Pathogenesis of Postprandial Hyperlipemia in Rats Fed Ethanol-Containing Diets

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ABSTRACT To study the mechanism of the increase in serum lipoproteins which occurs in rats fed alcohol chronically, and especially to assess the role of the intestine, the effects of acute and chronic ethanol administration on lymph and plasma lipids were compared in rats with and without intestinal lymph fistulae. In rats not previously given alcohol, the administration of one dose of a diet containing ethanol (3 g/kg) produced a significant increase in lymph flow, lipid output, and incorporation of dietary fat into lymph lipids when compared with the effects of a control diet containing isocaloric carbohydrate. However, no hyperlipemia developed after ethanol. By contrast, previous feeding of ethanol for several weeks modified the acute effects of ethanol on both lymph and serum lipids. Compared with control animals pair-fed with isocaloric carbohydrate-containing diets, rats which had been fed a diet with 36% of total calories as ethanol for 3–4 wk developed postprandial hyperlipemia when given a single dose of the ethanol-containing or even the ethanol-free diet. This was associated with an increased incorporation of labeled dietary fat and of intravenously injected [^{3}H]lysine into plasma lipoproteins of $d < 1.006$. However, postprandial lymph flow and lipid output were not higher in rats fed alcohol chronically than in their pair-fed controls. Moreover, when rats with lymph fistulae were given intravenous (i.v.) infusions of lymph lipids (to substitute for the diverted intestinal lymph), the ethanol-fed animals still developed hyperlipemia. Incorporation of i.v. lysine into $d < 1.006$ plasma lipoproteins also remained significantly increased. Thus, under these conditions, alcoholic hyperlipemia does not result from changes in intestinal lymph lipids. Two main factors appear to be involved: the acute effects of ethanol on hepatic lipid metabolism and the development of an increased capacity for lipoprotein synthesis during chronic ethanol feeding. The latter most likely occurs in the liver and it is postulated that it is linked to the associated changes in the hepatic endoplasmic reticulum.

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INTRODUCTION

Our previous studies (1) showed that postprandial hyperlipemia induced by chronic ethanol-feeding in the rat is due to increased production of plasma lipoproteins (predominantly the very low density fraction) rather than to changes in fat absorption or in removal of plasma lipids. However both the site and the mechanism of the increased lipoprotein production remained unknown. A number of observations indicated that the most likely site for this effect is the liver. However, it has been suggested that the intestine could also contribute significantly since plasma very low density lipoproteins (VLDL) [1] are partly derived from the intestine (2–4), and under certain experimental conditions, ethanol has been reported to increase the intestinal synthesis of triglycerides (5) and their output into the lymph (6).

The present investigation was designed to assess the role of intestinal lymph and the importance of previous alcohol-feeding in the development of postprandial alcoholic hyperlipemia.

METHODS

Materials. [*Carboxyl*-^{14}C]tripalmitin (13.5 mCi/mmol), [1-^{14}C]palmitic acid (5.4 mCi/mmol) and uniformly labeled L-[^{3}H]lysine (3 Ci/mmol), of radiochemical purity greater than 98%, were purchased from New England Nuclear Corp., Boston, Mass. Radioactive tripalmitin was diluted in chloroform, samples were dried under nitrogen and redissolved in the diets described below at a concentration of 1 μCi/ml. L-[^{3}H]lysine (diluted with 0.9% NaCl to 100 μCi/ml) was administered intravenously at a dose of 10 μCi/

[1] *Abbreviation used in this paper:* VLDL, very low density lipoproteins.

100 g body weight. To prepare labeled lymph lipids for intravenous infusion, [1-^{14}C]palmitic acid was diluted in chloroform, dried under nitrogen, and redissolved in the control diet described below at a concentration of 5 μCi/ml. This diet was administered by gastric tube at a dose of 6 ml/100 g body weight every 6 h to rats raised on Purina chow and in which mesenteric lymph fistulae had been prepared. 88% of the radioactivity in the lipids of the drained lymph was present in the chylomicron fraction (separated by ultracentrifugation at $3 \times 10^8 g$-min at 10°C, in a Beckman-Spinco SW41 swinging bucket rotor, Beckman Instruments, Inc., Fullerton, Calif.). The labeled total lymph or chylomicrons were pooled and infused as described below.

