Dietary ethanol is associated with reduced lipolysis of intestinally derived lipoproteins

Henry J. Pownall

Department of Medicine, Baylor College of Medicine and The Methodist Hospital, 6565 Fannin, MS A601, Houston, TX 77030

Abstract An acute oral fat load produces transient lipemia that is enhanced by the simultaneous ingestion of ethanol. This phenomenon has been reinvestigated in six normal subjects who consumed three different fat loads with and without ethanol. The fat loads consisted of saturated fat, polyunsaturated fat, or polyunsaturated fat enriched with omega-3 (n-3) fatty acids. Each fat load contained retinol as a marker for intestinally derived lipoproteins. Plasma levels of triglycerides, retinyl palmitate, and nonesterified fatty acid species were determined at several time points after consumption. Increasing the size of a saturated fat load increased postprandial lipemia and delayed retinyl palmitate clearance. Postprandial lipemia and plasma retinyl palmitate concentrations were lower when omega-3 or omega-6 (n-6) polyunsaturated fat was substituted for saturated fat. Preprandial ethanol increased postprandial lipemia, an effect that was most profound with the saturated fat load. Concurrently, oral ethanol also increased postprandial retinyl palmitate. Addition of ethanol to the fat loads had no effect on the plasma concentrations of fatty acids derived from peripheral tissue but appeared to decrease the plasma concentration of free fatty acids of dietary origin. These data support the hypothesis that preprandial ethanol is associated with impaired chylomicron hydrolysis that may be due to inhibition of plasma lipoprotein lipase. These findings are important to the search for the molecular mechanism of ethanolic hypertriglyceridemia and to the development of dietary guidelines for its control. —**Pownall, H. J.** Dietary ethanol is associated with reduced lipolysis of intestinally derived lipoproteins. *J. Lipid Res.* 1994. **35:** 2105–2113.

Supplementary key words hypertriglyceridemia • plasma free fatty acids • postprandial lipemia

Immoderate ethanol consumption is associated with abnormal triglyceride (TG) metabolism that can lead to hypertriglyceridemia (HTG) and fatty liver disease (1). The molecular pathologies of these disorders involve several organs and multiple proteins, whose expression in response to ethanol may be genetically determined. The lipolytic activities in plasma and adipose tissue liberate fatty acids that can be used for hepatic production of the TG secreted in very low density lipoproteins (VLDL). An acute amount of ethanol creates an increase in hepatic TG that might occur through the coordinate effects of ethanol on several metabolic pathways. Ethanol is metabolized in preference to fatty acids as a source of energy (2), thereby causing accumulation of dietary or endogenously synthesized fat in the liver. There is a concurrent increase in *sn*-glycerol-3-phosphate, the initial acyl acceptor in TG synthesis (3). Moreover, ethanol increases hepatic secretion of lipoproteins in subjects who have a limited capacity to clear TG from plasma (4). Ethanol has been found to increase plasma nonesterified fatty acids (NEFA) (5, 6), although data on this key point conflict (7–10). Herein we describe the interactions of ethanol with different kinds of oral fat loads and the effects on plasma concentrations of NEFA, TG, and chylomicrons.

MATERIALS AND METHODS

Study subjects

Potential subjects were screened to exclude those who had diabetes mellitus, plasma cholesterol levels >200 mg/dl, fasting plasma TG >100 mg/dl, hypertension, a history of renal or hepatic disease, or body weight greater than the 1960 Metropolitan Table Standards for a Medium Frame. Individuals who exercised regularly, used prescription medication or tobacco, or consumed more than 75 ml of ethanol per day were also excluded. The resulting study population contained six male subjects. The pre-entry lipid data are summarized in **Table 1.**

Dietary fat load

At intervals of more than 2 weeks, various ethanol and/or fat loads were given to each subject after a 12-h fast. Participants were advised not to consume ethanol

Abbreviations: HDL-C, high density lipoprotein-cholesterol; LPL, lipoprotein lipase; RP, retinyl palmitate; TG, triglycerides; HTG, hypertriglyceridemia; VLDL, very low density lipoproteins; NEFA, nonesterified fatty acids; LR, lipolytic response.

TABLE 1. Entry level body weight and plasma lipid levels

	Mean (SD)
Triglycerides, mg/dl[a]	93 (39)
Total cholesterol, mg/dl	162 (36)
HDL-C, mg/dl	48 (14)
Body mass index[b]	23 (0.9)
Weight, kg	69 (9)

[a]The mean ± SD for the fasting TG level measured at the beginning of each of the eight tests was 102 ± 52 mg/dl.
[b]Body mass index is expressed in (weight in kg)/(height in m)^2.

during the 24 h before the fat tolerance test. The sizes of the fat loads were calculated to provide 65, 33, and 22 g fat/m^2 body area; these values corresponded to 100%, 50%, and 33% fat loads, respectively. Heavy whipping cream was the source of the saturated fat load, and Microlipid (Chesebrough-Pond's, Inc., Greenwich, CN) was the source of the polyunsaturated fat load. Each fat load contained 100,000 units of vitamin A. An omega-3 polyunsaturated fat load was prepared by substituting 20 g of ProMega (Parke-Davis, Morris Plains, NJ) for an equal weight of polyunsaturated fat. The fatty acid analysis of each fat load was determined by gas chromatography of the methyl esters that were liberated by transesterification of an aliquot with methanolic base and boron trifluoride (**Table 2**). The 50% fat load tests were repeated with the addition of 13 fluid ounces (370 ml) of dry white wine (40 ml ethanol). The entire fat load and ethanol were consumed in 15 min. Control tests were performed to determine the effect of ethanol without fat and the effect of fasting.

