Alcohol Produces Dose-Dependent Antiatherogenic and Atherogenic Plasma Lipoprotein Responses (43395)

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Abstract. **A comprehensive assessment of lipoprotein compositional/metabolic response to incremental caloric ethanol (EtOH) doses ranging from low to moderate to high was undertaken using male squirrel monkeys. Control monkeys were maintained on a chemically defined, isocaloric liquid diet, while experimental primates were fed increasing doses of alcohol (6, 12, 18, 24, 30, and 36% of energy) substituted isocalorically for carbohydrate at 3-month intervals. Liver function tests and plasma triglyceride were normal for all animals. Plasma cholesterol showed a transient increase at the 12% caloric dose that was attributed solely to an increase in high density lipoprotein (HDL). A more pronounced increase in plasma sterol, beginning at 24% and continuing to 36% EtOH, was the result of increments in both HDL and low density lipoprotein (LDL) cholesterol, although the contribution by the latter was substantial primarily at the 36% dose. Plasma apolipoprotein elevations (HDL apolipoprotein A-I, LDL apolipoprotein B) generally accompanied the lipoprotein lipid increases, although the first atherogenic response for LDL became manifest as a significant increase in apolipoprotein B at 18% EtOH calories. Postheparin plasma lipoprotein lipase was not affected by dietary alcohol, whereas hepatic triglyceride lipase activity showed significant increases at higher (24 and 36%) EtOH doses. Plasma lecithin-cholesterol acyltransferase activity was normal at the 6 and 12% EtOH doses, but exhibited a significant reduction beginning at 18% and continuing to 36% EtOH. Alterations in these key lipoprotein regulatory enzymes may represent the underlying metabolic basis for the observed changes in lipoprotein levels and our earlier findings of HDL_2/HDL_3 subfraction modifications. Results from our study indicate that in squirrel monkeys, moderate (12%) EtOH caloric intake favors an antiatherogenic lipoprotein profile (↑HDL, normal LDL levels, and lecithin-cholesterol acyltransferase activity), whereas higher doses (24–36%) produce both coronary-protective (↑HDL) and atherogenic (↑LDL) responses. Moreover, the 18% EtOH level represents an important transition dose which signals early adverse alterations in lipoprotein composition (↑apolipoprotein B) and metabolism (↓lecithin-cholesterol acyltransferase).** [P.S.E.B.M. 1992, Vol 200]

An increasing body of evidence indicates that there is a causal relationship between moderate alcohol consumption and reduced risk of coronary heart disease (1–6). The underlying mechanism responsible for alcohol's coronary protective effect may be its ability to induce elevations in antiatherogenic high density lipoproteins (HDL) (7–12), which are responsible for removing cholesterol from vascular tissue and facilitating sterol delivery to the liver for excretion in bile (13, 14). By contrast, excessive, prolonged alcohol consumption and accompanying severe liver disease results in diminished HDL (10), putatively as a result of the liver's inability to synthesize and secrete these particles (15) or because of their accelerated clearance from the plasma compartment (16). Moreover, chronic alcoholism may actually increase the risk of cardiovascular disease (3, 4, 17).

While some authors suggest that HDL may in-

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crease 0.03–0.05 mmol/liter for every 10 g of ethanol (EtOH) imbibed per day (10), two recent clinical trials did not demonstrate an HDL cholesterol effect in subjects consuming low (12.6 g) to moderate (35 g) amounts of alcohol daily for 3 to 8 weeks (18, 19). Other investigations were unable to observe any changes in HDL apolipoprotein A-I in men and women consuming 22–44 g of alcohol per day (20). Moreover, atherogenic low density lipoproteins (LDL) show an even more variable pattern as a function of alcohol intake, including no effect (21–23), positive (17, 24–31), and negative (32–34) associations. Although the exact reasons for these discrepancies are unknown, they may be related to inherent difficulties in the design of human alcohol-lipoprotein studies. For example, it is difficult, if not impossible, to control for a number of dietary-lifestyle variables, especially in studies with free-standing populations, which can confound interpretation of experimental results. Cases in point include: the level of ethanol intake, which is often underreported when based on recall or dietary records (10); the amount of physical activity (35) and consumption of dietary fat (saturated, polyunsaturated, and cholesterol), both of which can markedly influence lipoprotein profiles (36); energy intake, which can alter the amount of body fat and, hence, HDL levels (37) and which can include ethanol calories added to nonalcoholic calories in light drinkers (38), or added to but also partially substituted for other energy sources in the diet of moderate to heavy drinkers (38); and binge-drinking patterns, which fail to induce an increase in HDL but which may instead produce an elevation in LDL cholesterol and its protein component, apolipoprotein B (7, 8, 39).

Besides these problems, human alcohol-lipoprotein studies have traditionally involved only one or two experimental ethanol doses using nonalcoholics for short time periods (18, 21, 40–45), or an evaluation of chronic alcoholics with hepatic dysfunction (46–48) and abnormalities in lipoprotein metabolism (11). Moreover, ethical considerations preclude the use of human subjects for experimental studies involving consumption of incremental doses of alcohol ranging from very light to moderate to excessive intake for prolonged periods. And finally, only a limited number of trials have attempted to provide a complete characterization of the lipoprotein response to variable EtOH doses by measuring both the lipid and protein components of the major lipoprotein classes (31, 32, 34, 40), as well as important enzymatic parameters in lipoprotein metabolism (21, 22, 31, 32, 40, 44, 45).