Animal procedures. Male rat littermates of a Sprague-Dawley strain from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.), weighing 120-180 g, were fed previously described liquid diets (7) in pairs or groups of three. The diets supplied 18% of total calories as proteins, 35% as lipids, 11% as carbohydrates, and 36% either as additional carbohydrate (control diet) or as ethanol (alcohol diet). Alcohol diet was given to one rat (alcohol rat) in each pair or trio and the other one or two rats (control rats) were limited to isocaloric amounts of control diets. Ethanol was introduced gradually into the diet, reaching the full concentration of 5 g/100 ml on the 5th day. The feeding was continued for 3–4 wk. As already previously reported for similarly treated rats (8) the rate of growth in the alcohol-fed rats was smaller than in their pair-fed controls (2.1±0.2 vs. 3.2±0.2 g/day; $P < 0.01$). In all these animals, the effects of a dietary load administered by gastric tube in a dose of 6 ml/100 g body weight was tested. In the case of the alcohol diet, this load was equivalent to a dose of ethanol of 3 g/kg body weight. To ensure an equal rate of food intake during the last 24 h before the tests, oral pair-feeding was replaced by gastric intubations, the last of which (3 ml of diet/100 g body weight) was given 3 h before the tests.

The effects of acute and chronic administration of ethanol on postprandial serum lipoproteins were assessed in 9 trios. The diets were labeled with [*carboxyl*-^{14}C]tripalmitin. At the start of each test, the alcohol diet was given to the alcohol rat (chronic ethanol administration) and to one of the controls (acute ethanol administration); the other control received the control diet. [^{3}H]lysine was then injected intravenously. Blood was collected from the tail at 30-min intervals or was drained from the aorta under ether anesthesia at 90 min. The animals were then sacrificed and the livers removed for lipid analysis.

To dissociate the effects of chronic alcohol-feeding from those of an acute dose of ethanol, nine pairs of rats were given a final dietary load of fat without ethanol. Both the alcohol-fed rats and the controls were given control diet labeled with [^{14}C]tripalmitin intragastrically and radioactive lysine was injected intravenously. 90 min later, blood was collected from the aorta and the liver excised. To ensure complete disappearance of ethanol from the blood at the time of the final intubation, six additional pairs were fed only control diet for 20 h before the tests.

The effects of ethanol on intestinal lymph were studied in another series of 10 pairs of rats. Cannulae made of polyethylene tubing (ID 0.58 mm–OD 0.96 mm) were inserted into the superior mesenteric lymph duct according to the procedure of Bollman, Cain, and Grindlay (9), into the stomach using the nasogastric route by the method of Epstein (10) and into the femoral vein. Whenever accessory intestinal lymphatics were visualized, they were ligated. The operations were performed under anesthesia with pentobarbital (30 mg/kg body weight intraperitoneally) and the animals were restrained in Bollman cages (11). The liquid diet with which each animal had been fed chronically was given at a rate of 1 ml/h per 100 g body weight for 24 h after the operation. Then the animals were given intragastrically 6 ml/100 body weight of this diet with [^{14}C]tripalmitin (1 μCi/ml) added. The lymph was collected during the hour preceding the administration of labeled diet and hourly for 3 h thereafter. 6 h after the administration of the first labeled diet, the alternative diet was given with [^{14}C]tripalmitin (ethanol to the control rat and vice versa) and lymph collection continued. In some animals, the experiment was repeated the following day with inversion of the order in which the diets were administered. The administration of the diet labeled with [^{14}C]tripalmitin was followed by an intravenous injection of [^{3}H]lysine and blood samples were taken from the tail 30, 60, 90, 120, and 180 min thereafter.