The fat tolerance tests were conducted in the General Clinical Research Center of The Methodist Hospital. Before consuming the fat load, each subject was fitted with a venous catheter from which 5 ml of blood was collected periodically. The first collection was immediately before the fat load was consumed; additional collections were at 0.5 h, 1 h, and every hour thereafter up to 8 h after consumption. On the morning of the day of the test, a saline lock with a 3-way stopcock was placed in one upper extremity for blood collection. The line was kept open with a saline drip of 30 ml/h and flushed with saline after each sample was collected. During the collection period, the participants were involved in passive activities. They consumed no food and only noncaloric drinks during this period. The protocol was reviewed by the institutional review boards of Baylor College of Medicine and The Methodist Hospital. All subjects gave informed consent and received $400 after completing all of the tests.

Lipid assays

At each time point after the fat load, the blood was analyzed for ethanol, TG, NEFA, and retinyl palmitate (RP), which labels intestinally derived particles (11). Although there is some exchange of RP with other lipoproteins, the amount is small during the first 8 h after consumption of a fat load (12). Enzyme-based assays (Boehringer-Mannheim, Indianapolis, IN) were used to measure plasma TG and ethanol. RP was measured by high performance liquid chromatography; retinyl acetate was used as an internal standard (13). The plasma RP was expressed as the ratio of retinyl palmitate to retinyl acetate. Inspection of the absorption spectra of other peaks in the chromatogram showed that they did not contain the retinyl chromophore, a result that suggests that nearly all of the retinyl esters that were formed on the three diets contained the palmitate moiety. NEFA levels were determined by a gas chromatographic method that also gave the fatty acid composition (14).

VLDL isolation and fatty acid analysis

After removal of chylomicrons by low speed ultracentrifugation, the VLDL were isolated by flotation for 18 h at d 1.006 g/ml at 45,000 rpm in a Beckman Ti60 rotor. The lipids were extracted and the TG was isolated by thin-layer chromatography. The fatty acid compositions of the VLDL-TG were determined by gas chromatographic analysis of the methyl esters liberated by transesterification with boron trifluoride in methanolic base (15).

Statistical tests

Comparisons of individual points were conducted using Student's *t*-test for paired variables. The ethanol-induced differences between the lines defining the time dependence of TG and RP were determined by multiple regression using dummy variables (16). The data were analyzed as a linear combination of the equations for the two lines that were compared. The equation for the

TABLE 2. Composition of the oral fat loads[a]

	Omega-3 Polyunsaturated Fat Load	Polyunsaturated Fat Load	Saturated Fat Load
Carbohydrate (g)	17	17	24
Protein (g)	1	1	5
Cholesterol (mg)	0	0	165
Fat (g)	65	65	65
Fatty acids (% of total fatty acids)			
14:0	1.94	0.24	12.28
16:0	8.54	7.58	36.51
16:1	2.87	0.09	1.33
18:0	2.47	2.59	18.12
18:1	12.23	12.45	26.89
18:2	59.93	76.92	4.87
20:4	0.00	0.00	0.00
20:5	8.51	0.00	0.00
22:6	3.50	0.12	0.00

[a]Based on the 100% load, which contained 65 g of fat.

second line was multiplied by a dummy variable of 0 for the first equation and 1 for the second equation. All of the data were analyzed by multiple regression and the parameters for analysis of variance were calculated. An F-test was applied to determine whether the additional terms were justified. The null hypothesis, which stated that the curves were coincident, was rejected on the basis of a small P value.

RESULTS

Effect of fat load dose on postprandial lipemia

With the smallest saturated fat load (33% = 22 g fat/m² body area), the lipemia peaked after 3 h and returned to baseline values after 8 h (**Fig. 1,** panel A). Similarly, the RP for this fat load peaked at 4 h and declined to near the baseline level at 8 h (Fig. 1, panel B). Increasing the saturated fat load to 50% (33 g fat/m² body area) did not significantly change the magnitude of the lipemia, but the peak time for lipemia was shifted from 3 to 4 h after consumption. The appearance and clearance of RP was not affected significantly by this increase. In contrast, increasing the fat load from 50% to 100% (65 g fat/m² body area) had a dramatic effect on both postprandial lipemia and plasma RP clearance. The fat load had been doubled, but the increase in plasma TG was much more than twofold greater and the peak lipemia was shifted to between 5 and 6 h. Eight h after consumption of the 100% saturated fat load, the lipemia still exceeded baseline plasma TG levels. Concurrently, the maximum value for plasma RP was delayed: it was not reached until the end of the fat load test (Fig. 1, panel B).

