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ETHANOL ENHANCES DE NOVO SYNTHESIS OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL[1]

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Abstract. Male squirrel monkeys fed ethanol at variable doses were used to assess whether alcohol enhances de novo synthesis of high density lipoprotein (HDL) cholesterol in vivo. Monkeys were divided into three groups: 1) Controls fed isocaloric liquid diet; 2) Low Ethanol monkeys fed liquid diet with vodka substituted isocalorically for carbohydrate at 12% of calories; and 3) High Ethanol animals fed diet plus vodka at 24% of calories. High Ethanol primates had significantly higher levels of HDL nonesterified cholesterol than Control and Low Ethanol animals while serum glutamate oxaloacetate transaminase was similar for the three treatments. There were no significant differences between the groups in HDL cholesteryl ester mass or specific activity following intravenous injection of labeled mevalonolactone. By contrast, High Ethanol monkeys had significantly greater HDL nonesterified cholesterol specific activity with approximately 60% of the radioactivity distributed in the HDL_3 subfraction. This report provides the first experimental evidence that ethanol at 24% of calories induces elevations in HDL cholesterol in primates through enhanced de novo synthesis without adverse effects on liver function.

Experimental studies with primates (1) and clinical trials with humans (2,3) have demonstrated that ethanol consumption is associated with elevations in HDL cholesterol and putative protection against coronary artery disease (1,2). Despite these observations, little is known about the threshold dose at which this elevation first occurs or the metabolic mechanism(s) responsible for this increase. Two current hypotheses suggest that alcohol may raise HDL levels by: 1) direct stimulation of hepatic lipoprotein synthesis and secretion secondary to ethanol's induction of microsomal enzyme activity (2,3); and/or 2) by enhancing extra-hepatic lipoprotein lipase activity which promotes transfer of surface components from very low density lipoproteins (VLDL) and chylomicrons during lipolysis to nascent HDL_3 particles (2,4). HDL_3, which may be secreted directly from the liver, is then converted to spherical HDL_2 via the lecithin cholesterol acyltransferase reaction (4,5). Taskinen et al. (6) demonstrated increased lipoprotein lipase activity and elevated HDL_2 cholesterol concentrations in male alcoholics in support of the latter conjecture. However, little experimental data is available to substantiate the enhanced de novo synthesis hypothesis. The present study was therefore designed to determine whether variable doses of ethanol fed to atherosclerosis susceptible squirrel monkeys enhance in vivo synthesis of HDL cholesterol from radiolabeled precursor.

Materials and Methods. Fifteen yearling male Bolivian squirrel monkeys were purchased from South American Primates, Inc.

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(Miami, FL) and randomly assigned to three treatment groups consisting of five monkeys/group: Controls fed isocaloric, chemically defined Juvenile Primate Liquid Diet #19 (18.4% protein, 29.4% fat and 52.2% carbohydrate) purchased from BioServ Inc. (Frenchtown, NJ); and Low and High Ethanol animals fed liquid diet with 100 proof vodka substituted isocalorically for carbohydrate at 12 and 24% of total calories, respectively. Monkeys were housed individually and given 80 ml (0.87 Kcal/ml) of diet twice daily.

Blood was collected monthly from the femoral vein of fasted monkeys into tubes containing EDTA·Na_2, plasma was isolated, and total cholesterol was measured colorimetrically (7). Serum glutamate oxaloacetate transaminase (SGOT) was monitored using the Reitman-Frankel method (Dade Diagnostics, Inc., Miami, FL). HDL was separated by heparin-manganese precipitation (8) and its nonesterified cholesterol component was measured by gas liquid chromatography with 3% SP-2250 on 100/200 Supelcoport (Supelco Inc., Bellefonte, PA), under isothermal conditions (267°C) and with cholestane as an internal standard (9). Total HDL cholesterol was determined in a similar manner following saponification with KOH (9) and esterified cholesterol was calculated by difference.

