Pathogenesis of the hypertriglyceridemia at early stages of alcoholic liver injury in the baboon

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Abstract To study the mechanism of alcoholic hypertriglyceridemia, baboons were pair-fed liquid diets containing 50% of energy as ethanol or as additional carbohydrate for 5–16 months. Alcohol-fed animals developed hypertriglyceridemia and early stages of alcoholic injury, namely fatty liver with or without perivenular fibrosis. In the fasting state, the triglyceride content was sixfold higher in very low density (VLDL) and intermediate density (IDL) lipoproteins and twofold higher in low density (LDL) and high density (HDL) lipoproteins. The increase in VLDL was markedly exaggerated in the postprandial state. To investigate the source of these increases, we determined net output or removal of serum triglycerides during circulation through either splanchnic or extrasplanchnic (lower extremities) vascular beds. In the splanchnic territory, there was net output of triglycerides in VLDL and net removal from the other lipoproteins. In alcohol-fed baboons, the output of VLDL-triglycerides into the hepatic (but not into the portal) vein tripled. This increase was mainly due to production of VLDL particles that were larger and had a flotation (S_f > 400) different from the S_f 20–400 which predominated in controls. This was associated with increased splanchnic removal of labeled chylomicron- or VLDL-triglycerides. In the lower extremities, there was an arteriovenous difference in VLDL-triglyceride concentration and this was increased in the alcohol-fed animals. Thus, the primary mechanism of the hypertriglyceridemia in alcohol-fed baboons was increased production of large, chylomicron-like VLDL by the liver, whereas both the extrasplanchnic extraction of VLDL-triglycerides and the splanchnic extraction of triglycerides from chylomicron- and VLDL-remnants were secondarily enhanced. — **Savolainen, M. J., E. Baraona, M. A. Leo, and C. S. Lieber.** Pathogenesis of the hypertriglyceridemia at early stages of alcoholic liver injury in the baboon. *J. Lipid Res.* 1986. **27:** 1073–1083.

Supplementary key words VLDL • chylomicrons

Alcohol consumption is one of the most common causes of hypertriglyceridemia (1), an effect that is particularly prominent in the postprandial state (2, 3). The greatest triglyceride elevation occurs in lipoproteins of very low density (VLDL), including chylomicrons or chylomicron-like particles that have also been observed in the fasting state (1, 4). In alcohol-fed rats, hyperlipemia persists even when the lipid output in the mesenteric lymph is equalized with that of pair-fed controls (5) or when the removal of serum triglycerides is blocked with Triton WR-1339 (6), suggesting enhanced hepatic production of serum triglycerides. However, the observation that the clearance of chylomicron-cholesteryl esters is decreased in ethanol-fed rats (7, 8) raised the alternate possibility that alcohol produces a defect in the hepatic uptake of chylomicron remnants. This study was therefore undertaken to investigate more directly whether the ethanol-induced hypertriglyceridemia is due to enhanced hepatic production of VLDL triglycerides, decreased hepatic removal of lipoprotein remnants, or a combination of both mechanisms, by measuring the transhepatic changes in serum triglycerides. To this effect, we chose the baboon, a nonhuman primate with striking similarities to man with regard to alcoholic liver damage (9) and to lipoprotein metabolism except that, as for most animals, HDL represent a larger fraction of total lipoproteins than in man (10). Moreover, we found that this species reproduces most of the qualitative features described for human alcoholic hyperlipemia.

METHODS

Materials

[9,10-^{3}H(N)]Oleoylglycerol (112 Ci/mmol) and tripalmitoyl [2-^{3}H]glycerol (2.74 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Reagents for the enzymatic determination of serum triglycerides and cholesterol were purchased from Beckman Instruments, Inc. (Palo Alto, CA) and those for phospholipids from Wako Chemical Industries (Osaka, Japan). The detailed composition of the liquid diets has been reported (9). They contained 1% fiber, and provided 1 kcal per ml, 18% of total energy as protein, 21% as fat, 11% as carbohydrate, and 50% either as ethanol or as additional carbohydrates.

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

Animal procedures

Ten female and eight male baboons (*Papio hamadryas*), 3 to 5 years old, were matched into pairs according to sex, approximate age, and body weight, and pair-fed the liquid diets described above for 5 to 16 months. In the alcohol-fed animals, the mean daily consumption of the diets was 62 ± 4 ml/kg body weight. This consumption is equivalent to an ethanol intake of 4.34 ± 0.28 g/kg body weight per day. After a few weeks of pair-feeding, a difference in the pattern of food intake appeared; the alcohol-fed animals consumed their diets evenly during the 24-hr feeding period, whereas the pair-fed controls ingested their rations during a few hours after the diet was made available to them. To equalize the duration of fasting prior to the measurements, the diet was removed from the cage of the alcohol-fed baboons at the beginning of the 18-hr fasting period, whereas in the case of the pair-fed controls, 10 ml of the control diet per kg body weight was given 4 hr before the beginning of this period. To assess for possible effects of the intermittent feeding pattern in the controls, total serum lipids and triglycerides were also measured after fasting in five baboons fed ad libitum monkey chow diet (Ralston Purina Co., Saint Louis, MO). The animals were anesthetized with ketamine-HCl (10 mg/kg).