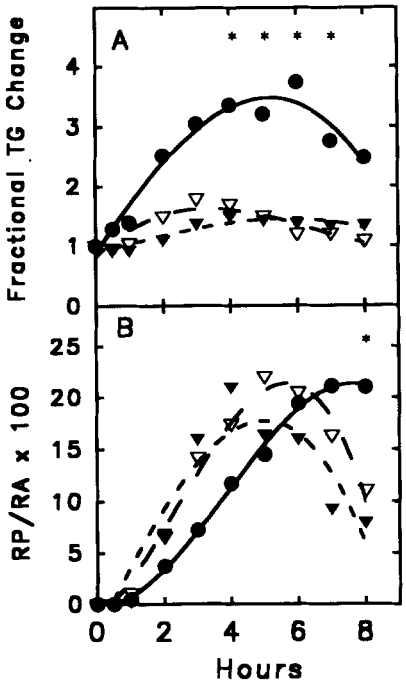

Fig. 1. Effect of various doses of saturated fat on plasma TG (A) and RP (B). Fat loads were (●--●) 100%, (▽--▽) 50%, and (▼ ·· ▼) 33%. The postprandial changes in the TG levels are expressed as fractional increases, whereas changes in RP are given as the ratio of retinyl palmitate to retinyl acetate. Asterisks indicate the time points after the 100% (65 g/m² body area) fat load that were significantly different ($P < 0.05$) from those found after the 33% and 50% fat loads. Differences between the effects of 33% fat load and the 50% fat load were not significant. Curves are plotted as third-order lines of regression.

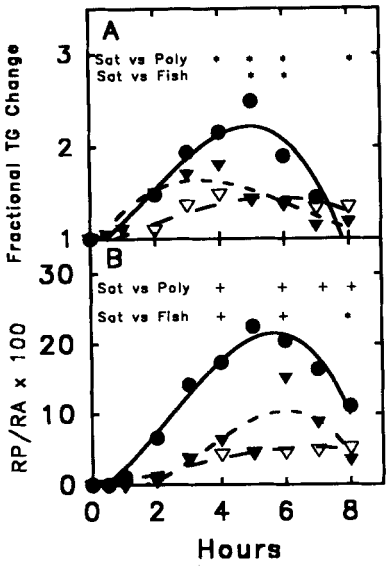

Fig. 2. Effect of fatty acid composition of oral fat loads (50 g/m² body area) on (A) plasma TG and (B) plasma RP. Fat loads were: (●--●) sat. diet, (▽---▽) poly. diet and (▼ ·· ▼) omega-3 diet. Asterisks ($P < 0.05$) and crosses ($P < 0.01$) indicate the time points in the saturated fat load that were significantly different from those of the polyunsaturated and omega-3 polyunsaturated fat loads. There were no significant differences between the effects of the polyunsaturated and omega-3 polyunsaturated fat loads on either plasma TG and RP. The lines are plotted as third-order lines of regression.

In some of our tests with large fat loads, two peaks for lipemia were observed. The second peak may have been due to hepatic utilization of NEFA derived from chylomicron hydrolysis for VLDL-TG synthesis and secretion. To avoid complicated analysis of the changes in multiple peaks, we selected the 50% fat load, which was not associated with bimodal lipemia. The effects of the saturated, polyunsaturated, and omega-3 polyunsaturated fat loads on postprandial lipemia are compared in **Fig. 2,** panel A. These data show that the lipemic responses found after the polyunsaturated and omega-3 polyunsaturated fat loads peaked earlier and were lower in magnitude than those observed following the saturated fat load. Plasma RP levels after the saturated fat load were also much higher than those after the polyunsaturated or the omega-3 polyunsaturated fat loads (Fig. 2, panel B).

Plasma TG levels did not change during the 8-h fasting interval that followed the overnight fast. In contrast, consuming only ethanol after an overnight fast caused a mild

but significant elevation of plasma TG (**Fig. 3**, panel A). Without exception, oral ethanol amplified the lipemia observed during fasting or when added to an oral fat load containing saturated, polyunsaturated, and omega-3 polyunsaturated fat. Figure 3, panels B–D, shows the effects of consuming ethanol with saturated, polyunsaturated, and omega-3 polyunsaturated fat loads. In all three cases, consumption of ethanol with the fat load increased the magnitude and duration of lipemia. This effect was greatest with the saturated fat load: the magnitude of lipemia was increased by a factor of two and the lipemic maximum was shifted from 3 h to 5 h. The accumulation of ethanol in plasma was also affected by addition of the fat load to the oral ethanol dose. In the absence of oral fat, plasma ethanol peaked at 50 ± 10 mg/dl at 0.5 h and had a clearance half time of 3 h; a combination of ethanol with 50% saturated fat gave a peak plasma ethanol of 35 ± 5 mg/dl with a half time of 4 h (data not shown).

Invariably, postprandial plasma RP levels were increased by the addition of ethanol to the fat loads (**Fig. 4**). The increases were greatest when ethanol was added to the polyunsaturated and omega-3 polyunsaturated fat loads. In addition, ethanol appeared to increase the time required for plasma RP to reach its peak value. The greatest differences between the control and the ethanol-supplemented fat loads were apparent at the later time points.

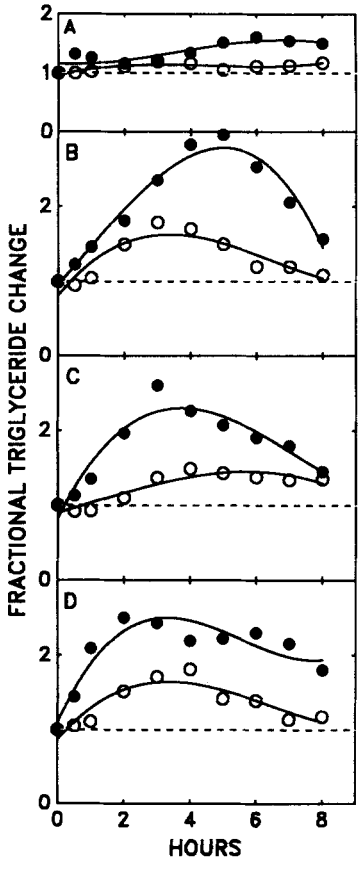

Fig. 3. Effect of ethanol on plasma TG; (A) fasting; (B) sat. diet; (C) poly. diet; (D) omega-3 diet; (○) control; (●) + ethanol. Data were best fitted to third-order lines of regression; curves for control and ethanol tests were significantly different (A, $P < 0.001$; B, $P < 0.01$; C, $P < 0.05$; D, $P < 0.0001$).

