Plasma Lipids of Dogs During and After Chronic Ethanol Administration.* (30067)

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Short-term administration of ethanol in animals has been reported to lead to increased concentrations of plasma unesterified fatty acids and triglycerides, as well as to an accumulation of liver lipids(1). Relatively little information is available about lipid changes associated with chronic ethanol ingestion(2, 3). It is known, however, that marked alterations in the various classes of blood lipids occur in patients with chronic alcoholism (4,5).

The present study was designed to investigate plasma lipids in dogs during and after a 12-week period of ethanol administration.

Materials and methods. The 8 adult-mongrel dogs used in this study ranged in body weight from 14 to 24 kg. They were housed in individual cages and observed for at least 2 weeks before administration of ethanol began. For a period of 12 weeks each animal then received a daily dose of 4 g ethanol/kg body weight as a 33% solution in water given *via* gastric tube 16 to 18 hours after the previous meal. Administration of ethanol was then stopped and the animals were followed up for an additional 4 weeks. During the observation period and throughout the 16 weeks of the experiment, the animals were maintained on a diet consisting of water, liver, beef and Purina Dog Chow. Blood samples were drawn from an external jugular vein without stasis before the first administration of ethanol for control determinations, at 1, 2, 4, 8, and 12 weeks during ethanol ingestion and at 1, 2 and 4 weeks following withdrawal of ethanol. During the period of ethanol administration, the blood samples were drawn 24 hours after the previous dose, at which time no detectable amount of ethanol could be found in the blood. The samples were placed in ice water immediately after withdrawal and were processed in a refrigerated centrifuge within 2 hours after collection. The plasma was analyzed for triglycerides(6), unesterified fatty acids(7), phospholipids(8) and total cholesterol(9). In addition, plasma volume (T-1824 space) was measured so that the total amount of lipids in the circulation could be determined. At the end of the experiment the animals were sacrificed by an overdose of sodium pentobarbital and the right lobe of the liver and the pancreas were excised. Portions of these tissues were stained with hematoxylin and eosin and, in addition, liver sections were also stained with Sudan IV. Liver triglyceride concentration was determined by the method of Butler *et al*(10). The method of paired observations in which each animal served as its own control was used for statistical analysis of the data.

Results. Plasma triglyceride concentrations were increased 51% to 86% from control during the 12 weeks the animals received ethanol. When ethanol was discontinued, the triglyceride levels rapidly returned to the control value (Fig. 1). Plasma unesterified fatty acid (UFA) concentrations were reduced, especially at the first week of ethanol administration, and returned to the normal range during the recovery period (Fig. 2). There was also a sustained increase in lipid phosphorus concentration (29% to 47%) during the 12-week ethanol period which was followed by a return to the initial levels after cessation of ethanol (Fig. 3). Similar changes were seen for plasma total cholesterol concentration during and after the withdrawal of ethanol (Fig. 4), with increases from control ranging from 51% to 106%

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followed by a more gradual return to the control value.

Body weight, plasma volume and total amount of circulating lipids calculated from the plasma volume and lipid concentration are presented in Table I. The body weight for the 8 dogs did not change appreciably throughout the experiment. Total plasma volume increased slightly during the period of ethanol administration and returned to the control level during the first week of recovery. Since the plasma pool was larger, the total amounts of triglycerides, lipid phosphorus and total cholesterol were increased more than data from concentration alone would suggest. On the other hand, the plasma UFA concentration observed during ethanol administration decreased more than would be expected on the basis of dilution alone.

Gross and microscopic examination of hepatic and pancreatic tissues obtained 4 weeks after withdrawal of ethanol revealed no pathologic changes. Only a trace of stainable lipid material was found in the liver sections. Average value ± SE for liver triglyceride concentration at this time was 3.16 ± 0.54 mg/g fresh liver tissue, which was similar to the value of 3.06 ± 0.58 mg/g found for 7 control animals.

Discussion. Chronic administration of ethanol promptly produced marked increases in plasma concentrations of triglycerides, lipid phosphorus and total cholesterol, and a decrease in plasma UFA in the dog. These changes in blood lipids were, in general, maintained throughout the period of ethanol in-