To equalize the lipid contribution derived from the intestine, lymph was diverted in another 12 pairs of rats. Either labeled total lymph or labeled chylomicrons were then infused through a femoral vein catheter by means of a model 975 compact infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) delivering 3.3 ml/h. Chylomicrons or lymph were diluted with 0.9% NaCl to provide all rats with an equal lipid load of 66.6 mg/h per 100 g body weight. The administered load of lipid was based on previous data of fatty acid disappearance from the gastrointestinal tract of rats given similar diets (1). 30 min after initiating the intravenous infusion, the unlabeled diets (6 ml/100 g body weight) were given by nasogastric tube and [^{3}H]lysine was injected intravenously.

Lymph was obtained in three consecutive 30-min collection periods and then blood was drained from the aorta under ether anesthesia. The infusion of lymph or chylomicrons was discontinued 15 min before the blood collection. This period was chosen because previous data of chylomicron clearance showed that under these conditions, over 90% of the chylomicrons disappear from the plasma within 10 min (1, 12).

Analysis. Blood alcohol concentrations were measured according to the method of Bonnichsen (13). Plasma, lymph, or liver samples were extracted in chloroform-methanol and washed according to Folch, Lees, and Sloane-Stanley (14). Samples of these extracts, dried under nitrogen, were used for measuring total lipid content (15) or radioactivity (1). The VLDL fraction was obtained from plasma samples of 1–3 ml, layered under saline of density equal to 1.006 and centrifuged at 1.3×10^8 g-min, 10°C, in a Beckman-Spinco 40.3 fixed angle rotor. Lipid and protein content and labeling of this fraction were assessed as reported previously (1). In some animals, lipid or protein radioactivity was measured in 85 μl blood samples, obtained from the tail at various times after administration of the labeled precursors, and processed as described for VLDL in 3 ml of carrier plasma.

Statistics. The values obtained in the alcohol rats were compared with those obtained in their pair-fed controls and the mean of the individual differences was tested by the Student *t* test. In limited instances (indicated in the text) group analysis was used (16). The results are given as their means ±SEM.

RESULTS

After administration of a single dose of the alcohol-containing diet (corresponding to 3 g/kg ethanol),

TABLE I

*Effects of Acute and Chronic Administration of Ethanol on Plasma Lipoproteins of $d < 1.006$**

		Diet administered acutely	Number of rats	Plasma lipoproteins ($d < 1.006$)			
				Lipid		Protein	
				Content	Labeling	Content	Labeling
				mg/ml	*dpm/ml*	*μg/ml*	*dpm/ml*
Rats fed ethanol chronically	I	alcohol	9	1.46±0.26	13,428±4,190	130.1±29.8	394.1±43.3
	II	control	9	1.26±0.20	9,572±2,456	142.5±35.5	493.7±42.2
Pair-fed controls	III	alcohol	9	0.65±0.14	2,767±607	69.7±15.4	247.2±42.9
	IV	control	18	0.51±0.04	3,898±630	54.3±8.2	249.3±30.4

Rats fed ethanol for 3–4 wk (or pair-fed controls) were studied 90 min after the i.v. injection of [^{3}H] lysine and the intragastric administration of [carboxyl-^{14}C] tripalmitin-labeled diets containing either ethanol (3 g/kg) or isocaloric carbohydrate. Rats from groups I and III and nine of those in group IV were fed isocalorically in groups of three each. Rats from group II and the remaining nine in group IV were pair-fed (see Methods).

$P < 0.01$ or < 0.02 for differences between rats fed ethanol chronically and their corresponding pair-fed controls (paired analysis).

* Means ±SEM.

maximum blood ethanol concentrations were reached after 1–2 h. The peak values were not significantly different (group analysis) in control animals, in rat pretreated for 3–4 wk with the alcohol-containing diet and in the alcohol-fed rats subjected to lymph diversion. The levels observed 90 min after ethanol administration were 128.0±8.3, 153.4±14.2 and 159.6±17.6 mg/100 ml, respectively. The somewhat higher values in the alcohol-fed rats may have been due to a residual level of ethanol after the chronic administration of alcohol diet. The lymph ethanol concentration, measured 1 h after alcohol administration was 322.9±13.3 mg/100 ml.