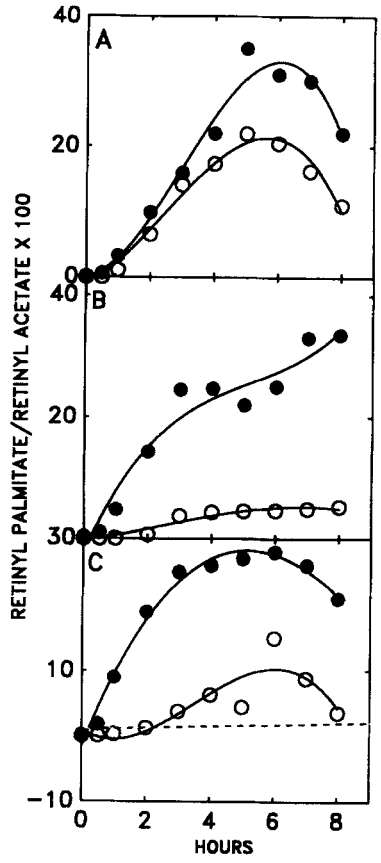

Fig. 4. Effect of ethanol on the appearance of plasma RP; (A) sat. diet; (B) poly. diet; (C) omega-3 diet. (○) control; (●) + ethanol. Data were best fitted to fourth-order lines of regression; curves for control and ethanol tests were significantly different (A, $P < 0.005$; B, $P < 0.01$; C, $P < 0.01$).

Comparison of panels A and B of **Fig. 5** shows that the addition of ethanol to an oral saturated fat load is associated with a significant reduction in the lipolytic response observed when the fat load was consumed without ethanol. After 4 h, the differences between the plasma NEFA for the saturated fat load and for the saturated fat load with ethanol were statistically significant for oleate and palmitate, the major fatty acids in the fat loads (**Table 3**). Similarly, the polyunsaturated fat load with ethanol demonstrated a greatly reduced lipolytic response (Fig. 5, panels C and D); this result was particularly apparent in the plasma levels of free linoleate, the major fatty acid in the fat load (Table 3). However, there was no significant change in the plasma levels of the NEFA species that were not components of the fat load, an effect that was independent of the addition of ethanol.

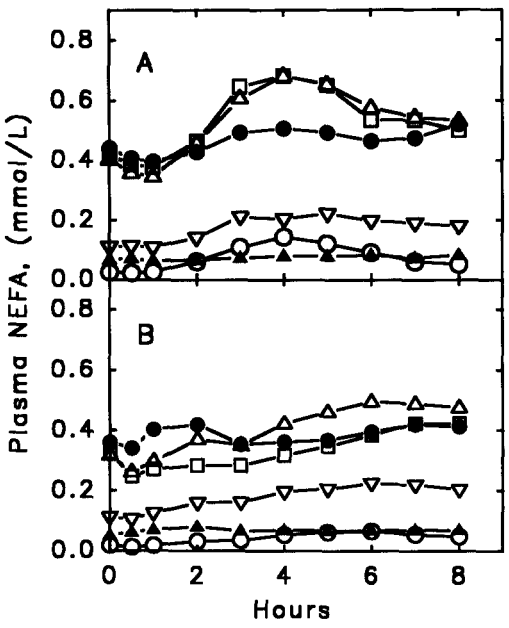

Fig. 5. Time dependence for the appearance of individual fatty acid species in plasma following an oral saturated fat load with and without the addition of ethanol. (A) sat. diet; (B) sat. diet + ethanol. The NEFA were myristate ($C_{14:0}$, ○), palmitate ($C_{16:0}$, △), stearate ($C_{18:0}$, ▽), oleate ($C_{18:1}$, □), linoleate ($C_{18:2}$, ●), and arachidonate ($C_{20:4}$, ▲).

The plasma concentrations of different NEFA species in plasma after ingestion of the various fat loads were different with and without ethanol. In the absence of ethanol, plasma NEFA reached the maximum level between 4 and 5 h (Fig. 5, panels A, C, and E). The major NEFA species found were those of the fat load: oleate and palmitate were the most abundant plasma fatty acids observed after the saturated fat load, and linoleate was the most prominent NEFA after the polyunsaturated fat loads. NEFA species that were not in the fat loads, such as arachidonate, did not change significantly over the 8-h test interval.

If the increases in the NEFA were only due to the hydrolysis of fat derived from the fat load, there should be a linear relationship between the difference between fasting and postprandial NEFA and the fatty acid composition of fat loads. We defined the lipolytic response (LR) as the difference between the amount of a given fatty acid found before the fat load and 4 h after its consumption (Table 3), which was the time point of maximal lipemia. We expressed this value as

$$LR = (PP\text{-}NEFA_i - F\text{-}NEFA_i) \qquad Eq.\ 1)$$

where $PP\text{-}NEFA_i$ and $F\text{-}NEFA_i$ represent the postprandial and fasting plasma concentrations of the ith fatty acid and $NEFA_i$ indicates $C_{14:0}$, $C_{16:0}$, etc. The percent lipolytic response (LR%) may be calculated from

$$LR\% = LR/[\Sigma(PP\text{-}NEFA_i - F\text{-}NEFA_i)] \times 100\% \qquad Eq.\ 2)$$

After the saturated fat load, there was a strong linear relationship between the percent lipolytic response and the fatty acid composition of the fat load (**Fig. 6**). The slope of the line is nearly unity, which suggests a correlation between the composition of the fat load and the composition of the additional fatty acids liberated by lipolysis. A similar relationship was found with the polyunsaturated and omega-3 polyunsaturated fat loads (data not shown). Thus, in the absence of oral ethanol, the compositions of the increases in plasma NEFA, as expressed by LR%, were nearly identical to those of the fat loads. In contrast, when the saturated fat load was consumed with ethanol, the correlation between LR% and the composition of the fat load was much less than unity (Fig. 6).