The progression of the alcoholic liver damage was followed by either needle or surgical biopsies performed at intervals of approximately 6 months. Since the hypertriglyceridemia of the alcohol-fed baboons markedly decreases with progression of the liver injury from fatty liver to the development of fibrosis and cirrhosis (11), this study on the hypertriglyceridemia was limited to animals at early stages of alcoholic liver injury. Following laparotomy, blood samples were obtained simultaneously from the main trunk of the portal vein and from the femoral artery. Then, 5–10 g of liver was excised for the analyses described below as well as other investigations. The volume of the liver sample was determined by displacement of saline in a graduated container and the specific gravity was calculated. Hepatic water was estimated as the difference between wet weight and after drying over anhydrous $CaSO_4$ (W. A. Hammond Drierite Co., Xenia, OH) to constant weight.

On another occasion, 2–3 months after the biopsies, the animals were fasted and anesthetized and venous blood was collected for labeling the VLDL-triglycerides, as described below. Two or 3 days later (in a random way), the animals were again fasted, given ketamine, and restrained on a radiography table. Two radiographic plates, one in antero-posterior and the other in a 60° right oblique position, were obtained for the assessment of the liver size, as previously reported (12). The liver volume was calculated from the three radiographic measurements recommended by Walk (13) and expressed per kg body weight, inasmuch as we previously found significant correlation between liver and body weight both in ethanol-fed and control baboons (12). The coefficient of variation of the difference between the calculated and the actual liver volume, measured at autopsy in seven baboons, was 11%.

To measure the hepatic blood flow, an intravenous loading dose (0.15 mg/kg body weight) of indocyanine green (Cardiogreen; Hanson, Wescott & Dunning, Inc., Baltimore, MD) dissolved in the solvent supplied and mixed with autologous plasma was given, followed by infusion of the dye at a constant rate (0.015 mg/kg body weight per min). Another cannula was introduced percutaneously into the femoral artery and advanced into the aorta. To obtain samples of hepatic venous blood, a polyurethane catheter (cobra type I; size 7F; USCI Cardiology & Radiology Products, Billerica, MA) was introduced in the femoral vein and advanced under fluoroscopy into the hepatic vein. Ten or more 3-ml blood samples were collected simultaneously from the aorta and the hepatic vein at various time intervals for 5 hr from restrained, nonanesthetized animals. The total blood collected was less than 10% of the blood volume of these baboons (860–960 ml). Plasma volume was determined by the dilution principle (14), using Evans blue dye (Harvey Laboratories, Inc., Philadelphia, PA). The hepatic plasma flow was calculated by the Fick principle from the product of the plasma volume and the arterio-hepatic venous differences in the concentration of indocyanine green (15). Since only animals with early alcoholic liver disease were selected for this procedure, the hepatic plasma flow should be considered equivalent to the splanchnic flow because there is no significant intra- or extra-hepatic shunting of blood at that stage. The product of hepatic (or splanchnic) plasma flow and the arterio-hepatic venous differences in triglyceride concentrations indicates net changes, since release and uptake affect the arterio-venous differences concomitantly. To measure triglyceride uptake independently of release, either dietary or VLDL-triglycerides were labeled as described below and their uptake was calculated from the arterio-hepatic venous differences in radioactivity, the specific activity in the arterial samples, and the flow. To this effect, an average of five simultaneous samples were collected from the aorta and the hepatic vein and analyzed in duplicate.

At the beginning and at the end of the period used for trans-splanchnic measurements, samples were also obtained simultaneously from the aorta and inferior vena cava immediately over the confluence of the iliac veins. This was done to assess arterio-venous differences in triglyceride concentrations during circulation through the lower extremities.

To assess postprandial changes, four pairs of baboons received, by gastric tube, 10 ml/kg body weight of the corresponding diets (labeled with tripalmitoyl [2-^{3}H]glycerol

in three of them). Blood samples were collected at 60-min intervals for 5 hr.

Labeling of dietary- and VLDL-triglycerides

Chylomicron-triglycerides were labeled "in vivo" by intragastric administration of the liquid diets mixed with tripalmitoyl [2-^{3}H]glycerol (1 μCi/ml of diet) to six baboons pair-fed either ethanol-containing diet or control diet.

In separate experiments, autologous VLDL-triglycerides were labeled "in vitro," under aseptic conditions, by the method of Fielding (16), and reinjected intravenously to four pairs of baboons. To this effect, fasting plasma of each baboon was incubated at 37°C for 3 hr with an equal volume of a 20% (w/v) aqueous solution of dimethyl sulfoxide, containing 250 μCi of tri[9,10-^{3}H]oleoylglycerol. After an overnight dialysis against NaCl-EDTA (d 1.006 g/ml), the plasma was layered under a d 1.006 g/ml NaCl solution and centrifuged at 100,000 *g* for 17 hr. The VLDL fraction was aspirated and injected intravenously. Since VLDL were cleared very rapidly, the simultaneity of the arterial and hepatic venous samples was assured by collecting the blood through tubes of equal lengths and diameters. In the d < 1.006 g/ml fraction, the arteriohepatic venous differences in radioactivity were divided by the triglyceride specific activity in arterial blood to calculate the amount of triglycerides removed in the splanchnic area.

Analysis of the liver samples

A liver sample (0.5–1 g) was homogenized in chloroform–methanol 2:1 (v/v) and the total lipids were extracted (17) and measured (18). Liver triglycerides were separated by thin-layer chromatography (18), eluted with ether, and measured by their ester bonds (19). Phospholipids were measured in the lipid extract by phosphorus assay (20) and hepatic cholesterol was measured by a modification (21) of the method of Searcy and Bergquist (22). Protein was measured by the method of Lowry et al. (23) and DNA by the method of Burton (24).