Because of these drawbacks, several investigators have elected to use nonhuman primates in EtOH-lipoprotein experiments because of their close phylogenetic relationship to humans, their clinical relevance to humans in terms of development of spontaneous (49, 50) or diet-induced atherosclerosis (51), and because of the strong homology that exists between human and nonhuman primate lipoprotein metabolic pathways (52, 53). Furthermore, nonhuman primates consistently develop an elevation in LDL at high alcohol doses (26, 54–56) which has obvious pathophysiological implications (57).

In an effort to overcome the above procedural difficulties associated with human trials, we developed a quantitative EtOH delivery system involving the administration of vodka to male squirrel monkeys (55, 56, 58–61). Using a nutritionally adequate, chemically defined liquid diet, these animals can be taught to drink increasing doses of EtOH substituted isocalorically for carbohydrate for chronic periods (55, 60). The present study was undertaken to provide the first comprehensive evaluation of lipoprotein compositional (lipid and protein) response to incremental caloric doses of alcohol ranging from low (6%) to moderate (12%) to high (24–36%) levels characteristic of alcoholics (62). Concurrent measurement of key metabolic correlates (lipoprotein and hepatic triglyceride lipase, lecithin-cholesterol acyltransferase) allowed us to describe the underlying molecular basis for the observed alterations in lipoprotein levels.

Materials and Methods

Thirty male squirrel monkeys (*Saimiri sciureus*) were randomly assigned in equal numbers to control or EtOH groups. Control primates were maintained on isocaloric Primate Liquid Diet No. 19 (BioServ, Inc., Frenchtown, NJ) throughout the 18-month study and thus provided a reference group to assess lipoprotein response over time without the influence of dietary EtOH. EtOH primates were fed progressively increasing doses of alcohol (6, 12, 18, 24, 30, and 36%) substituted isocalorically for carbohydrate for 3-month periods at each dose. One hundred proof vodka was chosen as the source of EtOH (6% calories = 1.18 g EtOH/day) because of its purity and low congener content, and because it represents a common alcoholic beverage consumed by humans. Diet No. 19 had a caloric density of 0.87 kcal/ml, a caloric distribution of 18.4% protein, 52.2% carbohydrate, and 29.4% fat with a polyunsaturated:saturated fatty acid ratio of 1.00 and a cholesterol content of 0.14 mg/kcal.

At the end of each treatment period, blood was collected from the femoral vein of fasted, unanesthetized monkeys into syringes containing disodium-EDTA, plasma was isolated, and aliquots were taken for determination of SGOT, γ-glutamyltranspeptidase (Dade Diagnostics, Inc., Miami, FL), and triglyceride (Sclavo Diagnostics, Wayne, NJ). After precipitation of lower density lipoproteins in another plasma aliquot with heparin-manganese (63), the supernatant was collected and HDL cholesterol (64) and phospholipid (65)

were measured colorimetrically. LDL cholesterol was determined using the formula: (very low density lipoprotein + LDL cholesterol) − plasma triglyceride/5 (66), which provides values nearly identical to those obtained for LDL separated from squirrel monkey plasma by ultracentrifugation (67, 68). An external reference standard (Monitrol; Dade Diagnostics) was used to correct for procedural variations in lipid analyses.

Plasma apolipoprotein A-I was measured using the single radial immunodiffusion method developed by Miles Laboratories (Elkhart, IN). Briefly, plasma samples were individually incubated with an equal volume of 0.2 *M* sodium cholate and 0.1% bovine serum albumin at 37°C for 1.5 hr. Immunodiffusion plates contained 0.1 g/ml of agarose, 0.001 g/ml of sodium azide, and 0.025 g of polyethylene glycol to enhance immunoprecipitation. Unknown plasma apolipoprotein A-I concentrations were determined from a standard calibration curve prepared by microscopic measurement of ring diameters. Quantitative, primate apolipoprotein A-I standard was isolated with a Bio-Gel A-0.5 *M* agarose bead column and its purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antisera to apolipoprotein A-I were prepared for us by Dr. K. C. Hayes at Brandeis University.

Plasma apolipoprotein B-100 was measured by the Tago "maximal diffusion" radial immunodiffusion method (Tago, Inc., Burlingame, CA) using human antiapolipoprotein B sera which cross-react with squirrel monkey apolipoprotein B antigen. Ring diameters were quantitated as described above with a Tago calibrated radial immunodiffusion plate reader. Apolipoprotein B measured in squirrel monkey plasma quantitatively represents LDL protein content because very low density lipoprotein (VLDL) is typically present in low concentration in this primate species (69).