In order to determine whether fatty acids of dietary origin are used for VLDL synthesis, the fatty acid compositions of the VLDL-TG were analyzed immediately before and 4 h after consumption of the omega-3 polyunsaturated fat loads with and without ethanol. The amounts of both eicosapentaenoate and docosahexaenoate in the VLDL-TG were increased following the fat load without

TABLE 3. Postprandial NEFA (μmol/ml) as a function of dietary fatty acid composition and ethanol (n = 6)

	Sat. Fat		Sat. Fat + Ethanol		Poly. Fat		Poly. Fat + Ethanol		Omega-3		Omega-3 + Ethanol	
Fatty Acid	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h
14:0	0.026	0.14	0.018	0.053[a]	0.037	0.030	0.016	0.02	0.016	0.026	0.016	0.012[a]
16:0	0.40	0.68	0.32	0.38[a]	0.36	0.42	0.34	0.34	0.32	0.41	0.31	0.30
16:1	0.046	0.08	0.030	0.03[a]	0.045	0.05	0.030	0.030	0.041	0.046	0.022	0.016[c]
18:0	0.11	0.21	0.11	0.16	0.11	0.11	0.12	0.12	0.076	0.10	0.11	0.11
18:1	0.42	0.68	0.33	0.26[b]	0.42	0.45	0.29	0.25[a]	0.30	0.40	0.20	0.18[c]
18:2	0.44	0.51	0.36	0.30[a]	0.43	0.96	0.37	0.64	0.39	0.94	0.32	0.49[a]
20:4	0.067	0.08	0.051	0.05[b]	0.079	0.11	0.07	0.06	0.053	0.070	0.069	0.069
20:5	0	0	0	0	0	0	0	0	0.0024	0.009	0.0031	0.006
22:6	0	0	0	0	0	0	0	0	0.010	0.015	0.017	0.018
Total	1.51	2.38	1.22	1.23[a]	1.48	2.12	1.23	1.45	1.22	2.01	1.07	1.19[a]

Fat vs. fat + ethanol; [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.005$.

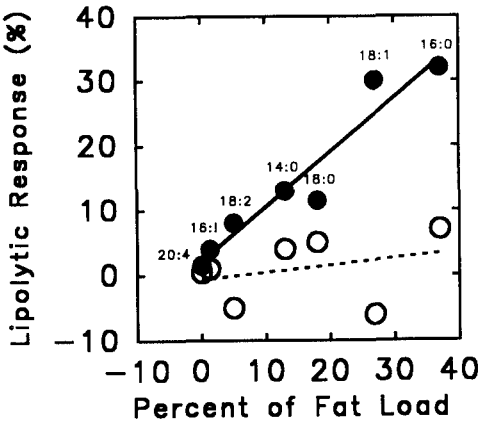

Fig. 6. Reduction in lipolytic response by ethanol following an oral fat load composed of saturated fat. Correlation of the lipolytic response (defined by equation 2, LR% = LR/[Σ(PP-NEFA − F-NEFA)] × 100%) is shown for saturated fat (●, R = 0.957) and saturated fat + ethanol (○, R = 0.299).

ethanol (**Table 4**). In contrast, the levels of these two fatty acids, which in these tests were exclusively of dietary origin, were significantly reduced when the ethanol was added to the fat load.

DISCUSSION

Increases in TG-rich lipoproteins that can cause the lipolytic capacity of plasma to be exceeded are expected to be a function of plasma lipoprotein lipase (LPL) levels and the rate of secretion of TG-rich lipoproteins (17–19). Others have shown that a change of diet has little effect on the plasma postheparin lipolytic activity (20, 21). In this study, the rates of clearance of plasma triglycerides after a fat-rich meal were a function of the quality and quantity of the fat load. Increasing the fat load from 33% to 50% resulted in a small increase in the magnitude of the lipemia. However, doubling the load from 50% to 100% increased the plasma triglycerides by more than a factor of five. Moreover, the peak lipemia was shifted from 3 h to 5 h when the fat load was increased to 100% (Fig. 1, panel A). Plasma retinyl palmitate showed a similar delay in clearance (Fig. 1, panel B). Cohen, Noakes, and Spinnler Benade (22) investigated the effects of fat dose on postprandial lipemia and obtained some results similar to ours. They also observed that the increased dose delayed the peak lipemia. However, they reported a response different from the one observed in our population: postprandial lipemia increased linearly with fat dose. For our study group, we observed that the capacity of the lipolytic machinery of plasma may be exceeded between 50% and 100% of the standard saturated fat load. This lipemia is due to chylomicrons and their remnants. To avoid the ambiguities of overloading the lipolytic capacity of plasma, the 50% fat load was used in the remainder of the tests.