Effects of ethanol-feeding on liver lipids. As expected, 90 min after the administration of a single dose of the ethanol-containing diet, the concentration of total hepatic lipid was significantly higher in rats which had been fed alcohol for 3–4 wk (115.4±9.4 mg/g) than in the controls given a single dose of the control diet (51.7±2.2 mg/g) ($P < 0.01$). The latter results were not significantly different from those obtained in control rats given a single dose of the ethanol-containing diet (48.6±1.9 mg/g). Diversion of lymph for 24 h significantly lowered the hepatic total lipid concentrations of all rats ($P < 0.01$, group analysis); the difference between alcohol rats (59.9±5.6 mg/g) and controls (41.1±1.1 mg/g) was also reduced but remained significant ($P < 0.01$). When lymph depletion was prevented by intravenous infusion of total lymph or chylomicrons, the hepatic total lipid concentration was not decreased significantly (92.9±6.2 mg/g in the alcohol-fed rats and 45.3±2.7 mg/g in their controls; group analysis).

The incorporation of [*carboxyl*-^{14}C]tripalmitin (given as part of the diet 90 min beforehand) into total hepatic lipids was also assessed. The results obtained after a single dose of control diet were essentially the same in control animals as in rats fed alcohol chronically (221,771±24,008 and 172,475±17,298 dpm/liver, respectively). Similarly after a single dose of alcohol-containing diet, the results did not differ significantly between rats previously fed alcohol and their controls (544,788 ±47,208 and 500,652±60,537 dpm/liver, respectively). However, regardless of the diet used for chronic treatment, an acute dose of alcohol-containing diet resulted in a greater hepatic lipid-labeling than that observed after a single dose of the control diet ($P < 0.01$).

Effects of ethanol-feeding on plasma lipids. In rats fed alcohol for 3–4 wk, a single intubation of either the alcohol or the control diet produced turbidity of the plasma. As observed previously (1), the main changes involved the VLDL's (Table I), although other fractions were similarly affected. The peak elevation in blood lipids occurred 90 min after intragastric feeding. Therefore, the results observed at 90 min were selected for a series of comparisons concerning the effects of either ethanol-containing or control diets on postprandial hyperlipemia both in rats fed ethanol chronically as well as in their pair-fed controls.

First, a comparison of the lipid content of the $d <$ 1.006 lipoproteins was made between the preprandial value and the 90 min postprandial peak obtained after administration of the ethanol diet to rats fed alcohol chronically. There was a 356% increase from 0.41 ±0.04 to 1.46±0.26 mg/ml ($P < 0.01$, group analysis). The corresponding protein values appeared to increase also but the effect was not significant on statistical analysis (from 62.4±12.6 to 130.1±29.8 μg/ml; group analysis, $n = 16$). Administration of control diet to the

pair-fed controls did not increase the lipid content of $d < 1.006$ lipoproteins significantly when the preprandial and postprandial values were compared (0.44±0.19 vs. 0.51±0.04 mg/ml), nor was there a significant change in the protein content (59.7±4.5 vs. 54.3±8.2 μg/ml).

Furthermore, the capacity to develop postprandial hyperlipemia was compared between ethanol-fed and control rats after a single dose of their respective diets. The lipid and protein content of $d < 1.006$ lipoproteins were significantly higher in alcohol-fed rats than in their pair-fed controls (Table I). The incorporation of both L-[^{3}H]lysine into the protein moiety and of dietary [*carboxyl*-^{14}C]tripalmitin into the lipid moiety of the lipoproteins were increased significantly in the rats fed alcohol chronically. The total lipid concentration was also significantly higher in the plasma of alcohol-fed rats (6.33±0.48 mg/ml) than in that of pair-fed controls (3.85±0.37 mg/ml, ($P < 0.01$).