Analysis of the blood samples

Blood was drawn into tubes containing 1 mg of ethylene diaminotetracetic acid (EDTA), tetrasodium salt, per ml of blood. The plasma was immediately separated by centrifugation at 680 *g* at 4°C, for 20 min. Several duplicate samples of 1.5 ml of plasma were layered under d 1.006 g/ml NaCl-EDTA solution and centrifuged at 0.6×10^6 *g*-min at 10°C in a 40.3 rotor (Beckman Instruments Inc., Palo Alto, CA) for flotation of chylomicrons or chylomicron-like (S_f > 400) particles. These particles were separated by tube slicing. The infranatants were adjusted to a density of 1.300 g/ml by addition of NaCl and KBr and transferred into 5-ml Quick-Seal® tubes (Beckman) and overlayered with the d 1.006 g/ml solution (0.15 M NaCl containing 0.01% EDTA). The tubes were sealed and centrifuged at 360,000 *g* at 10°C for 45 min in a Beckman L8-80 ultracentrifuge in a vertical rotor (model VTi 65), as described by Chung et al. (25). The centrifugation produced clear separation of VLDL (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml), LDL (d 1.019–1.063 g/ml), and HDL (d 1.063–1.210 g/ml), as identified by centrifugation of lipoprotein fractions isolated by sequential centrifugation (26) and labeled in vitro or stained with activated Fat Red 7B (27). After centrifugation, the fractions were recovered by piercing the bottom of the tube with a needle in a Beckman Fraction Recovery System and pushing the contents from below with a KBr solution of density 1.346 g/ml. The S_f > 400-, VLDL-, and IDL-triglycerides were recovered in approximately one-third of the initial volume of plasm. Both in alcohol-fed and control baboons, the recovery of serum triglycerides in the sum of lipoprotein fractions was virtually complete (109 ± 5% vs. 106 ± 7%, respectively; seven pairs; NS).

Negative staining of the lipoprotein fractions was carried out according to Clark et al. (28) at 37°C by maintaining the samples, staining solutions, grids, and forceps at the desired temperature on a slide warmer. A small drop of sample was placed on the copper grid and allowed to remain there for 30 sec. The grids were then stained with approximately 20 drops of 2% sodium phosphotungstate, pH 7.4, at 37°C and rinsed in the same solution. Negatively stained samples were immediately examined in a 10C Zeiss electron microscope. Pictures were taken from all samples at the same magnification. On the print, we superimposed a transparent graph paper of known area divided into squares (10 × 10 mm). All the particles having spherical shape were counted and the diameters were measured on 100–200 particles.

Both in the plasma and in the isolated fractions, triglycerides were determined by the enzymatic method of Wahlefeld (29), using the end-point-method and the Beckman triglyceride UV reagent kit. We adjusted the sample volume to fall in the optimal range, since there was good linearity with sample volumes between 20 and 200 μl in the assay. The concentration of free glycerol was measured separately with glycerol kinase and subtracted from the triglyceride values. Most of the plasma glycerol was found in the d > 1.210 g/ml fraction, but 30–40% contaminated the HDL fraction. The cholesterol concentration was determined with cholesterol oxidase (30). Phospholipids were determined after hydrolysis by phospholipase D as free choline by the choline oxidase reaction (31). Proteins were measured either by the method of Lowry et al. (23) in LDL and HDL, and by the *o*-phthalaldehyde reaction (32) in the triglyceride-rich fractions after delipidation (33).

Statistics

All values are expressed by their means ± the standard

error of the mean (SEM). Values obtained in the alcohol-fed animals were compared to those obtained in their pair-fed controls and the mean of the individual differences was tested by the Student's *t* test (paired comparisons) (34). Paired *t* test was also used to assess the significance of arterio-venous differences, extractions, and outputs. When more than one control was used in the comparison or when changes over time were studied, the significance of such changes was assessed by one-way analysis of the variance (ANOVA) (34).

RESULTS

Effects of alcohol feeding on blood ethanol concentration and body weight

The consumption of the ethanol-containing diet produced blood ethanol levels detectable at all times during the 24-hr period. The concentrations were greater 2 hr after the serving of the daily ration (38 ± 5 mM) than immediately prior to the serving of the next ration (20 ± 6 mM), despite the constant availability of the ethanol-containing diet. After the 18-hr fasting period, there was no detectable ethanol in the blood. During the feeding of alcohol-containing diets for an average of 8.1 ± 1.5 months, there were no changes in body weight from an initial weight of 12.3 ± 0.3 kg to a final weight of 11.2 ± 0.7 (n = 9; NS). During the same period, the isocaloric substitution of ethanol for additional carbohydrate in the pair-fed controls resulted in weight gain from 12.7 ± 0.6 kg to 13.7 ± 0.7 (n = 9; $P < 0.02$ on paired comparison). Thus, at the time of the liver and blood measurements, the pair-fed controls were slightly heavier than the alcohol-fed despite isocaloric feeding. Since we found no significant differences in hepatic or serum changes between male and female baboons, or the animals fed for various times within the 5–16-month period, their results were pooled.