Plasma lecithin-cholesterol acyltransferase (LCAT) was measured as described by Stokke and Norum (70) and modified by Lacko *et al.* (71). In addition, postheparin plasma samples were collected at selected EtOH doses from fasted primates 10 min after the intravenous injection of 100 IU/kg body wt of heparin (Lipo-Hepin; Riker Laboratories, Inc., Northridge, CA), which quantitatively releases lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) into the blood. Triglyceride hydrolysis was quantitated using tri-(1-^{14}C)-oleoylglycerol as described by Musliner *et al.* (72). Liberated ^{14}C-oleic acid was extracted as outlined by Belfrage and Vaughan (73) and measured with a Beckman liquid scintillation spectrometer. Lipoprotein lipase was calculated as the difference between total postheparin lipolytic activity measured in the presence of 0.19 *M* NaCl and the activity of HTGL assayed in 1.0 *M* NaCl. LPL and HTGL activities in the EtOH group were calculated as μmoles of fatty acid liberated/ml/hr of postheparin plasma and expressed as a percentage of control values set at 100%.

Data were expressed as the mean ± SE. Mean values were analyzed for significant differences ($P < 0.05$) by analysis of variance and Duncan's multiple range test.

Results

Alcohol at increasing caloric percentages had no significant effect on two measures of liver function. SGOT ranged from 7 to 30 IU/liter in control animals and from 8 to 34 IU/liter in the EtOH group. Means ± SE for γ-glutamyltranspeptidase at selected doses were: control, 94 ± 15 units/ml; 12%, 76 ± 3; 24%, 87 ± 4; and 36%, 113 ± 10. Similarly, no significant alteration in plasma triglyceride was observed in control (range, 57–103 mg/dl) versus EtOH (range, 69–105) primates.

Prior to the start of the study, there were no significant differences in plasma cholesterol between the control (289 ± 17 mg/dl) and pre-EtOH (283 ± 7 mg/dl) treatment groups, although these values were generally higher than what we had reported previously for control monkeys fed Liquid Diet No. 19 (190–233 mg/dl) (56, 58–60). Thereafter, plasma total cholesterol showed a transient elevation at the 12% dose and then a dramatic increase beginning with 24% and continuing to 36% EtOH (Fig. 1). At the 12% level, the cholesterol elevation was attributed primarily to an increase in HDL cholesterol (Fig. 2), while at the 24, 30, and 36% doses, the plasma sterol increase was a result of increments in both HDL (Fig. 2) and LDL (Fig. 3) cholesterol. Across time, plasma cholesterol in control primates at the 6% EtOH treatment period was significantly ($P < 0.05$) higher than plasma cholesterol for controls at all other periods, with the exception of the 36% EtOH treatment period (Fig. 1). HDL cholesterol in control monkeys at the 36% EtOH period was sig-

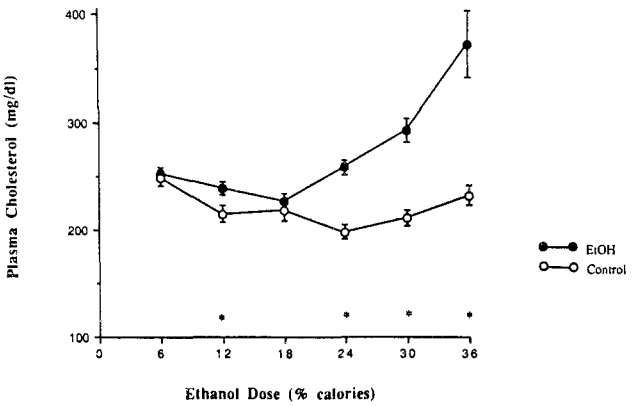

Figure 1. Effect of ethanol dose on plasma cholesterol concentration. Values represent mean ± SE for 15 monkeys/group expressed as mg/dl. Asterisk indicates a significant difference ($P < 0.05$) between EtOH (●) and control (○) groups.

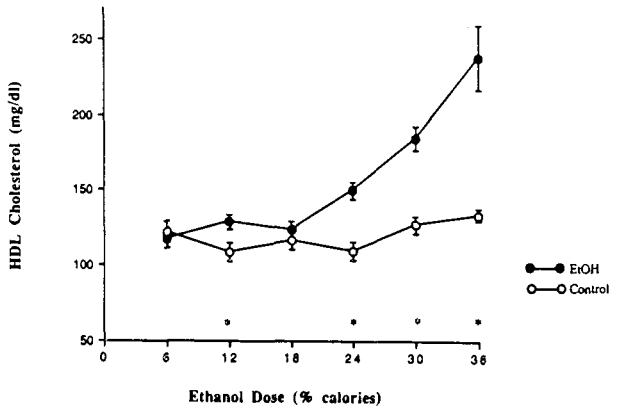

Figure 2. Effect of ethanol dose on high density lipoprotein cholesterol concentration. Values represent mean ± SE for 15 monkeys/group expressed as mg/dl. Asterisk indicates a significant difference ($P < 0.05$) between EtOH (●) and control (○) groups.