In vitro polyunsaturated fats are hydrolyzed more rapidly than saturated fats (23, 24). This finding correlates with the observation that clearance of plasma TG derived from dietary polyunsaturated fats is more rapid than clearance of those derived from saturated fats (Fig. 2). The increase in plasma RP after the saturated fat load was significantly greater than that after either of the polyunsaturated fat loads. Concurrently, there was an increase in the plasma NEFA which had a fatty acid composition that corresponded to that of the fat load (Fig. 5). Therefore, the increase in plasma TG is likely to be of dietary origin. If the higher plasma RP levels correspond to chylomicrons or their remnants (12), the source of the increased lipemia is due to either more rapid secretion of chylomicrons after the saturated fat load or a lower rate of clearance of chylomicrons composed of saturated fat. As the quality of the diet has little effect on intestinal absorption and the plasma postheparin lipolytic activity (20, 21), the increase in plasma RP level is probably due to a mechanism that makes the chylomicrons formed after the polyunsaturated diets more susceptible to lipolysis (23, 24).

Postprandial lipemia is even more profound when an oral fat load is supplemented with intoxicating quantities of ethanol (25–28). Our data support the hypothesis that oral ethanol inhibits lipolysis of chylomicrons, although the mechanism by which this occurs is not clear. The most compelling evidence derives from the effects of ethanol on the levels and compositions of plasma triglycerides and NEFA after a saturated fat load. In the absence of ethanol, a saturated fat load induces a distinct lipemia (Fig. 3, panel B) with the liberation of NEFA having the composition of the fat load (Fig. 5). Addition of ethanol to the saturated fat load was associated with increased lipemia (Fig. 3, panel B) without the appearance of plasma NEFA of dietary origin (Fig. 6). Thus, the ethanol-induced amplification of postprandial lipemia and the concomitant reduction in plasma NEFA with the same composition as the fat load make it likely that ethanol enhances postprandial lipemia by inhibiting LPL activity against intestinally derived lipoproteins. A fortuitous ethanol-induced increase in the rate of NEFA uptake might equal that of

TABLE 4. Percent omega-3 fatty acid content of VLDL (n = 6)

Hour Post Ingestion	Eicosapentaenoate		Docosahexanoate	
	Control	Ethanol	Control	Ethanol
0	0.2	0.0	0.5	0.0
4	0.5	0.4	0.5	0.0
8	2.8	0.8[a]	1.7	0.2[b]

[a] $P < 0.05$; [b] $P < 0.01$, paired Student's t-test; other differences were not significant.

chylomicron hydrolysis, thereby leading to the reduction in postprandial NEFA. However, plasma levels of arachidonate, which was not in the fat load, can be used as a marker for endogenous free fatty acids. As panels A and B of Fig. 6 indicate, there was no change in the arachidonate levels over the entire 8 h after fat loads consumed with or without ethanol. Therefore, it does not appear that transport of fatty acids between plasma and adipose tissue is greatly changed by consumption of ethanol. Over short time intervals, RP is a reliable marker for intestinally derived lipoproteins (12); the data from measurement of this marker are consistent with a delay in clearance of chylomicrons and their remnants that could be due to inhibition of chylomicron lipolysis.

The ethanol-induced enhancement of lipemia after a polyunsaturated fat load was less profound than that found after a saturated fat load. Moreover, ethanol did not totally suppress the release of postprandial NEFA after a polyunsaturated fat load. However, the amount of free linoleate found 4 h after the fat load consumed with ethanol was significantly lower than that observed for the polyunsaturated fat load consumed without ethanol. These data are consistent with our other observations and the data of others (23, 24) that show that polyunsaturated fats are better substrates for LPL (Fig. 3). Although LPL activity against intestinally derived triglycerides is lower when ethanol is added to a polyunsaturated fat load, there is apparently enough activity to hydrolyze the more reactive substrates, which contain polyunsaturated fat.

The mechanism of lipemia after co-consumption of ethanol and fat is not known. One possibility is that acetate derived from ethanol oxidation is used for additional hepatic lipogenesis. If the additional fatty acids are incorporated into VLDL-TG and secreted, plasma TG levels would be expected to increase. These additional VLDL could be competitive inhibitors of the LPL-mediated hydrolysis of intestinally derived lipoproteins. A number of pieces of evidence argue against this interpretation. First, Fig. 3A shows that in the absence of a fat load, the increase in postethanolic triglycerides does not occur until the sixth hour. In contrast, the postprandial differences between the plasma triglycerides observed with and without consumption of ethanol are already apparent by the second hour (Fig. 3, panels B–D). Thus, increased VLDL-TG is not likely to be the cause of chylomicronemia at the earlier time points. The linear relationship between postprandial lipemia and fasting TG levels also makes this mechanism improbable (23). Our data (Fig. 3, panel A) show an increase in postethanolic TG of about 50%. A simple competitive inhibition should be associated with only a 50% increase in postprandial lipemia. However, the enhanced lipemia exceeds 200% (Fig. 3, panels B and C). Therefore, the evidence is not totally consistent with a model for simple competitive inhibition by VLDL. In addition, stable isotope measurements show that, compared to dietary fat intake, endogenous synthesis of fatty acids makes a relatively minor contribution to VLDL-palmitate (29, 30).