In addition, a comparison was made of the capacity of both ethanol-fed and control rats to develop hyperlipemia when given one dose of control diet. The plasma $d < 1.006$ lipoprotein content and labeling were significantly higher in rats previously fed alcohol for 3–4 wk than in the pair-fed controls (Table I). Total plasma lipids were also increased significantly (5.91±0.78 vs. 3.50±0.28 mg/ml; $P < 0.01$). Moreover, in a separate group of six alcohol-fed rats in which alcohol was excluded from the diets during the 20 h preceding the experiments, the lipid content of $d < 1.006$ lipoproteins 90 min after administration of control diet was again higher (0.63±0.05 mg/ml) than in the controls (0.47 ±0.04 mg/ml) ($P < 0.05$). No significant changes in the protein content of this lipoprotein fraction were observed in this group of rats.

Finally, the effect of a single dose of the alcohol-containing diet was compared with that of the control diet when both were given to control rats. After administration of the ethanol-containing diet, total plasma lipids (3.50±0.28 mg/ml) were not higher than after the control diet (3.85±0.37 mg/ml). Similarly, the plasma lipoproteins of $d < 1.006$ were not significantly different (Table I).

Effects of ethanol-feeding on intestinal lymph flow. In all animals, the administration of the control diet produced a prompt and significant increase in the volume of lymph collected. The effect persisted for approximately 3 h. The inclusion of ethanol in the diet increased the lymph flow even further, both in rats chronically fed alcohol and in their pair-fed controls (Fig. 1). However, whatever diet was given acutely, the postprandial lymph flow was always lower in the rats previously fed alcohol chronically than in their controls. Even when a single dose of alcohol diet was given to rats chronically fed ethanol, the volume of lymph collected was not greater than that obtained from control rats given a single dose of control diet.

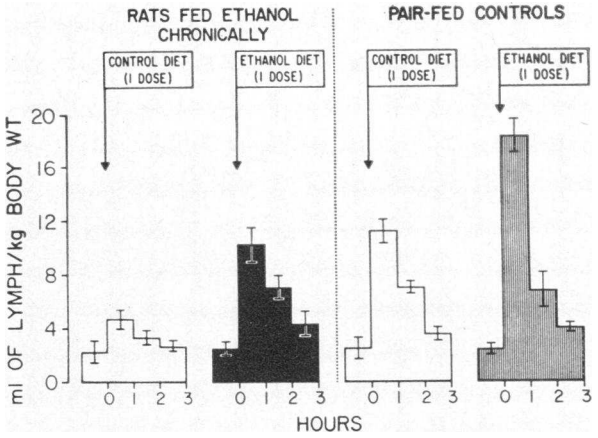

FIGURE 1 Changes in mesenteric lymph flow in 10 pairs of rat chronically pair-fed ethanol or isocaloric carbohydrate (controls) after intragastric administration of a single dose of control diet or alcohol diet (3 g ethanol/kg body wt).

Effects of ethanol-feeding on intestinal lymph lipids. When control rats were given one dose of the alcohol-containing diet (acute ethanol administration), lymph lipid output was 69% higher than after one dose of the control diet. By contrast, in rats fed alcohol chronically, the lymph lipid output was not greater after a dose of alcohol diet than after a dose of control diet, nor was it greater than in controls given a single dose of control diet (Table II).

Even more striking differences between the responses to acute and chronic administration of ethanol were found when the incorporation of dietary [*carboxyl*-^{14}C] tripalmitin into lymph lipids was measured (Fig. 2). This incorporation was significantly enhanced as well as accelerated when ethanol was given to control rats, but not when ethanol was given to rats chronically fed alcohol.

Effects of ethanol-feeding on plasma lipids in rats with lymph fistulae. 24 h of lymph drainage resulted in a lowering of plasma lipids and prevented the development of postprandial alcoholic hyperlipemia. In rats fed alcohol for 3–4 wk and then given one dose of the alcohol-containing diet to which labeled tripalmitin had been added, the lipid content and labeling of plasma $d < 1.006$ lipoproteins (90 min after the administration of the label) were 0.23±0.07 mg/ml and 503±37 dpm/ml, respectively, whereas the corresponding values for the pair-fed control rats given one dose of the control diet were 0.28±0.09 mg/ml and 732±55 dpm/ml. However, despite lymph diversion, the incorporation of intravenously injected [^{3}H]lysine into the protein moiety of plasma $d < 1.006$ lipoproteins was significantly ($P < 0.01$) greater in the rats given alcohol chronically

