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ETHANOL INDUCED ALTERATIONS IN LOW AND HIGH DENSITY LIPOPROTEINS[1]

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Abstract. Male squirrel monkeys fed ethanol (ETOH) at variable doses were used to determine whether alcohol modifies levels of plasma low density lipoproteins (LDL) in addition to increasing high density lipoproteins (HDL). Because we earlier showed that high alcohol consumption enhances lipoprotein cholesterol synthesis, experiments were also performed to further assess whether ETOH alters lipoprotein clearance and plasma transfer processes in vivo. Monkeys were divided into three groups: Controls fed isocaloric liquid diet; and Low and High ETOH animals fed liquid diet with vodka substituted isocalorically for carbohydrate at 12 and 24% of calories, respectively. High ETOH primates had significantly more LDL lipid and protein while serum glutamate oxaloacetate transaminase was similar for the three groups. Although removal of ^{3}H LDL cholesteryl ester (CE) from the plasma compartment was not affected by dietary ETOH, transfer of LDL CE to HDL was impaired in the High ETOH group suggesting a mechanism for the enlarged circulating pool of LDL. Transfer of ^{14}C HDL CE to lower density lipoproteins was similar for the three groups. However, ETOH at both doses delayed clearance of radiolabeled HDL CE from circulation. Thus besides enhancing synthesis of lipoproteins, ETOH at a moderately high dose (24% of calories) influences lipoprotein levels in primates by modifying lipid transfer processes (LDL) as well as by altering clearance (HDL) without adversely affecting liver function.

Elevations in low density lipoprotein (LDL) are associated with risk of coronary heart disease (1) while increased levels of high density lipoproteins (HDL) may have a protective effect (2). Although recent attention has focused on ETOH induced elevations in HDL and reduced coronary artery occlusion (3), the effect of low-moderately high chronic alcohol consumption on LDL levels is less clear. For example, Taskinen et al. (4) reported reduced LDL in alcoholics with normal liver function while Goldberg et al (5) and Crouse and Grundy (6) showed no alterations in this lipoprotein class in nonalcoholics following acute vs chronic periods of ETOH intake, respectively. LDL is however, elevated in rats (7) and pigtailed macaques (8) given dietary ETOH

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at 36% of calories and in alcoholics with fatty liver (9). We recently reported increases in very low density-low density lipoprotein (VLDL-LDL) cholesterol in atherosclerosis susceptible squirrel monkeys fed ETOH at 24% of calories (10). One goal of the present study was therefore to determine whether this VLDL-LDL increase was the result of elevations in LDL or both lower density lipoprotein classes.

Theoretically, ETOH-induced changes in lipoprotein levels could result from modifications in synthesis, clearance or plasma transfer processes (1,11). Essentially nothing is known about ETOH's effect on lipid transfer proteins which play an important role in the redistribution of lipids among VLDL, LDL, and HDL (11,12). On the other hand, an earlier rat experiment (7) and our primate study (13) have demonstrated that ETOH may increase HDL by stimulating de novo synthesis. By contrast, a recent investigation has provided indirect evidence that acute ingestion of alcohol causes an elevation in HDL as a result of delayed clearance (5). The second goal of the present study was therefore to determine whether chronic intake of ETOH influences the rate of removal of LDL and HDL from the plasma compartment or exchange of their lipid components in vivo.

Materials and Methods. Fifteen yearling male Bolivian squirrel monkeys were assigned to three treatment groups consisting of 5 monkeys/group: 1) Controls fed isocaloric, chemically defined Juvenile Primate Liquid Diet #19 purchased from BioServ, Inc. (Frenchtown, NJ); 2) Low ETOH animals fed liquid diet with 100 proof vodka substituted isocalorically for carbohydrate (sucrose, maltose-dextrin, dextrin) at 12% of total calories; and High ETOH monkeys given liquid diet plus vodka substituted isocalorically and representing 24% of total calories. Diet #19 had a caloric density of 0.87 kcal/ml, a caloric distribution of 18.4% protein, 29.4% fat, and 52.2% carbohydrate, and a polyunsaturated/saturated fatty acid ratio of 1.0. Feeding protocol, primate housing, monthly blood collections, and plasma cholesterol and serum glutamate oxaloacetate transaminase (SGOT) assays were performed as previously described (10,13). After 24 months of treatment, VLDL and LDL were isolated by sequential ultracentrifugation (14). Lipoprotein cholesterol, protein and phospholipid were measured as described earlier (15).