Effects of alcohol feeding on the liver

The livers of the alcohol-fed baboons were larger than those of the controls, especially when expressed per body weight (**Table 1**). There was a striking accumulation of triglycerides, to the extent of decreasing the water space and the concentrations of other components (such as protein) per gram of liver. There was a tendency for an increase in total liver protein, but at variance with other series (which included more advanced stages of alcoholic liver injury (11)), hepatic protein per kg body weight was not significantly increased after alcohol feeding in the present group. Histologically, there was marked steatosis and ballooning of perivenular hepatocytes, mild increase in the number of mesenchymal cells and, in two of the alcohol-fed baboons, deposition of fibrous tissue around the terminal hepatic venules (perivenular fibrosis).

Effects of alcohol feeding on serum lipids and lipoproteins

The feeding of ethanol-containing diets resulted in hypertriglyceridemia in the fasting state: 1009 ± 141 μmol/l of plasma, compared either to their pair-fed controls (412 ± 49 μmol/l) or to baboons fed chow diet ad libitum (318 ± 47 μmol/l). Readministration of the corresponding diets (10 ml per kg body weight) by gastric tube exaggerated the hypertriglyceridemia in the alcohol-fed animals, whereas it produced no significant changes in the pair-fed controls. Significant postprandial changes occurred only in lipoproteins of $d < 1.006$ g/ml, which include chylomicrons and VLDL (**Fig. 1**).

After an 18-hr fast (**Table 2**), alcohol-fed baboons had more protein and lipid in all lipoprotein fractions than controls. Proportionally, the greatest difference was observed in the triglycerides of the lipoprotein fractions of density less than 1.019 g/ml (VLDL and IDL). Particularly striking was the sevenfold increase in lipoproteins with flotation characteristics resembling chylomicrons in fasted alcohol-fed animals as compared to controls. Even in LDL and HDL, the concentration of triglycerides doubled in the alcohol-fed baboons.

Effects of alcohol feeding on net splanchnic release or uptake of lipoprotein-triglycerides

In spite of the fact that the livers of the alcohol-fed animals were larger than those of controls, the blood flow through the splanchnic vascular bed was similar (31.6 ± 5.4 ml/kg body weight per min vs. 27.5 ± 5.4, in the controls). Arterial and hepatic venous concentrations of triglycerides in various lipoproteins differed by 15–37%, a change greater than the coefficient of variation (6.3%) for duplicate triglyceride determinations performed in the same animal. The arterio-hepatic venous differences indicated that, during one passage through the splanchnic territory, there was net release of VLDL-triglycerides into the hepatic vein and net uptake of LDL- and HDL-triglycerides from the arterial blood (**Table 3**). Alcohol feeding resulted in a fourfold increase in the net release of triglycerides in $d > 1.006$ g/ml lipoproteins. The net release of VLDL-triglycerides correlated with the magnitude of the hypertriglyceridemia (**Fig. 2**). The $d < 1.006$ g/ml lipoproteins released in the hepatic vein of the alcohol-fed baboons had decreased buoyant density and a fourfold increase in the lipid/protein ratio (10.3 ± 2.7, compared to 2.6 ± 0.9 in the controls; n = 6 pairs; $P < 0.01$); thus, most of the increased release was accounted for by the lipid moiety. Indeed, most of the difference in VLDL was due to the appearance of $S_f > 400$ lipoproteins (resembling chylomicrons) in the hepatic venous blood of the alcohol-fed animals. Morphometrically, these $S_f > 400$ particles were not only increased in number but also in size: 22% of these particles

TABLE 1. Effects of alcohol feeding on body weight, liver size, and composition[a]

Measure	Alcohol-fed Baboons	Pair-fed Controls	P^b
Initial body weight (kg)	12.3 ± 0.3	12.7 ± 0.6	NS
Final body weight (kg)	11.2 ± 0.7	13.7 ± 0.7	< 0.01
Liver weight/body weight (g/kg)	37.3 ± 0.4	27.0 ± 0.2	< 0.01
Hepatic triglycerides			
mg/g of liver	148 ± 19	26 ± 7	< 0.001
g/kg body weight	5.5 ± 0.7	0.7 ± 0.2	< 0.001
Hepatic cholesterol			
mg/g of liver	2.47 ± 0.61	3.30 ± 0.50	NS
g/kg body weight	0.10 ± 0.03	0.09 ± 0.01	NS
Hepatic phospholipids			
mg/g of liver	33.0 ± 3.2	40.9 ± 3.0	NS
g/kg body weight	1.2 ± 0.2	1.1 ± 0.2	NS
Hepatic protein			
mg/g of liver	122 ± 7	137 ± 4	< 0.02
g/kg body weight	4.55 ± 0.58	3.69 ± 0.30	NS
Hepatic DNA			
mg/g of liver	1.9 ± 0.1	1.9 ± 0.1	NS
g/kg body weight	0.071 ± 0.001	0.052 ± 0.005	< 0.05
Hepatic water			
mg/g of liver	623 ± 26	695 ± 25	< 0.001
g/kg body weight	19.2 ± 0.8	16.2 ± 0.8	< 0.01

[a] Means ± SEM of 18 baboons pair-fed liquid diets containing 50% of calories either as ethanol or as additional carbohydrate for 8.1 ± 1.5 months (5–16 months).
[b] Paired comparisons; NS, not significant.

had a diameter greater than 90 nm, whereas no particles that big were found in the controls (**Fig. 3**). They were also observed, although less frequently, in the arterial blood of the ethanol-fed baboons.

In three pairs of baboons, portal and arterial blood were simultaneously collected during laparotomy. There was no difference in triglyceride concentrations between the two sampling sites (**Table 4**).