TABLE II

*Effects of Acute and Chronic Administration of Ethanol on Intestinal Lymph Lipid Output**

	Diet administered acutely	Before feeding	After feeding		
			1st h	2nd h	3 rd h
Rats fed ethanol chronically	alcohol	8.2±1.5	23.8±4.2	15.0±3.0	12.6±0.4
	control	8.8±2.0	23.0±6.9	17.9±4.6	12.7±1.8
Pair-fed controls	alcohol	13.9±2.3	48.7±6.5‡	22.3±2.4§	14.1±1.8‖
	control	13.8±5.0	28.6±6.8‡	13.1±2.4§	6.1±0.5‖

10 pairs of rats fed ethanol for 3–4 wk (or pair-fed controls) were studied after intragastric administration of a single dose of diet with ethanol (3 g/kg) or isocaloric carbohydrate.

* Mg/h per 100 g body wt; means ±SEM.

‡ $P < 0.05$ (paired analysis).

§ $P < 0.02$ (paired analysis).

‖ $P < 0.01$ (paired analysis).

(439±72 dpm/ml) than in the controls (283±46 dpm/ml). By contrast, the incorporation of the lysine into lipoproteins of the diverted lymph was not increased: the protein radioactivity in $d < 1.006$ chylous lipoproteins recovered during the 90 min after intragastric feeding was 1339±205 dpm in the alcohol-fed rats and 1298±242 dpm/ml in their pair-fed controls.

When lymph depletion was prevented by giving the lymph diverted animals intravenous loads of either chylomicrons or complete lymph, postprandial alcoholic hyperlipemia was again observed. 90 min after administration of one dose of the ethanol-containing diet to the alcohol-fed rats, the total plasma lipid concentration was 8.48±1.35 mg/ml. The corresponding value for controls given one dose of the control diet was 4.75±0.35 mg/ml ($P < 0.01$). The results obtained after infusion of chylomicrons did not differ from those obtained after total lymph and therefore all results were treated as a single group. The recovery of chylous [^{14}C]fatty acids in the lipid moiety and the incorporation of intravenous [^{3}H]lysine into the protein moiety of the plasma lipoprotein fraction of $d < 1.006$ were also greatly enhanced by ethanol-feeding (Table III). Part of the intravenously administered lipid label reappeared in the lymph. The lymph lipid radioactivity was significantly greater 60–90 min after the ethanol-containing than after the control diet (3,750±810 vs. 890±95 dpm/100 g body weight; $P < 0.01$). No significant difference was seen during the 1st h.

The lymph lipid output during the 90 min collection period was comparable in the ethanol-fed rats and in the controls (46.5±8.1 vs. 48.9±8.2 mg/100 g body weight), in keeping with the results observed in lymph diverted animals not given lipids intravenously.

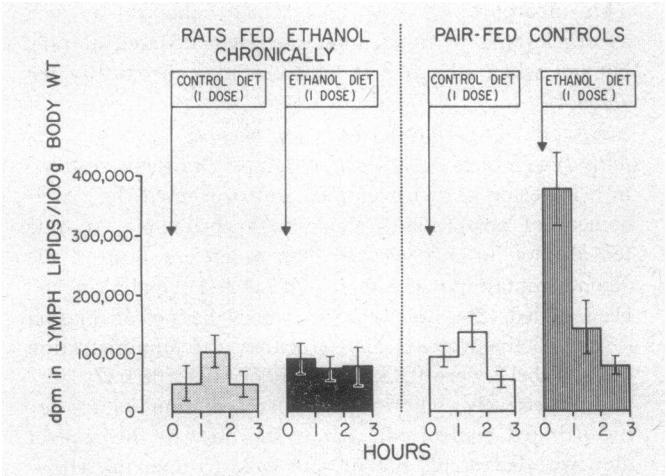

FIGURE 2 Incorporation of dietary [*carboxyl*-^{14}C]tripalmitin into intestinal lymph lipids in 10 pairs of rat chronically pair-fed ethanol or isocaloric carbohydrate (controls) after intragastric administration of a single dose of control diet or alcohol diet (3 g ethanol/kg body wt).