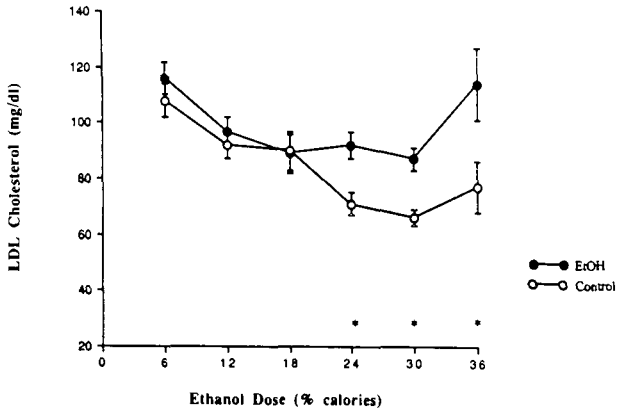

Figure 3. Effect of ethanol dose on low density lipoprotein cholesterol concentration. Values represent mean ± SE for 15 monkeys/group expressed as mg/dl. Asterisk indicates a significant difference ($P < 0.05$) between EtOH (●) and control (○) groups.

nificantly greater than that for controls at the 12% and 24% EtOH periods (Fig. 2). LDL cholesterol in control animals at the 6% EtOH period was significantly greater than that for controls at all other periods (Fig. 3). Controls at the 12% and 18% EtOH periods also had higher LDL cholesterol values than controls at the 24% and 30% periods. The above plasma lipid alterations in control monkeys may reflect seasonal variations over the course of the 18-month study period.

Plasma apolipoprotein levels generally followed the pattern of lipoprotein cholesterol, with significant increases in HDL apolipoprotein A-I (Fig. 4) accompanying the elevations in HDL cholesterol beginning at the 24% dose, which indicated that alcohol caused an increase in the entire HDL particle mass (lipid + protein). Absolute mean concentrations ± SE (mg/dl) of apolipoprotein A-I for control monkeys at the time points corresponding to the 6, 12, 18, 24, 30, and 36% EtOH doses were 180.6 ± 9.1, 212.7 ± 16.1, 277.0 ± 33.8, 291.7 ± 16.6, 303.0 ± 35.7, and 263.6 ± 19.3, respectively. Apolipoprotein B first showed an increase

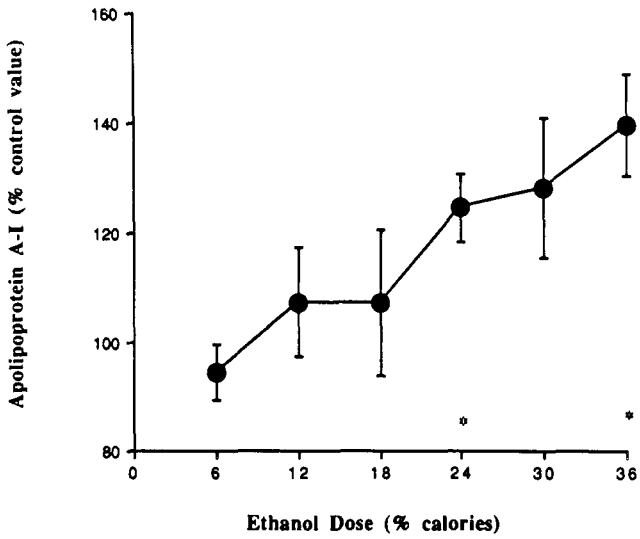

Figure 4. Effect of ethanol dose on plasma apolipoprotein A-I concentration. Values represent mean ± SE for 15 monkeys/group expressed as percentage of control values set at 100. Asterisk indicates significant difference ($P < 0.05$) between EtOH and control groups.

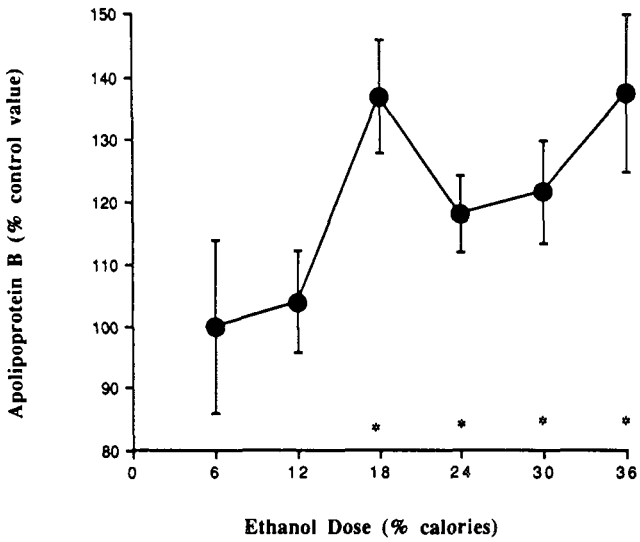

Figure 5. Effect of ethanol dose on plasma apolipoprotein B concentration. Values represent mean ± SE for 15 monkeys/group expressed as percentage of control values set at 100. Asterisk indicates significant difference ($P < 0.05$) between EtOH and control groups.

at 18% EtOH and remained significantly elevated at the higher (24–36%) alcohol doses (Fig. 5). Absolute mean concentrations ± SE of apolipoprotein B for control monkeys at the time points corresponding to the 6, 12, 18, 24, 30, and 36% EtOH doses were 80.9 ± 5.3, 83.3 ± 5.2, 90.1 ± 4.5, 98.4 ± 4.9, 113.6 ± 7.9, and 98.5 ± 11.2, respectively.