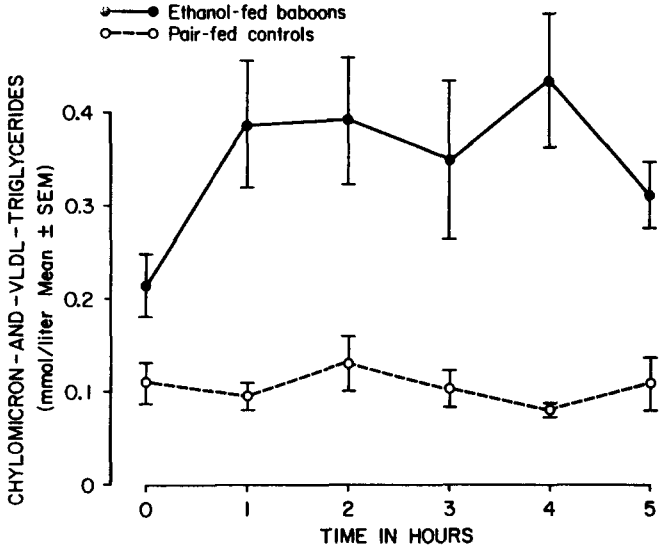

Fig. 1. Postprandial changes in serum chylomicron- plus VLDL-triglycerides in four pairs of baboons fed 50% of calories either as ethanol or as additional carbohydrate for 5–16 months and given an intragastric dose (10 ml/kg body weight) of their respective diets. The fasting hypertriglyceridemia of alcohol-fed animals was markedly exaggerated postprandially ($P < 0.01$, ANOVA), whereas no postprandial changes occurred in the controls.

Effects of alcohol feeding on splanchnic uptake of triglycerides from dietary chylomicrons and endogenous VLDL particles

The uptake was measured by labeling the triglycerides of either chylomicrons or VLDL. The administration of either label resulted in arterio-hepatic venous differences in radioactivity. Using this difference to calculate triglyceride removal, we found a marked increase in splanchnic triglyceride uptake from remnants of either chylomicrons or VLDL by the alcohol-fed baboons (**Table 5**). In the fasting state, the greater removal of VLDL-triglycerides by the alcohol-fed animals was proportional to the greater load of these lipids entering the splanchnic area; therefore, the fractional removal remained unchanged. Similar relationships occurred postprandially in the controls. However, in the alcohol-fed baboons, the marked postprandial increase in chylomicrons and VLDL was not matched by a proportional increase in uptake, resulting in a significantly lower fractional removal.

Effects of alcohol feeding on extra-splanchnic metabolism of lipoprotein-triglycerides

Simultaneous sampling from the aorta and inferior vena cava revealed significant arterio-venous differences in the concentrations of triglycerides in VLDL, but not in LDL or HDL fractions (**Table 6**). The arterio-venous

TABLE 2. Effects of alcohol feeding on plasma lipoproteins after 18 hr fasting[a]

	VLDL				
	$S_f > 400$	S_f 20–400	IDL	LDL	HDL
A. Alcohol-fed baboons					
Triglycerides (μmol/l plasma)	159 ± 52[b]	230 ± 67[b]	176 ± 49[b]	271 ± 39[b]	427 ± 30[b]
Cholesterol (μmol/l plasma)	18 ± 1	160 ± 29[b]	398 ± 41[b]	959 ± 158	1676 ± 173[c]
Phospholipids (μmol/l plasma)	4 ± 1	44 ± 14	151 ± 23[b]	509 ± 91[d]	1220 ± 140[d]
Protein (mg/l plasma)	14 ± 13[d]	25 ± 10	62 ± 5[b]	222 ± 13[d]	1644 ± 144
B. Pair-fed controls					
Triglycerides (μmol/l plasma)	23 ± 5	40 ± 4	30 ± 5	130 ± 19	240 ± 40
Cholesterol (μmol/l plasma)	12 ± 5	28 ± 6	109 ± 14	887 ± 202	1218 ± 85
Phospholipids (μmol/l plasma)	1 ± 1	11 ± 2	59 ± 7	310 ± 19	810 ± 120
Protein (mg/l plasma)	8 ± 1	19 ± 7	27 ± 6	166 ± 21	1314 ± 134

[a] Means ± SEM of 12 to 18 baboons pair-fed liquid diets containing 50% of calories either as ethanol or as additional carbohydrate for 8.1 ± 1.5 months. Alcohol-fed baboons had fatty liver with or without perivenular fibrosis, but without septal fibrosis or cirrhosis.
[b] $P < 0.01$; paired comparisons.
[c] $P < 0.02$; paired comparisons.
[d] $P < 0.05$; paired comparisons.

differences were significantly greater in the alcohol-fed baboons than in the pair-fed controls. Although the absolute removal was greater in the alcohol-fed animals, the fractional removal was the same in both groups.

