerides were significantly higher than in normal subjects at all concentrations of blood alcohol ($P < .01$). Cholesterol concentrations during intoxication were not significantly different in any of the groups. Elevations in free fatty acid were not significantly different for the normal and carbohydrate-induced type IV groups, except at blood alcohol concentrations between 151 mg to 200 mg/100 ml ($P < .05$). However, in both groups elevations in free fatty acids were significantly higher than in the primary type IV group, except at the lowest blood alcohol concentrations (0 to 50 mg/100 ml) ($P < .05$, $.01$).

These data suggest that the triglyceride response in alcoholics during drinking is highly correlated with the presence of a preexisting lipoprotein disorder. The mechanisms underlying the expression of this alcohol-induced hyperlipidemia are unclear. It is known that ethanol may increase the levels of circulating lipoproteins. This occurs as a function of increased production (*12*) of lipoproteins that are probably formed in the endoplasmic reticulum of hepatic cells (*13*).

Triglyceride has been found to be the major lipid fraction of the alcoholic fatty liver (*2*). Although alcohol-induced elevations of serum triglycerides and accumulation of triglyceride in the fatty liver could be coincidental, that these phenomena frequently occur at the same time suggests related pathophysiological processes (*2*, *4*). Since individuals with a *primary* type IV abnormality of serum lipoprotein function develop very high concentrations of serum triglyceride after prolonged periods of alcohol consumption, they may also accumulate large amounts of triglyceride in liver. Confirmation of this hypothesis would require simultaneous determination of serum and liver triglycerides (by the use of liver biopsy procedures) in primary type IV alcohol addicts during intoxication.

The origin of the elevated concentrations of triglyceride that follow alcohol intake by primary type IV subjects is not completely explained by known mechanisms of lipid metabolism. However, the differences in concentrations of free fatty acid in the serum between normal and primary type IV subjects during prolonged periods of alcohol consumption indicates that either mobilization, esterification, or utilization are different between the two groups. It is possible that the higher concentrations of free fatty acid in the normal subjects reflects a lower rate of esterification in the liver with concomitantly lower concentrations of triglyceride.

Fatty infiltration of the liver may occur in some patients without evidence of hyperlipemia (*14*). However, if individuals with a primary type IV disorder are at higher risk for the development of hyperlipemia and the alcohol-induced fatty liver, it would be possible to identify them through screening techniques by means of analysis of serum lipoprotein profiles. Finally, since the type IV pre-beta lipoprotein disorders are associated with pancreatitis and premature atheromatosis (*3*), the possible role of alcohol abuse in the genesis of these disease states should be reexamined.

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