After one year of treatment, fasted, unanesthetized monkeys were injected intravenously with 183-250 uCi of RS-(5-^{3}H) mevalonolactone (specific activity 13.8 Ci/mmol) (New England Nuclear, Boston, MA). Blood samples were collected at 5, 20, 40 min, 1, 2, 3, 4, 5, 6 and 24 hr and plasma was immediately separated. Monkeys were allowed free access to diet after the 40 minute time point. HDL was isolated as described above and immediately extracted with chloroform:methanol (2:1 v/v) (10). Nonesterified and esterified cholesterol were separated by thin-layer chromatography (11) and radioactivity was measured with a liquid scintillation spectrometer. HDL cholesterol and cholesteryl ester mass and radioactivity were corrected to a plasma volume calculated as 4% of body weight and specific activity was expressed as percent of injected dose. HDL subfractions in the two hour plasma sample were separated by polyacrylamide gel electrophoresis (11). Bands were subjected to scanning densitometry, then cut, digested with NCS solubilizer (Amersham Corp., Arlington Heights, IL) and radioactivity was monitored. 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 was no significant difference in the mean body weights between the Control (825 ± 69g), Low (890 ± 74) and High (675 ± 62) groups or in SGOT (range 82-90-IU/ml). However, High Ethanol animals circulated significantly more total plasma cholesterol (286 ± 9 mg/dl) and HDL nonesterified cholesterol (49 ± 4) than Low Ethanol (221 ± 5, 36 ± 2) and Control (233 ± 7, 39 ± 3) primates. There were no significant differences between the three groups in HDL cholesteryl ester mass (range: 134-152 mg/dl) or specific activity. By contrast, High Ethanol monkeys incorporated significantly more ^{3}H mevalonolactone into HDL nonesterified cholesterol beginning at two hours after injection, continuing for the next 4 hours, and at 24 hours (Fig. 1). It is unlikely that the HDL cholesterol specific activity patterns reported in Fig. 1 were the result of ethanol related alterations in hepatic mevalonic acid pool size and/or exchange of radiolabeled mevalonate with endogenous pools since previous *in vivo* and *in vitro* cholesterol synthesis experiments with rats (36% dietary ethanol) using ^{14}C mevalonate argue against such changes (12).

Densitometric scans of HDL_2 and HDL_3 subfractions were similar for the three treatments which had a HDL_2/HDL_3 ratio range of 5.8-8.7. In addition, for all groups, only 39% of the radioactivity appeared in the HDL_2 subclass while the majority (61%) of the label was associated with HDL_3 suggesting that the nascent HDL_3 particles were preferentially labeled with newly synthesized cholesterol. This is important in view of recent reports which demonstrate that 40% of the total body cholesterol synthesis in squirrel monkeys occurs in the liver (13) and that the HDL_3 subfraction is selectively elevated in men who consume moderate amounts of alcohol (14). It is thus likely that the greater HDL nonesterified cholesterol

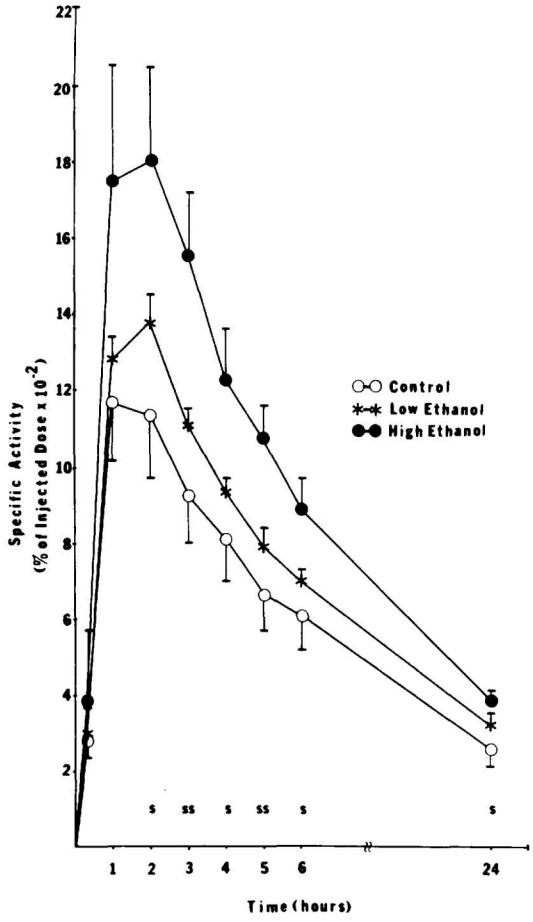

Fig. 1 in vivo synthesis of HDL nonesterified cholesterol following the injection of ^{3}H mevalonolactone. Time points represent mean ± SEM for 5 monkey/group. S indicates significant difference ($P < 0.05$) between High Ethanol and Control monkeys and SS difference between High Ethanol and both Control, Low Ethanol groups.

radioactivity in High Ethanol monkeys (Fig. 1) was the result of enhanced hepatic de novo synthesis.

Taken together, data from this study support previous in vivo (15) and liver perfusion (16) rat studies which suggest that increased HDL lipid synthesis results from intake of dietary ethanol at 36 - 37% of calories. The present study is unique, however, in elucidating a lower dose (24%) at which this ethanol effect occurs, and in providing the first in vivo experimental documentation that alcohol consumption specifically enhances de novo HDL nonesterified cholesterol synthesis in a nonhuman primate species with clinical relevance to man.

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