DISCUSSION

This study shows that alcoholic hypertriglyceridemia is primarily due to enhanced hepatic release of large, chylomicron-like VLDL. At the same time, both the splanchnic and the extra-splanchnic removal of plasma triglycerides were secondarily increased by chronic alcohol consumption. This was found at early stages of alcoholic liver injury in an animal model that reproduced the essential features of the hyperlipemia observed in human volunteers (35, 36). There was hypercholesterolemia as well as hypertriglyceridemia. Recently, we studied the mechanism of the hypercholesterolemia in these or similarly treated baboons (37). In the present study, we focused on the mechanism of the hypertriglyceridemia, which was found to affect all lipoprotein fractions. Compared to the pair-fed controls, the greatest increase in triglycerides occurred in lipoproteins with density less than 1.006 g/ml. In the fasting state, this increase accounted for 41% of the total increase in plasma triglycerides. The increases in IDL- and LDL-triglycerides accounted for an additional 31%. Two other changes that have been observed in man during alcohol consumption were also seen in the alcohol-fed baboons, namely

TABLE 3. Effects of alcohol feeding on splanchnic net release or uptake of triglycerides from lipoprotein fractions in the fasting state[a]

	VLDL[b]				
	$S_f > 400$	S_f 20–400	IDL	LDL	HDL
A. Alcohol-fed baboons					
Artery (μmol/l plasma)	94 ± 40	151 ± 27	102 ± 16	208 ± 19	272 ± 35
Hepatic vein (μmol/l plasma)	117 ± 33	173 ± 29	99 ± 16	202 ± 18	254 ± 34
HV-A difference (μmol/l plasma)	+23 ± 12	+22 ± 5	−4 ± 3	−6 ± 5	−18 ± 15
Net release (+) or uptake (−) (nmol/kg body weight per min)	+419 ± 193[c]	+395 ± 93[d]	−68 ± 57	−137 ± 99	−379 ± 283
B. Pair-fed controls					
Artery (μmol/l plasma)	29 ± 4	40 ± 6	35 ± 3	121 ± 18	186 ± 32
Hepatic vein (μmol/l plasma)	30 ± 4	55 ± 9	33 ± 4	100 ± 15	138 ± 18
HV-A difference (μmol/l plasma)	+1 ± 4	+15 ± 8	−2 ± 2	−21 ± 6	−49 ± 21
Net release (+) or uptake (−) (nmol/kg body weight per min)	+34 ± 82	+154 ± 35[d]	−55 ± 25	−285 ± 98	−502 ± 193[c]

[a] Means ± SEM for eight alcohol-fed baboons and their pair-fed controls. The net release or uptake of triglycerides was calculated by multiplying the hepatic venous-arterial (HV-A) differences in concentration by the splanchnic plasma flow.
[b] The net release of VLDL was significantly greater in alcohol-fed baboons than in controls ($P < 0.01$; paired comparison).
[c] $P < 0.05$; significance of net release or uptake.
[d] $P < 0.01$; significance of net release.

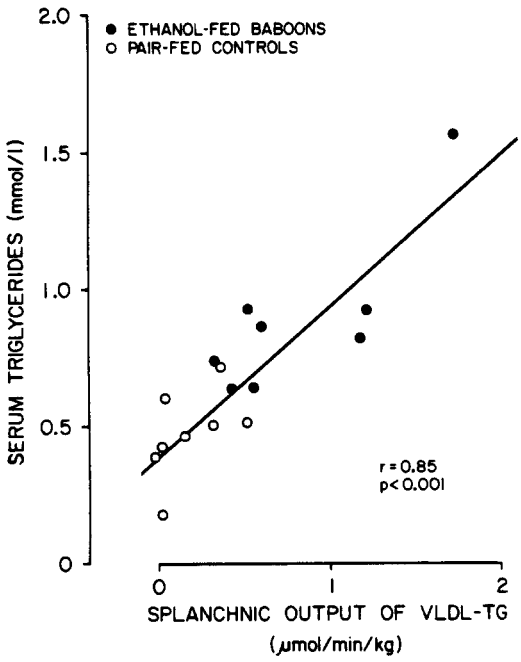

Fig. 2. Correlation between the magnitude of the hypertriglyceridemia and the splanchnic (hepatic) output of VLDL-triglycerides in baboons pair-fed 50% of calories either as ethanol or additional carbohydrate. The correlation (r = 0.83) is also significant ($<$ 0.02) when controls are excluded.

chylomicron-like particles in the fasting state (1, 4) and increased triglyceride in HDL (35). In the alcohol-fed baboons, the HDL-triglycerides doubled, accounting for 23% of the total increase in plasma triglycerides whereas, in men, this accounts for less than 10% of the hypertriglyceridemia (35). It must be pointed out, however, that some of the few differences between baboon and human lipoproteins are the lower VLDL- and LDL- and the higher HDL-triglyceride content in the baboon; the high LDL/HDL ratio appears to be unique to the human species (10). There is no evidence to suggest that this quantitative difference alters the mechanism by which alcohol increases serum triglycerides.

In baboons, as in men (2, 3), alcoholic hypertriglycleridemia was markedly exaggerated during the postprandial state and decreased rapidly during fasting. Therefore, the hypertriglyceridemia is mild or absent in the majority of hospitalized alcoholics, when examined in the fasting state (38, 39), whereas marked and persistent hypertriglyceridemia after alcohol consumption develops only in relatively few individuals (40, 41).

We limited our study to alcohol-fed baboons with early stages of liver injury (fatty liver with or without perivenular fibrosis) because we have observed that the hypertriglyceridemia markedly decreases or disappears with the development of septal fibrosis or cirrhosis (11, 42). In addition, our experimental design included control for the alterations in nutrient intake that are commonly associated with chronic alcohol abuse.