Lipoprotein lipase, expressed as a percentage of control values, showed a nonsignificant increase at 12% EtOH and remained at slightly elevated levels at the 24% and 36% doses (Fig. 6). By contrast, hepatic triglyceride lipase increased linearly with increasing levels

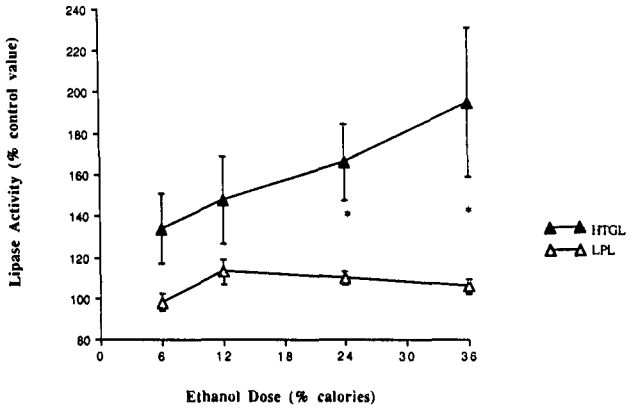

Figure 6. Effect of ethanol dose on postheparin plasma LPL and HTGL activity measured as μmoles fatty acid liberated/ml/hr. Values represent mean ± SE for 15 monkeys/group expressed as percentage of control values set at 100. Asterisk indicates significant difference ($P < 0.05$) between EtOH and control groups.

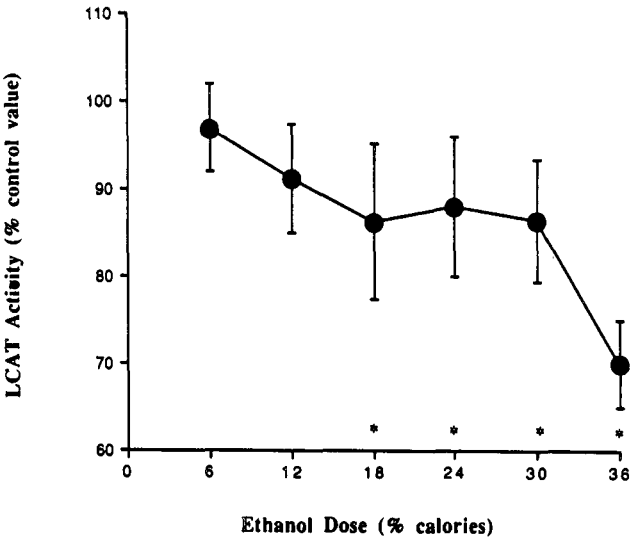

Figure 7. Effect of ethanol dose on plasma lecithin-cholesterol acyltransferase activity measured as percentage of esterification/min. Values represent mean ± SE for 15 monkeys/group expressed as percentage of control values set at 100. Asterisk indicates a significant difference ($P < 0.05$) between EtOH and control groups.

of dietary EtOH and achieved statistical significance at the 24% and 36% doses relative to controls (Fig. 6). Plasma LPL activity, measured in absolute units (μmol/ml/hr), was, on the average for all animals, seven times higher than corresponding HTGL values. Control LPL activities corresponding to the 6, 12, 24, and 36% EtOH dose periods were 23.0 ± 1.2, 25.8 ± 2.3, 21.1 ± 0.9, and 24.4 ± 1.0, respectively. Control HTGL activities for the same periods were 8.2 ± 2.0, 2.9 ± 0.4, 2.1 ± 0.3, and 1.9 ± 0.1, respectively.

Figure 7 shows that plasma LCAT activity, expressed as a percentage of control values, was not altered in monkeys fed 6% and 12% EtOH, but exhibited a significant reduction beginning at 18% and continuing to 36% EtOH. Control LCAT activities (% esterification/min) corresponding to the 6, 12, 18, 24, 30, and 36% EtOH dose periods were 0.151 ± 0.006, 0.170 ± 0.010, 0.152 ± 0.011, 0.134 ± 0.007, 0.140 ± 0.007, and 0.117 ± 0.007, respectively. By contrast, LCAT substrate, HDL phospholipid, increased linearly as a function of alcohol dose (Fig. 8). However, LCAT activity was inversely related to VLDL + LDL cholesterol (Fig. 9).

Discussion

Although γ-glutamyltranspeptidase was somewhat elevated at the 36% EtOH dose (as reported in Results),

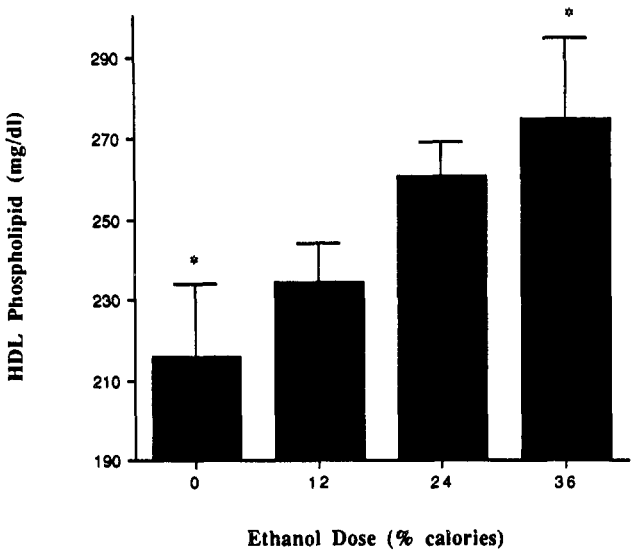

Figure 8. Effect of ethanol dose on high density lipoprotein phospholipid concentration. Values represent mean ± SE for 15 monkeys/group expressed as mg/dl. Asterisk indicates a significant difference ($P < 0.05$) between EtOH and control groups.