DISCUSSION

This study shows that although the acute administration of an ethanol-containing diet to rats not previously exposed to alcohol increases the flow and lipid content of intestinal lymph and the incorporation of labeled dietary fatty acids into lymph lipids, it nevertheless does not produce hyperlipemia. By contrast, in rats fed alcohol for 3–4 wk and then given a single dose of alcohol-containing diet, hyperlipemia did develop, whereas lymph flow and lipid output were not greater than in the controls given a diet containing an equal load of fat but devoid of alcohol. This suggests that changes in intestinal lymph produced by ethanol do not contribute significantly to postprandial alcoholic hyperlipemia in the rat. This interpretation is supported by the results obtained in rats receiving equal loads of lymph lipids (by intravenous infusion from a pooled source) together with drainage of the rats own intestinal lymph. Under these conditions, administration of ethanol to rats fed alcohol

TABLE III

*Effects of Ethanol on Plasma Lipoproteins of $d < 1.006$ in the Presence of a Constant Load of Intestinal Lymph Lipid**

	Plasma lipoproteins ($d < 1.006$)			
	Lipid		Protein	
	Content	Labeling	Content	Labeling
Rats fed ethanol chronically	2.53±0.25	10,378±2,435	303.6±85.2	1,006±247
Pair-fed controls	0.79±0.04	1,585±198	140.5±22.6	389±40
	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.02$
	(Paired analysis)			

Intestinal lymph was drained in 12 pairs of rats fed ethanol for 3–4 wk (or pair-fed controls). All rats received an identical i.v. lipid load labeled with [^{14}C], given either as chylomicrons (five pairs) or as total lympth (seven pairs), together with i.v. [^{3}H] lysine.

* Means ±SEM.

diets for 3–4 wk produced a rise in plasma lipoproteins of $d < 1.006$ similar to that seen in animals without fistulae.

Although intestinal lymph lipids appear to play no major role in the pathogenesis of postprandial alcoholic hyperlipemia, they still have a physiological function. Indeed, the postprandial hyperlipemia of rats chronically fed ethanol was prevented by the drainage of lymph. In accord with previous studies (17–19), an adequate supply of dietary lipids was found to be necessary for the development of alcoholic hyperlipemia. Thus intestinal lymph is important because it is the major route for adsorbed lipids.

Another role for the lymph was recently proposed (5, 6) namely that changes in intestinal lymph produced by ethanol could contribute significantly to alcoholic hyperlipemia. However, in these reports, the increases in intestinal lymph lipid were in VLDL rather than in chylomicrons and the lipid changes were also very much smaller than in the present study, presumably because only fasting animals were studied. Furthermore, the changes were only seen 16 h after a high dose of ethanol, at a time when the blood ethanol level had already fallen considerably and when recovery from a transiently fatty liver may have been occurring. Actually, the minor increase in lymph lipid noted in those studies may have been a consequence of the hyperlipemia rather than its cause. This interpretation is supported by our finding of enhanced reappearance of intravenously injected lipids in the lymph during the development of alcoholic hyperlipemia.

The possibility remains that lipids produced in the intestine could contribute to hyperlipemia through pathways other than the diverted lymph, but the absence of hyperlipemia in lymph-depleted animals given alcohol tends to rule this out. Thus, the mechanism of alcoholic postprandial hyperlipemia in the rat appears to be a nonintestinal one. Consistent with this interpretation is the finding that ethanol increased the incorporation of intravenously injected lysine into plasma lipoproteins of $d < 1.006$ without an associated increase in the labeling of the lymph lipoproteins. Since the liver is the only other known major site of plasma lipoprotein production, it is reasonable to postulate that the effect of ethanol takes place in this organ.