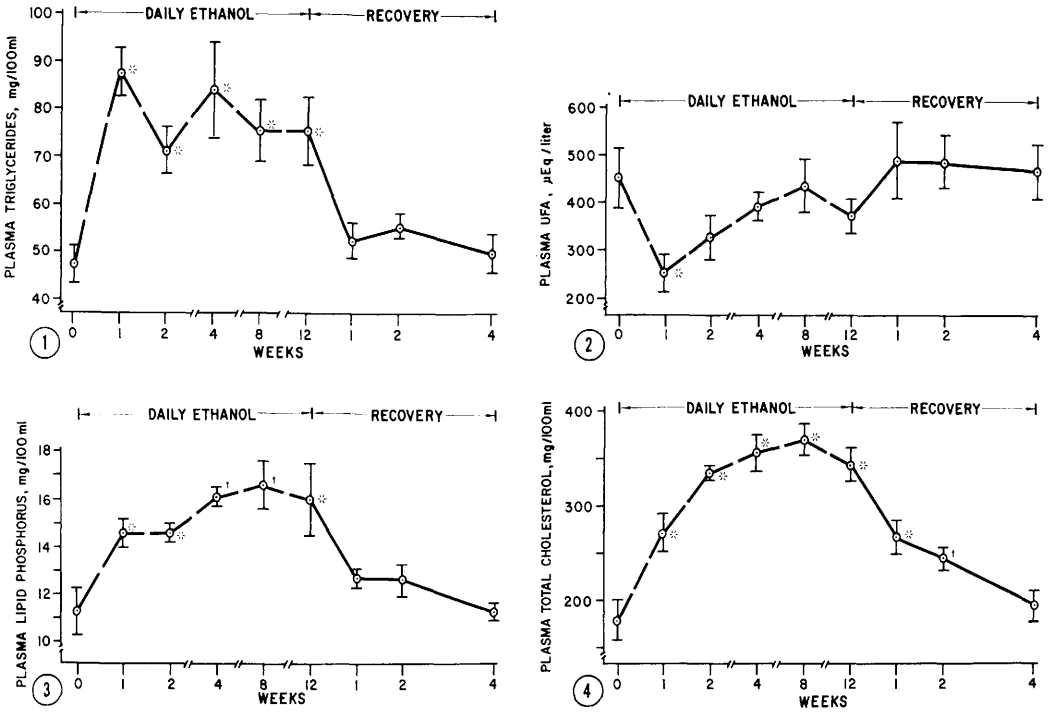

FIG. 1. Plasma triglyceride concentrations of 8 dogs during and after 12 wk of daily ethanol (4 g/kg) administration. Values expressed as means ± S.E. * $P<0.01$ when compared to control value on a paired sample basis.

FIG. 2. Plasma unesterified fatty acid concentrations of 8 dogs during and after 12 wk of daily ethanol (4 g/kg) administration. Values are expressed as means ± S.E. * $P<0.05$ when compared to control value on a paired sample basis.

FIG. 3. Plasma lipid phosphorus concentrations of 8 dogs during and after 12 wk of daily ethanol (4 g/kg) administration. Values are expressed as means ± S.E. * $P<0.05$, † $P<0.01$ when compared to control value on a paired sample basis.

FIG. 4. Plasma total cholesterol concentrations of 8 dogs during and after 12 wk of daily ethanol (4 g/kg) administration. Values are expressed as means ± S.E. * $P<0.01$, † $P<0.05$ when compared to control value on a paired sample basis.

TABLE I. Body Weight, Plasma Volume and Total Plasma Lipid Content of 8 Dogs During and After 12 Weeks of Daily Ethanol (4 g/kg) Administration.

Weeks	Body wt, kg	Total plasma volume, ml/kg	Total plasma volume, ml	Plasma triglycerides, mg	Plasma UFA, μEq	Plasma lipid phosphorus, mg	Plasma total cholesterol, mg
0	18.8 ± 1.2	49.8 ± 1.9	942 ± 82	454 ± 63	446 ± 97	103 ± 13	1708 ± 247
1	18.4 ± 1.0	53.5 ± 4.2	989 ± 106	854 ± 89†	231 ± 19*	146 ± 18*	2695 ± 351*
2	18.1 ± .9	55.7 ± 2.8†	1018 ± 94	733 ± 84*	318 ± 38	148 ± 12*	3414 ± 355†
4	17.8 ± .8	60.3 ± 4.0*	1074 ± 84	892 ± 102†	410 ± 30	172 ± 13†	3776 ± 282†
8	17.9 ± .7	54.3 ± 3.4	966 ± 57	732 ± 68†	415 ± 42	163 ± 19*	3604 ± 364†
12	18.7 ± .9	52.1 ± 2.6	970 ± 46	752 ± 90*	367 ± 48	156 ± 22*	3340 ± 250†
Recovery							
1	18.7 ± .9	49.8 ± 3.7	926 ± 61	490 ± 57	455 ± 74	117 ± 6	2451 ± 172*
2	18.9 ± 1.0	51.2 ± 2.8	965 ± 66	541 ± 51	455 ± 36	127 ± 13	2375 ± 201
4	19.0 ± 1.0	52.6 ± 1.7	1002 ± 59	503 ± 68	471 ± 72	103 ± 10	1964 ± 241

Values are expressed as means ± SE.
* $P < .05$, † $P < .01$ when compared to respective control values on a paired sample basis.

gestion and were rapidly corrected after ethanol administration was discontinued.