^{3}H LDL and ^{14}C HDL CE were prepared in vitro exactly as outlined by Portman et al (14). The injected ^{3}H LDL had 140µg total cholesterol, 113µg protein, a specific activity of 11,685,848 DPM/mg cholesterol, and a label distribution of 5% nonesterified cholesterol and 95% CE. Comparable values for ^{14}C HDL were 260µg, 632µg, 1,979,161 DPM/mg and 3% and 97%. Radiolabeled LDL and HDL were combined and injected intravenously into fasted, unanesthetized monkeys. Blood samples were collected at 2,5,10,20, 30,90, and 120 min, HDL was separated from VLDL-LDL (16), and aliquots from each fraction were removed for liquid scintillation counting. HDL radioactivity was corrected to a plasma volume calculated as 4% of body weight. Mean values for the three groups were analyzed for significant differences ($P<0.05$) by analysis of variance and Duncan's Multiple range test.

Results and Discussion. There were no significant differences between the groups in SGOT (range 80-91 IU/ml) suggesting that chronic alcohol intake did not disturb liver function. Body weights of Control, Low and High ETOH monkeys were 650±63, 745±50 and 638±27g, respectively, at the onset of the study and 900±51, 960±34 and 695±79g after 24 months of treatment. High ETOH animals had significantly more plasma cholesterol and triglyceride (289±9, 106±5mg/dl) than Low ETOH (221±3, 94±3mg/dl) and Control (233±7, 86±3mg/dl) primates. VLDL cholesterol (range 3-4mg/dl) and protein (range 11-14mg/dl) were similar for the three groups. However, High ETOH monkeys had more LDL lipid and protein than the other treatment groups (Table 1). This finding represents the first demonstration of ETOH induced elevations in LDL in nonhuman primates without accompanying liver disease at

TABLE I

Effect of Ethanol (ETOH) Consumption on Low Density Lipoprotein (LDL) Composition

	Treatment Groups		
Constituent	Control	Low ETOH (12%)	High ETOH (24%)
Cholesterol	82±5[a,b]	86±5	109±11[b]
Phospholipid	52±4[b]	53±2[b]	68±6[b]
Protein	69±5[b]	75±4[b]	94±8[b]

[a]Values represent means ± SEM for 5 monkeys/group expressed as mg/dl.

[b]High ETOH mean significantly different ($P<0.05$) from means of other groups with superscript b.

a lower caloric dose (24%) than previously reported (8). However, the observed LDL increase in our High ETOH monkeys must also be viewed in light of the concurrent elevation in anti-atherogenic HDL which we recently reported in these animals (13). Earlier studies documented elevations in LDL lipid in alcoholics with fatty liver (9) and in rats and monkeys (7,8) fed ETOH at 36% of calories. On the other hand, LDL apoprotein B is not altered in pigs fed ETOH as 26% of calories (17) or in alcoholics without hepatitis (18). Other studies involving nonalcoholics have shown a wide range of alcohol-LDL responses including no effect (19), positive (20) and negative (21) associations. The exact reason for these discrepancies is unclear.

Elevations in LDL could result from enhanced synthesis, impaired clearance or alterations in plasma transfer processes. We recently showed that High ETOH primates incorporated more ^{3}H mevalonolactone into VLDL-LDL cholesterol compared to Controls suggesting that accelerated production may contribute to LDL elevations (10). Delayed clearance did not contribute to the LDL increase in the present study since the rate of removal of ^{3}H LDL CE from the plasma compartment was similar for the three treatments. However, although the transfer of ^{14}C CE from HDL to VLDL-LDL was not altered by ETOH, the reciprocal movement of ^{3}H CE from LDL to HDL was impaired in both alcohol groups (Fig. 1). Bidirectional transfer of CE between LDL and HDL is facilitated by lipid transfer proteins (11,12). Data in Fig. 1 provide the first account of an ETOH associated disturbance in this process and suggest that besides enhanced synthesis, diminished transfer activity may also increase the pool of circulating LDL in High ETOH monkeys. Impaired lipid transfer may in turn be related to depressed lecithin:cholesterol acyl transferase