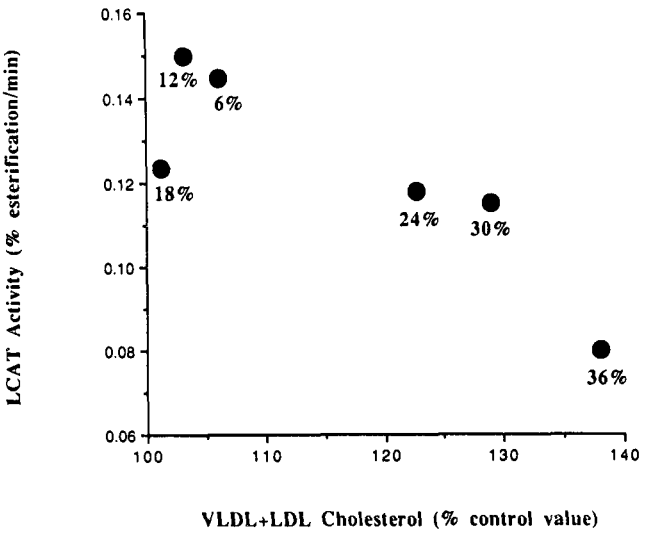

Figure 9. Relationship between lecithin-cholesterol acyltransferase activity and VLDL + LDL cholesterol. Values represent mean values for LCAT activity measured as percentage of esterification/min plotted against mean VLDL + LDL cholesterol (% control values) for each alcohol dose (% calories).

there were no significant differences between treatment groups at any alcohol level for this variable or SGOT, which are currently considered the best indices of alcohol-induced liver damage (74). Absence of liver injury may reflect the short time period (3 months) primates were maintained at each dose. However, squirrel monkeys continue to show normal liver function tests even when fed 24% EtOH for prolonged periods (2 years), suggesting that this species may be somewhat resistant to hepatic damage following consumption of high alcohol doses (60).

Fasting plasma triglyceride was unaltered by EtOH treatment through 36% of calories (Results). Very low density lipoprotein lipid plus protein mass in this species is also not affected by high dose alcohol (60). Since hypertriglyceridemia prevails at the fatty liver stage of alcohol abuse (24), this is taken as additional evidence of normal liver function in our animals even at high alcohol doses. Moreover, since lipoprotein lipase activity remained stable throughout the range of alcohol doses (Fig. 6), our data indicate that EtOH did not impede this enzyme in clearing VLDL and chylomicron triglyceride from the circulation. By contrast, plasma total cholesterol showed a transient elevation at the 12% dose and then a marked increase beginning with 24% and continuing to 36% EtOH (Fig. 1). We demonstrated recently that this hypercholesterolemic response can become manifest in squirrel monkeys fed 24% EtOH in as little as 2 weeks and encompasses an elevation in both HDL and LDL (56). Increases in LDL include increments in both lipid and protein which may remain elevated for up to 2 years with continued drinking (60). Thus, squirrel monkeys and other nonhuman primates (pigtailed macaques, baboons) appear to show a reproducible elevation in plasma cholesterol following consumption of EtOH at 24–50% of calories, suggesting that the lipoproteins of these species may be particularly responsive to alcohol-mediated changes (26, 54, 55). Humans, on the other hand, exhibit a much more varied plasma cholesterol response to dietary alcohol. While results from a predominant number of clinical trials involving nonalcoholics consuming low-moderate EtOH doses (6, 19, 40–42, 75, 76) and investigations with chronic alcoholics (32, 48, 77, 78) demonstrate low or normal plasma cholesterol levels, a limited number of other studies of nonalcoholics given moderately high amounts of alcohol (22, 31) and alcoholics (79) show a significant elevation in circulating total cholesterol. In alcoholics at least, the presence or absence of a hypercholesterolemic response may reflect the stage of liver damage, since hyperlipemia, in general, is more common in patients with fatty liver than cirrhosis (24).