One apparent discrepancy between the ethanol-induced hypertriglyceridemia in baboons and in human volunteers is the persistence of the lipid changes in the animal model. This difference can be attributed to the smaller ethanol doses that can be given to the volunteers. Indeed, feeding 36–43% of calories as ethanol to alcoholics (43) or 20–30% to normal volunteers (35, 44) produced hyper-

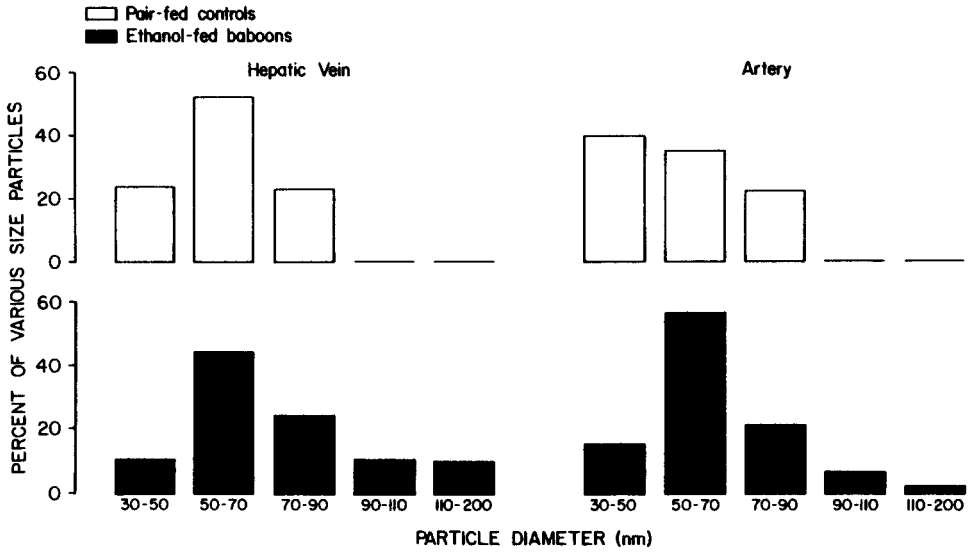

Fig. 3. Frequency distribution of particle diameters in the hepatic venous and arterial S_f > 400 serum lipoproteins. In alcohol-fed baboons, large particles (with diameter greater than 90 nm) were released in the hepatic venous blood and found, although less frequently, in arterial blood during the fasting state. Such large particles were not found in the controls.

TABLE 4. Comparisons between arterial and portal concentrations of lipoprotein-triglycerides[a]

	VLDL				
	$S_f > 400$	S_f 20-400	IDL	LDL	HDL
A. Alcohol-fed baboons					
Arterial blood	53 ± 25	78 ± 37	87 ± 35	168 ± 47	334 ± 72
Portal blood	50 ± 18	57 ± 23	98 ± 43	192 ± 59	378 ± 81
B. Pair-fed controls					
Arterial blood	25 ± 12	24 ± 8	31 ± 5	100 ± 36	200 ± 54
Portal blood	23 ± 7	29 ± 6	37 ± 6	106 ± 30	219 ± 37

[a]Mean (μmol/l plasma) ± SEM in six baboons pair-fed liquid diets with or without ethanol, in which simultaneous samples were obtained from portal vein and femoral artery during laparotomy. Hypertriglyceridemia occurred in the alcohol-fed animals, but there were no consistent differences in triglyceride concentrations between arterial and portal blood in any of the lipoprotein fractions.

triglyceridemia which decreased or disappeared after a few weeks of ethanol intake, in association with enhanced activity of extrahepatic lipoprotein lipase (44). However, in the baboon fed 50% of calories as ethanol (which is the average ethanol intake by alcoholics), the hypertriglyceridemia persisted for at least 16 months of alcohol feeding, provided the liver injury had not progressed to significant fibrosis. The alcohol-fed baboons showed enhanced extra-splanchnic extraction of plasma triglyceride, but without changes in fractional removal; this was obviously insufficient to clear the hypertriglyceridemia.

The increased extraction of VLDL-triglycerides by extra-splanchnic tissues suggests that the alcohol-induced hypertriglyceridemia originates in the splanchnic organs. In the postprandial state, this could be due to decreased splanchnic extraction of remnants of either chylomicrons or VLDL, to increased hepatic release of triglycerides, or to both. To distinguish between these possibilities, we measured extraction and release of triglycerides during circulation of the lipoproteins from the aorta to the hepatic vein. In fasting volunteers, with or without hyperlipemia, a small net transhepatic release of triglycerides has been documented (45, 46). In the baboon, however, we found net hepatic release of triglycerides only in the alcohol-fed animals, but not in the controls. This was due to considerable extraction of triglycerides from fractions other than VLDL, which equaled or exceeded the hepatic release of VLDL-triglycerides. By contrast, in the alcohol-fed animals, the release of VLDL-triglycerides was fourfold greater, whereas the splanchnic extraction of triglycerides from LDL and HDL tended to be smaller than in controls. Most of the triglycerides released into the hepatic venous blood of alcohol-fed baboons appeared in a lipoprotein fraction with a fourfold higher lipid/protein ratio than normal VLDL and which displayed flotation characteristics resembling those of chylomicrons. More-

TABLE 5. Effects of alcohol feeding on splanchnic extraction of chylomicron- and VLDL-triglycerides[a]

	Amount Entering the Splanchnic Area[b] (μmol/min per kg body wt)	Amount Removed[c] (μmol/min per kg body wt)	Fractional Removal[d] %
A. Alcohol-fed baboons			
Fasting	3.8 ± 0.4[e]	1.0 ± 0.3	29.2 ± 10.0
Postprandial	26.0 ± 7.2[f]	2.8 ± 0.9	10.8 ± 1.6[f]
B. Pair-fed controls			
Fasting	1.6 ± 0.6	0.4 ± 0.1	25.3 ± 2.3
Postprandial	2.5 ± 0.8	0.8 ± 0.3	30.8 ± 2.8

[a]Mean ± SEM of four pairs of fasted baboons in which autologous VLDL-triglycerides were labeled "in vitro" with tri[9,10-^{3}H]oleoylglycerol and reinjected to the same animals, and three pairs of animals after intragastric feeding of the corresponding diets labeled with tripalmitoyl[2-^{3}H]glycerol.