The increase in the concentration of $d < 1.006$ plasma lipoproteins after ethanol most likely results from enhanced lipoprotein synthesis rather than from delayed removal of chylomicrons. Indeed, it was observed 15 min after stopping the infusion of lymph lipids and it has been shown previously that more than 90% of injected chylomicrons are removed from the circulation within this time; moreover, the removal of chylomicrons was found not to be inhibited by ethanol-feeding (1, 12). Furthermore, when doubly-labeled chylomicrons ([^{3}H] in the glycerol moiety and [^{14}C] in the fatty acids) were infused, it was found that after their disappearance, ethanol enhanced the reappearance into plasma VLDL of the fatty acid, but not the glycerol label (1). In addition, the lipid changes in VLDL coincided with a significant increase in the incorporation of intravenously-injected lysine into the protein moiety of these lipoproteins resulting in an increased specific activity. All these findings are consistent with increased lipoprotein synthesis rather than chylomicron retention. Although in the present study a higher lipid to protein ratio was observed in the $d < 1.006$ lipoproteins after ethanol, this does not appear to have been due to a slowing of the rate of chylomicron removal secondary to the hyperlipemia. Indeed, chylomicron removal was previously tested under conditions in which there was also considerable alcoholic postprandial hyperlipemia and it was found not to be affected by this degree of hyperlipemia (1). Thus the increased lipid to protein ratio in

rats with postprandial alcoholic hyperlipemia could be due to formation of new lipoproteins of greater lipid content rather than to retention of chylomicrons.

Uptake of dietary lipids by the liver has been shown to be unaffected by ethanol (12), but alcohol is known to produce a number of alterations in hepatic lipid metabolism, such as increased fatty acid synthesis (20, 21) and decreased fatty acid oxidation (20, 22). The latter changes may explain why the accumulation of labeled dietary fatty acids into liver lipids is greater after an acute administration of the alcohol-containing diet than after the control diet, whether or not the rats had been previously given ethanol. Increased availability of fatty acids could theoretically promote lipoprotein synthesis. However, these acute effects of ethanol on lipid metabolism are not by themselves sufficient to explain the hyperlipemia since the latter was only observed in rats given alcohol chronically and not in animals given alcohol for the first time. This suggests that in some way, chronic feeding of ethanol promotes the hepatic capacity for lipoprotein production. This interpretation is supported by the finding that even when a dietary lipid load without ethanol was given to rats fed alcohol chronically, hyperlipemia also developed. Moreover, when ethanol was given to these rats with elimination of the dietary lipid load by means of lymph diversion, increased incorporation of radioactive lysine into the plasma lipoproteins of $d < 1.006$ was still observed. Of course, the acute effects of ethanol on hepatic lipid metabolism, especially decreased fatty acid oxidation, may play a contributory role by making more substrate available for lipoprotein synthesis. This possibility is supported by the observation that the magnitude of the postprandial hyperlipemia of rats fed ethanol chronically was less when alcohol had been withheld from the diet for the 20 h preceding the experiments. Thus both the acute effects of ethanol on hepatic lipid metabolism and an increased capacity to produce lipoproteins appear to contribute to the development of postprandial alcoholic hyperlipemia. It is noteworthy that despite their capacity to display hyperlipemia, ethanol-fed rats still developed a fatty liver. This fat accumulation in the liver most likely indicates that the increased lipid load exceeded the mechanisms for lipid disposal such as lipoprotein secretion.

The mechanism of what appears to be an increased capacity to produce lipoproteins after prolonged ethanol-feeding is unknown, but could be linked to the proliferation (23) and increased activity (24) of the hepatic smooth endoplasmic reticulum. Indeed, this structure is the main site of fatty acid esterification (25) and lipoprotein production (26). Furthermore, Joly, Feinman, Ishii, and Lieber (27) have recently shown that chronic ethanol-feeding is associated with increased activity of hepatic L-α-glycerophosphate acyltransferase, a microsomal enzyme involved in fatty acid esterification. It remains to be tested whether other drugs that "induce" hepatic microsomes share with ethanol the proposed ability to increase the hepatic capacity to produce serum lipoproteins.

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