Similar to plasma cholesterol, alcohol's influence on LDL cholesterol levels in humans is often variable and ranges from no effect (21–23) to increases (17, 24–31) or decreases (32–34, 80). In addition, apolipoprotein B, LDL's major protein component, may either increase (30) or decrease (33, 80) when alcohol is included in the diet of nonalcoholics. The exact reason for these disparate lipoprotein responses is not known, although alcohol dose may be an important factor, since two recent studies demonstrate significant increases in LDL protein in volunteers given 60 g (30, 31) but not 30 g of EtOH per day (31). Nonhuman primates, on the other hand, consistently show elevations in LDL cholesterol and/or protein at EtOH doses ranging from 18 to 50% of total daily calories (26, 54, 55, 60). In squirrel monkeys, in particular, plasma apolipoprotein B remains at abstinent levels in animals fed 12% EtOH, but shows a significant elevation beginning at 18% and continuing to 36% EtOH (Fig. 5). Since LDL cholesterol is also low in monkeys fed the 12% dose, but elevated at 24% alcohol (Fig. 3), the intermediate 18% level may represent an important transition dose to alcohol levels with potentially detrimental effects on circulating lipoproteins. Furthermore, it appears that at 24% of calories, one underlying metabolic factor in the LDL increase in EtOH-fed squirrel monkeys may be accentuated LDL production (59), although disturbances in the transfer of cholesteryl ester between LDL and HDL (60) and alterations in LDL catabolism (34) may also be important.

Consistent with a large number of other animal (25, 26, 54) and human (10, 11, 17, 81) studies, squirrel monkeys show a dose-related increase in HDL cholesterol beginning at the 12% EtOH caloric level and continuing to the 36% dose (Fig. 2). Interestingly, the squirrel monkey plasma cholesterol response-curve for dietary EtOH (Fig. 1) is largely a reflection of change in HDL cholesterol (Fig. 2). However, at 12% EtOH, the total plasma cholesterol increase is mainly attributable to an increment in HDL cholesterol (Fig. 1 and 2), while at higher doses (24–36%), both LDL (Fig. 3) and HDL (Fig. 2) contribute to the cholesterol increase, although the former plays a major role only at the 36% dose. Moreover, as reported in many other studies (31–33, 37, 44, 45, 81), an elevation in HDL's major protein component, apolipoprotein A-I, accompanies the lipid increase (Fig. 4), indicating that alcohol induces a change in the entire lipoprotein particle mass (32, 33, 45). In squirrel monkeys, the first significant increase in apolipoprotein A-I occurs at 24% EtOH (Fig. 4), which is somewhat after the initial HDL cholesterol increase at 12% EtOH (Fig. 2).

A recent controversy has emerged concerning which HDL subfraction is altered in response to alcohol consumption (81–84). This has important clinical implications because elevated HDL_2 is more often cited as the particular HDL subfraction linked to decreased coronary risk (44, 85–87), although recent investigations indicate that HDL_3 may preferentially promote cellular cholesterol efflux (88) and independently confer

coronary protection (89). While a number of studies involving acute (21), light to moderate (40, 44, 90, 91), or heavy (32, 45, 46, 77, 92–94) drinking have reported increments in predominantly the HDL_2 subclass, other trials have demonstrated increases in both subfractions (12, 31, 33, 80, 95–104), and later work has documented a selective elevation in HDL_3 following moderate alcohol intake (83, 84, 105–109). More recently, several investigators have concluded that alcohol may alter HDL subfractions variably depending upon dose (31, 45, 80, 84). In squirrel monkeys, the HDL_2 versus HDL_3 response is clearly related to alcohol dose (55, 110). At 12% of EtOH calories, these animals exhibit an HDL_2:HDL_3 cholesterol ratio that is significantly greater than in controls (0%) or monkeys fed higher (24 and 36%) doses (55). However, with additional amounts of alcohol included in their diet (24 and 36% calories), HDL_3 cholesterol and total mass (lipid + protein) show a proportionate increase along with HDL_2, resulting in an overall decline in the HLD_2:HDL_3 ratio (55). Thus, if the HDL_2:HDL_3 ratio is a better predictor of coronary risk than total HDL, as suggested by several investigators (111, 112), then a reduction in this ratio and a commensurate increase in LDL cholesterol (Fig. 3) and apolipoprotein B (Fig. 5) at the higher alcohol doses in squirrel monkeys would lead to a less favorable lipoprotein profile compared with animals fed more moderate (12%) EtOH levels.

In an attempt to understand the metabolic basis of the above EtOH-induced changes in circulating lipoprotein levels, we measured the activity of three important enzymes in the lipoprotein metabolic scheme: (i) lecithin-cholesterol acyltransferase; (ii) lipoprotein lipase; and (iii) hepatic triglyceride lipase. LCAT was of particular interest because any disruption in plasma cholesterol esterification, as in familial LCAT deficiency, may have potentially profound effects on cell membranes, lipoprotein composition-metabolism, reverse cholesterol transport, and atherogenesis (113–116). We showed earlier that dietary EtOH at moderate doses (12% of calories) in squirrel monkeys results in normal or elevated acyltransferase activity, whereas a higher dose (24%) produces a significant decrease in cholesterol esterification (61). It was, therefore, important to determine whether a significant decline in LCAT activity became apparent at an intermediate dose between 12 and 24% EtOH, and whether cholesterol esterification was further impaired at substantially higher levels of alcohol intake (30–36% of calories).