It is unlikely that inadequate nutrition was responsible for these changes since (1) the animals were observed to eat the food provided which was nutritionally well-balanced and sufficient in quantity, (2) the most marked lipid changes occurred during the first week of both ethanol administration and recovery, and (3) body weight was maintained throughout the period of ethanol administration. It is also doubtful whether the effect of ethanol on plasma lipids could be attributed to the additional caloric intake provided by the ethanol, since no detectable change occurred in plasma lipids during administration of isocaloric amounts of carbohydrate or fat to human subjects(11,12). It is also unlikely that natural fluctuations in blood lipid levels were responsible for these changes, since Rosenfeld *et al*(13) reported minimal changes in blood lipids for dogs over a 2-month period. It should be again emphasized that the blood samples were always drawn at a time when no detectable amount of alcohol could be found in the blood. Consequently, it is probable that the observed changes in plasma lipids represent the chronic effect of ethanol, rather than the transient effects of acute ethanol intoxication.

Changes in plasma volume occurring after ethanol administration could mask the presence or extent of plasma lipid alteration. Since in the present study plasma volume was elevated, the observed increased concentrations of plasma triglycerides, lipid phosphorus and cholesterol were not the result of hemoconcentration. Furthermore, the fall in UFA concentration could not be explained on the basis of hemodilution alone (Table I).

A decrease in plasma UFA levels similar to that found in the present experiment has also been observed in short-term studies in man(11); however, in rats, either increases (14) or no change(15) in plasma UFA have been observed. The ethanol-induced metabolic processes leading to a decrease in plasma UFA are not definitely known. Such a decrease may result either from a decreased mobilization of UFA from the adipose tissue or from their increased uptake by the liver or peripheral tissues.

The marked and sustained increase in plasma triglycerides in the present study is in agreement with the observation of Klatskin(2) that elevated plasma triglyceride levels occurred in rats receiving ethanol for 12 days. Hypertriglyceridemia has been a consistent finding in alcoholic patients(5,12). In rats, plasma triglyceride concentration has been reported to be unchanged(15) or increased(16) following administration of ethanol. Again, the metabolic processes leading to the rise of plasma triglycerides after ethanol ingestion are not definitely known. It could result from a decreased removal of triglycerides from the circulation, perhaps due to a reduction of lipoprotein lipase activity (12), or from an increased synthesis and release of triglycerides by the liver(17). Without turnover rates for the various triglyceride pools, the relative importance of either

of these mechanisms cannot be defined.

The significant increases for plasma lipid phosphorus and cholesterol found in this study are in contrast to data reported on chronic experiments with ethanol-treated rats (2). This could be due to the different species and to the higher ethanol dose employed in our experiment. However, Grande *et al* (3) reported significant increases in serum cholesterol concentration in both normal men and dogs following daily ingestion of ethanol for 2 to 3 weeks. Again, no definite explanation can be offered for the effect of ethanol on plasma phospholipid and cholesterol concentrations in the dog.

Hyperlipemia in chronic alcoholics has been considered to result from liver injury (4) or pancreatitis(5). In the present study no gross or histopathologic changes were observed in either of these tissues obtained 4 weeks after cessation of ethanol administration. In our previous studies, in which samples were obtained immediately after 8 to 12 weeks of ethanol, pancreatitis was never observed and the liver sections revealed only a trace of stainable lipid material(18). On this basis, it is doubtful that even reversible changes occurred in the pancreas and liver during the recovery period in this study. Therefore, it appears that, in the dog, at least, ethanol may exert a direct effect on lipid metabolism since the marked alterations in plasma lipids occurred without demonstrable hepatic or pancreatic damage. This is further supported by the relatively rapid onset of the lipid changes, which were quite marked after the first week of ethanol administration, and their prompt return toward baseline levels after ethanol was discontinued.

Summary. The response of plasma lipids to daily administration of ethanol (4 g/kg body weight) for 12 weeks and following the cessation of ethanol administration was investigated in dogs. Chronic administration of ethanol resulted in marked and sustained increases in plasma concentrations of triglycerides, lipid phosphorus and total cholesterol, and in a decrease of plasma unesterified fatty acids. There was a slight increase in plasma volume. Upon discontinuing administration of ethanol the plasma lipids returned to baseline levels.

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