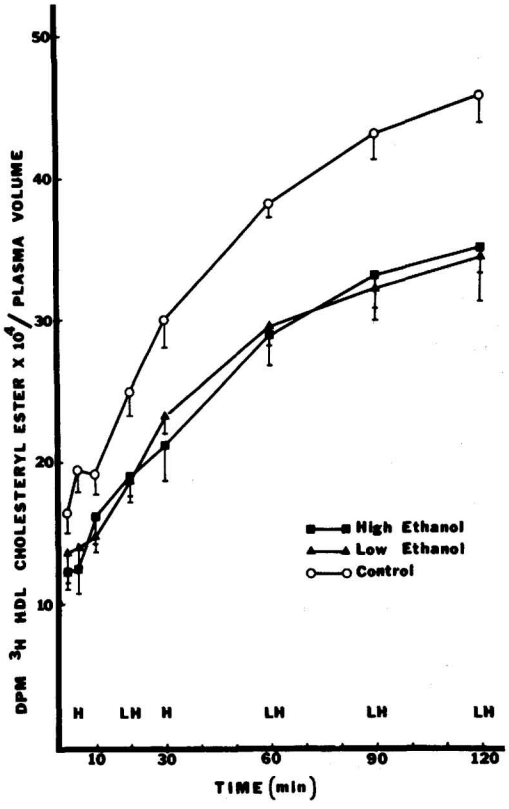

Fig. 1 In vivo transfer of plasma ^{3}H LDL cholesteryl ester to HDL. Time points represent mean ± SEM for 3-5 monkeys/group. L, H, and LH indicate significant difference ($P<0.05$) between Controls and Low ETOH, High ETOH and both Low, High ETOH groups, respectively.

(LCAT) which we observed in High ETOH animals (22) since the activity of LCAT and transfer proteins are closely coordinated (11).

Although alcohol did not interfere with the transfer of lipid from HDL to the lower density lipoproteins, ETOH related alterations in synthesis and clearance are two explanations for the elevations in HDL cholesterol we earlier observed in High ETOH animals (13). Similar to VLDL-LDL, the 24% ETOH regimen also enhanced HDL cholesterol synthesis (10,13). Furthermore, Fig. 2 shows that ETOH at both caloric doses caused a delay in HDL CE clearance from the plasma compartment. When Low and High ETOH values in Fig. 2 were combined as a single alcohol group, t-test analysis revealed significant differences compared to Control means at 10,20,60,90 and 120 min.

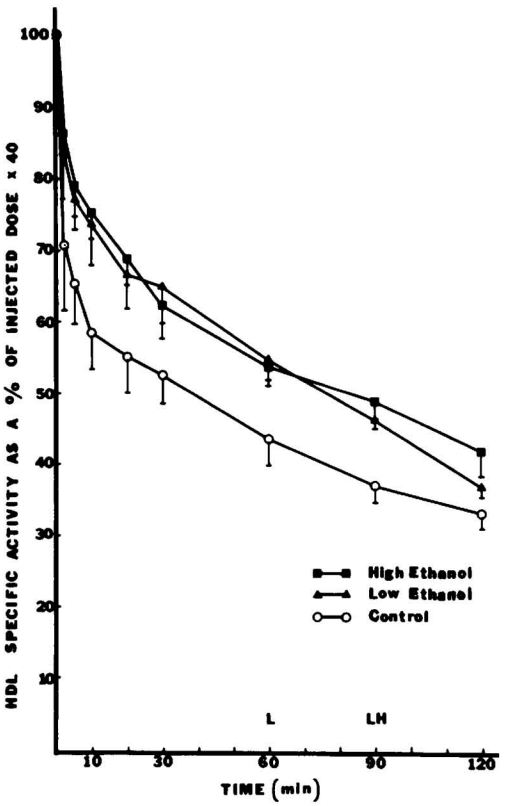

Fig. 2 In vivo clearance of ^{14}C HDL cholesteryl ester from the plasma. Time points and statistical significance same as in Fig. 1 legend.

The mechanism by which ETOH slows clearance of HDL from circulation may be related to the activity of hepatic triglyceride lipase (HTGL) which plays a key role in removing HDL from the blood, degrading its lipids and facilitating its delivery to the liver (4,5). Goldberg et al (5) showed that elevations in HDL in nonalcoholics during acute ETOH intake resulted from inhibition of HTGL. Furthermore, Redgrave and Martin (23) suggested that impaired hepatic uptake was responsible for delayed chylomicron remnant clearance in rats fed ETOH at 36% of calories.

In summary, our results are novel in that they show that besides contributing to elevations in anti-atherogenic HDL, ETOH at 24% of calories also increases atherogenic LDL. Accelerated synthesis as well as ETOH associated impairment in CE transfer from LDL to HDL may both contribute to the enlarged circulating pool of LDL in High ETOH animals. In addition, we have provided the first direct in vivo evidence in primates that alcohol at moderately high doses increases HDL levels by alterations in two important molecular mechanisms: 1) enhanced de novo synthesis (13) and 2) delayed plasma clearance.

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