As noted in Figure 7, LCAT activity remained in the normal, control range in monkeys fed 6 and 12% EtOH, but showed a significant reduction beginning at 18% and continuing to 36% EtOH. Reduced acyltransferase activity in monkeys fed the 24 and 36% doses and concurrent increases in HDL_3 (55) may reflect an impaired conversion of $HDL_3 \rightarrow HDL_2$ (117). In addition, we demonstrated earlier that cholesterol esterification remains depressed in squirrel monkeys fed 24% EtOH, even after 2 months of abstinence (118). However, severe LCAT deficiency, analogous to what has been reported in patients with alcoholic hepatitis (47), was never observed in our primates. Interestingly, reduced LCAT activity at the higher alcohol doses occurred despite elevated concentrations of LCAT substrate (HDL phospholipid, Fig. 8) and LCAT activator (apolipoprotein A-I, Fig. 4). More specifically, HDL phosphatidyl choline mass and accompanying linoleic acid levels are actually elevated in monkeys fed high dose (24%) alcohol (61), indicating that EtOH consumption in this species does not lead to lipoprotein essential fatty acid depletion, which is sometimes observed in humans following chronic alcohol intake (119).

We suggested earlier that reaction product (lysophosphatidyl choline) inhibition of LCAT (61) or a direct influence of circulating EtOH (120) may contribute to diminished transesterification at high alcohol doses. However, more recent studies have emphasized that overloading of VLDL and LDL with cholesteryl esters may also inhibit the LCAT reaction (121). The latter observation is consistent with the inverse relationship between LCAT activity and VLDL + LDL cholesterol, as a function of alcohol dose, presented in Figure 9. Regardless of the mechanism involved, these data reaffirm the importance of measuring plasma LCAT activity as a biochemical marker of alcohol-related alterations in HDL metabolism in the absence of overt liver dysfunction (61).

The final two lipoprotein regulatory enzymes measured in the present study were lipoprotein lipase and hepatic triglyceride lipase. LPL, located on the capillary endothelium of extrahepatic tissues, degrades the triglyceride core of VLDL and chylomicrons as their surface components are transferred to HDL_3, which is then converted to HDL_2 by the action of LCAT (40, 81, 122–124). HTGL is located on the plasma membranes of hepatic endothelial cells and is responsible for hydrolyzing the phospholipid and triglyceride components of VLDL and chylomicron remnants, HDL_2, and LDL (21, 24, 40, 124–126). Depending on ethanol dose, acute versus chronic period of intake, species studied, and degree of liver damage, a wide range of responses has been reported for these two enzymes, including no effect, depressed, and accentuated activity (15, 21, 22, 24, 31, 32, 40, 44, 45, 82, 127, 128).

Nikkila (129) and Taskinen *et al.* (40, 80) have attempted to clarify these time-dose discrepancies by suggesting that acute intake of alcohol may have a transient inhibitory effect on LPL that returns to normal levels after 10 h. Subsequent short-term episodes of drinking enhance LPL activity, which is more dra-

matically increased with prolonged heavy consumption of alcohol (32, 40). The latter may be a compensatory response in the alcoholic to accentuated hepatic VLDL synthesis (40).

Similarly, acute intake of alcohol may cause a temporary inhibition of HTGL (21, 40), which may later increase in activity in the chronic drinker without evidence of liver dysfunction (32, 92). However, as alcoholic hepatitis develops, a marked depression in HTGL is observed (128, 130).

The concentration of HDL (specifically HDL_2) in plasma appears to be controlled by the balance of LPL versus HTGL activity, with LPL enhancing HDL_2 synthesis and HTGL promoting its clearance from circulation (32, 40, 129, 131). Two important consequences of HTGL lipolytic activity may include hepatic degradation of HDL lipids and delivery of sterol for biliary excretion (21, 40, 124, 126, 131, 132), and reconversion of HDL_2 to HDL_3, which can then be returned to the blood (131, 133, 134). Thus, a consideration of the data presented in Figure 6 and our earlier studies (55, 110) suggests that in squirrel monkeys, the sustained, higher absolute (μmol/ml/hr) activity of LPL would favor net HDL_2 synthesis and concurrently maintain plasma triglyceride (VLDL) at normal levels (Results) (60, 80, 129). This intravascular HDL production along with the enhanced *de novo* hepatic HDL synthesis we also observed (58) would lead to higher plasma HDL (HDL_2) levels at elevated alcohol doses (Fig. 2) (55). On the other hand, increased relative, but lower absolute, HTGL activity (Fig. 6) would permit HDL synthesis via LPL while promoting delivery of HDL_2 to the liver and enhancing its conversion to HDL_3. The latter hypothesis is consistent with the increased neutral sterol and bile acid excretion and elevated HDL_3 levels we reported earlier in monkeys fed 24–36% EtOH calories (55, 59). More importantly, elevated HTGL would not only indicate normal hepatic function, since the activity of this enzyme is depressed with liver injury (24, 128), but may also signal enhanced LDL production at the 24 and 36% doses (Figs. 3 and 5), because HTGL is responsible for the final conversion of VLDL remnants to LDL in the liver (132–134, 136–138).

We have provided the first detailed description of the relationship between alcohol dose and lipoprotein composition-metabolism using a nonhuman primate model whose pathophysiological response to dietary EtOH resembles that seen in humans. In addition, we have delineated alcohol doses in squirrel monkeys that clearly produce antiatherogenic (12%) versus coronary-prone (24%) lipoprotein profiles, and identified an intermediate dose (18%) that represents an important transition to alcohol levels with decidedly unfavorable effects on circulating lipoproteins.

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