[b]Density < 1.006 g/ml arterial triglyceride concentration × splanchnic plasma flow.

[c]Arterio-hepatic venous difference in d < 1.006 g/ml radioactivity divided by triglyceride specific activity in that fraction.

[d]Amount of triglycerides removed divided by amount entering the splanchnic area × 100.

[e]$P < 0.05$; paired comparison.

[f]$P < 0.01$; paired comparison.

TABLE 6. Effects of alcohol feeding on arterio-venous differences in lipoprotein-triglycerides during circulation through the lower extremities of fasting baboons[a]

	VLDL[b]	LDL	HDL
A. Alcohol-fed baboons			
Aortic blood	157 ± 31	245 ± 28	248 ± 29
Inferior vena caval blood	139 ± 28	249 ± 20	249 ± 27
Arterio-venous difference	+17.1 ± 5.4[c]	−3.6 ± 2.6	−0.8 ± 15
B. Pair-fed controls			
Aortic blood	49 ± 6	107 ± 18	177 ± 24
Inferior vena caval blood	43 ± 6	110 ± 19	176 ± 26
Arterio-venous difference	+5.4 ± 2.1[d]	−1.9 ± 4.0	+0.6 ± 5.3

[a] Mean μmol/l plasma ± SEM in 16 baboons pair-fed liquid diets with or without ethanol, in which simultaneous samples were obtained from the aorta and the inferior vena cava immediately above the confluence of the iliac veins.
[b] The A-V difference in VLDL-triglycerides was significantly greater in alcohol-fed baboons than in controls ($P < 0.05$; paired comparison).
[c] $P < 0.01$; significance of the arterio-venous difference.
[d] $P < 0.05$; significance of the arterio-venous difference.

over, this fraction contained particles that had a diameter larger than 90 nm; such particles were not present in the controls.

These chylomicron-like particles were also present in the arterial and portal blood, although in smaller concentrations than in the hepatic venous blood. Thus, these chylomicron-like particles must have originated in the liver of the alcohol-fed animals. The mechanism of this effect remains unknown. An increase in VLDL size has also been produced by high carbohydrate diets in patients with carbohydrate-induced hypertriglyceridemia (47), suggesting that the large VLDL could represent an appropriate response to enhanced triglyceride synthesis. Our previous observation that alcohol consumption delays the secretion of other export proteins (albumin and transferrin) from the liver (48) also raises the alternate possibility that these large VLDL may result from prolonged intracellular transit time and associated excessive growth of the secretory particle.

Since the net transhepatic changes reflect the balance between release and extraction of triglycerides from lipoproteins of similar buoyant densities, we also measured the splanchnic extraction of radioactive triglycerides from chylomicrons labeled "in vivo" and from the fasting VLDL fraction labeled "in vitro." In either case, more triglycerides were extracted from the splanchnic blood (presumably by the liver) in the alcohol-fed baboons than in the controls. To minimize hepatic recirculation of the label into the released VLDL-triglycerides, the chylomicron-triglycerides were labeled in the glycerol moiety, of which only a minor fraction is reutilized for triglyceride synthesis in the liver (the major fraction being derived from glucose) (49). The extraction of VLDL-triglycerides was measured within 30 min, at which time newly synthesized triglycerides do not yet appear in the blood. Thus, we found no evidence to support an alteration in the hepatic extraction of remnants as a primary mechanism for the hypertriglyceridemia in the alcohol-fed animals, at least at these early stages of alcoholic liver injury. Despite the increased removal of triglyceride from remnants, accumulation of these products in the blood may occur in alcohol-fed animals during the postprandial state if the removal is exceeded by the increased availability of chylomicrons plus VLDL, since these lipoproteins compete for common removal mechanisms from the plasma. A similar competition could account for the decreased clearance of labeled chylomicron-triglycerides or cholesteryl esters observed both in men (35) and animals (7, 8) given alcohol chronically. The increased triglycerides in IDL and LDL are probably products of the incomplete hydrolysis of VLDL-triglycerides by extrahepatic lipoprotein lipase. The reason for the increase in HDL-triglycerides is less obvious. Inasmuch as we only measured net transhepatic changes in this fraction, the apparent decrease in splanchnic extraction of HDL-triglyclerides in the alcohol-fed animals could reflect enhanced hepatic release of triglyceride-rich HDL, decreased hydrolysis by hepatic triglyceride lipase, or both.

In conclusion, our observations indicate that alcoholic hypertriglyceridemia observed at early stages of alcoholic liver injury results primarily from increased hepatic release of large, chylomicron-like VLDL. Although the removal of these particles and their remnants from the circulation is increased by chronic alcohol consumption, during the postprandial state, the production of triglycerides exceeds the capacity for removal, resulting in secondary accumulation of catabolic products of these lipoproteins in the plasma and exacerbation of the postprandial lipemia.

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