The enhanced lipemia could also be due to the hepatic utilization of fatty acids of dietary origin for VLDL-TG synthesis and secretion, an effect reported for fat loads administered without ethanol (31). If this were the case, the composition of the VLDL-TG would include those fatty acids derived from the fat load. However, as Table 4 indicates, ethanol reduces the amount of eicosapentaenoate and docosahexaenoate in VLDL-TG. A possible explanation for this result is that there is less hydrolysis of chylomicrons, which are the only source of these two fatty acids in our studies.

Although ethanol-induced HTG has been variously attributed to alterations in intestinal absorption and secretion, inhibition of lipolysis in adipose tissue, decreased β-oxidation, and increased lipogenesis (1), other studies have shown that ethanol significantly reduces plasma NEFA without affecting the rate of NEFA removal from the plasma compartment (10, 32). This effect was assigned to the ethanol-induced inhibition of the release of NEFA from adipose tissue, presumably through an effect on hormone-sensitive lipase. An alternative explanation that is also consistent with these observations is that ethanol indirectly inhibits LPL lipolysis of VLDL. The same mechanism associated with inhibited VLDL lipolysis in fasting subjects might be involved in the inhibition of chylomicron lipolysis in subjects who consume ethanol with a fat load. Although physiological concentrations of ethanol have no effect on in vitro lipolysis (27, 33), there is no consensus on the effects of oral ethanol on LPL activity. Postheparin lipolytic activity has been reported to decrease (34, 35) or to remain unchanged (26, 28) after acute ethanol intake. Mishra et al. (36) reported that a constant infusion of ethanol is associated with a decrease in plasma apolipoprotein B-100; they assigned this effect to a decreased conversion of VLDL to LDL. They also observed an ethanol-associated delay in the release of postheparin LPL that could also be interpreted as a lower plasma LPL activity. Our results are consistent with, but do not prove, a decrease in LPL activity.

The effects of ethanol on lipolysis are mediated by acetate, one of its oxidation products (7, 10). However, the addition of acetate did not produce a measurable difference in fatty acid release when an artificial emulsion containing apoC-II was treated with pure bovine milk lipoprotein lipase (L. C. Smith, private communication). The effects of acetate on LPL may differ in vivo, where the enzyme is bound to the capillary endothelium. There is some evidence that the plasma of individuals that are susceptible to ethanolic lipemia contains a factor that inhibits lipoprotein lipase (37). The activation or release of this or another inhibitory factor into plasma might be elicited by acetate, but this question remains open.

Although the mechanism is unknown, preprandial ethanol and the magnitude and duration of lipemia are clearly associated. Impairment of the lipolysis of intestinally derived lipoproteins appears to be the cause of this association. This effect is particularly acute following an oral load of saturated fat. One of the main medical concerns for subjects who are susceptible to ethanol-induced HTG is the risk for pancreatitis and fatty liver disease. HTG and the attendant low plasma levels of high density lipoprotein-cholesterol (HDL-C) are also risk factors for atherosclerosis (38, 39), although no clear mechanistic connection between elevated TG and vascular disease has been proved. However, elevation of the late phase of postprandial lipemia is reportedly a better predictor of coronary artery disease than plasma HDL-C (40). As a consequence, it has been recommended that elevated plasma TG ($>$ 200 mg/dl) be treated by diet, exercise and drugs taken singly or in various combinations. Our data show that the anti-lipolytic effects of ethanol are lower with a diet composed of polyunsaturated fat. Therefore, it may be prudent to consider reduction of the intake of dietary saturated fat in patients who consume ethanol and who are susceptible to diet- or ethanol-induced lipemia. A more definitive recommendation will depend on additional studies in a larger population that includes hypertriglyceridemic subjects.

This work was supported by grants from the Alcoholic Beverage Medical Research Foundation and the National Institutes of Health (HL-27341). The technical assistance of Quein Pao, Elise Harper, and Shaena Choi is gratefully acknowledged. I extend special thanks to Patricia Pace, R. D., who managed the study population, and to Dr. Roger D. Knapp, who performed the statistical tests. These studies were conducted using the General Clinical Research Center of The Methodist Hospital.

Manuscript received 3 February 1994 and in revised form 12 July 1994.

REFERENCES

1. Lieber, C. S., and J-P. Pignon. 1989. Ethanol and dyslipoproteinemias. *In* Human Plasma Lipoproteins. J. C. Fruchart and J. Shepherd, editors. Walter de Gruyter & Co., Hawthorne, NY. 245–280.
2. Lieber, C. S., A. Lefevre, N. Spritz, L. Feinman, and L. M. DeCarli. 1967. Difference in hepatic metabolism of long- and medium-chain fatty acids: the role of fatty acid chain length in the production of the alcoholic fatty liver. *J. Clin. Invest.* **46:** 1451–1460.
3. Nikkila, E. A., and K. Ojala. 1963. Role of hepatic L-α-glycerophosphate and triglyceride synthesis in production of fatty liver by ethanol. *Proc. Soc. Exp. Biol. Med.* **113:** 814–817.
4. Chait, A., M. Mancini, A. W. February, and B. Lewis. 1972. Clinical and metabolic study of alcoholic hyperlipidæmia. *Lancet.* **2:** 62–64.
5. Lieber, C. S., N. Spritz, and L. M. DeCarli. 1969. Fatty liver produced by dietary deficiencies: its pathogenesis and potentiation by ethanol. *J. Lipid Res.* **10:** 283–287.
6. Savolainen, M. J., V. P. Jauhonen, and I. E. Hassinen. 1977. Effects of clofibrate on ethanol-induced modifications in liver and adipose tissue metabolism: role of hepatic redox state and hormonal mechanisms. *Biochem. Pharmacol.* **26:** 425–431.
7. Abramson, E. A., and R. A. Arky. 1968. Acute antilipolytic effect of ethyl alcohol and acetate in man. *J. Lab. Clin. Med.* **72:** 105–117.
8. Elko, E. E., W. R. Wooles, and N. R. DiLuzio. 1961. Alterations and mobilization of lipids in acute ethanol-treated rats. *Am. J. Physiol.* **201:** 923–926.
9. Jauhonen, V. P., M. J. Savolainen, and I. E. Hassinen. 1975. Cyclic AMP-linked mechanisms in ethanol-induced derangements of metabolism in rat liver and adipose tissue. *Biochem. Pharmacol.* **24:** 1879–1883.
10. Crouse, J. R., C. D. Gerson, L. M. DeCarli, and C. S. Lieber. 1968. Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man. *J. Lipid Res.* **9:** 509–512.
11. Zilversmit, D. B. 1976. Role of triglyceride-rich lipoproteins in atherogenesis. *Ann. NY Acad. Sci.* **275:** 138–144.
12. Krasinski, S. D., J. S. Cohn, R. M. Russell, and E. J. Schaefer. 1990. Postprandial plasma vitamin A metabolism in humans: a reassessment of the use of plasma retinyl esters as markers for intestinally derived chylomicrons and their remnants. *Metabolism.* **39:** 357–365.
13. Rudy, J. L., F. Ibarra, M. Zeigler, J. Howard, and C. Argyle. 1989. Simultaneous determination of retinol, retinyl palmitate, and α-tocopherol in serum or plasma by reversed-phase high performance liquid chromatography. *LC-GC* **7:** 969–971.
14. Lepage, G., and C. C. Roy. 1988. Specific methylation of plasma nonesterified fatty acids in a one-step reaction. *J. Lipid Res.* **29:** 227–235.
15. Morrisett, J. D., H. J. Pownall, R. L. Jackson, R. Segura, A. M. Gotto, Jr., and O. D. Taunton. 1977. Effects of polyunsaturated and saturated fat diets on the chemical composition and the thermotropic properties of human plasma lipoproteins. *In* The Chemistry and Biochemistry of Polyunsaturated Fatty Acids. R. I. Holman and W. H. Kunau, editors. AOCS Publishers, Champaign, IL. 139–161.
16. Kleinbaum, D. G., L. L. Kupper, and K. E. Muller. 1988. Applied Regression Analysis and Other Multivariate Methods. 2nd ed. PWS-Kent Publishing Co., Boston, MA. 260–274.
17. Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. 1988. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *J. Lipid Res.* **29:** 925–936.
18. Havel, R. J. 1965. Metabolism of lipids in chylomicrons and very low density lipoproteins. *Handb. Physiol.* **Sect. 5:** 499–504.
19. Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
20. Phillipson, B. E., D. W. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth. 1985. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **312:** 1210–1216.
21. Goodnight, S. H., Jr., W. S. Harris, W. E. Connor, and D. R. Illingworth. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis.* **2:** 87–113.
22. Cohen, J. C., T. D. Noakes, and A. J. Spinnler Benade.

1988. Serum triglyceride responses to fatty meals: effects of meal fat content. *Am. J. Clin. Nutr.* **47:** 825–827.
23. Weintraub, M. S., R. Zechner, A. Brown, S. Eisenberg, and J. L. Breslow. 1988. Dietary polyunsaturated fat of the ω-6 and ω-3 series reduce postprandial lipoprotein levels. *J. Clin. Invest.* **82:** 1884–1893.
24. Hulsmann, W. C., M. C. Oerlemans, and H. Jansen. 1980. Activity of heparin-releasable liver lipase: dependence on the degree of saturation of the fatty acids in the acylglycerol substrates. *Biochim. Biophys. Acta.* **618:** 364–369.
25. Brewster, A. C., H. G. Lankford, M. G. Schwartz, and J. F. Sullivan. 1966. Ethanol and alimentary lipemia. *Am. J. Clin. Nutr.* **19:** 255–259.
26. Verdy, M., and A. Gattereau. 1967. Ethanol, lipase activity, and serum-lipid level. *Am. J. Clin. Nutr.* **20:** 997–1003.
27. Barboriak, J. J., and R. C. Meade. 1968. Enhancement of alimentary lipemia by preprandial ethanol. *Am. J. Med. Sci.* **255:** 245–251.
28. Wilson, D. E., P. H. Schreibman, A. C. Brewster, and R. A. Arky. 1970. The enhancement of alimentary lipemia by ethanol in man. *J. Lab. Clin. Med.* **75:** 264–274.
29. Faix, D., R. Neese, C. Kletke, S. Wolden, D. Cesar, M. Coutlangus, C. H. Shackleton, and M. K. Hellerstein. 1993. Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. *J. Lipid Res.* **34:** 2063–2075.
30. Hellerstein, M. K., M. Christiansen, S. Kaempfer, C. Kletke, K. Wu, J. S. Reid, K. Mulligan, N. S. Hellerstein, and C. H. Shackleton. 1991. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J. Clin. Invest.* **87:** 1841–1852.
31. Schneeman, B. O., L. Kotite, K. M. Todd, and R. J. Havel. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc. Natl. Acad. Sci. USA.* **90:** 2069–2073.
32. Jones, D. P., E. S. Perman, and C. S. Lieber. 1965. Free fatty acid turnover and triglyceride metabolism after ethanol ingestion in man. *J. Lab. Clin. Med.* **66:** 804–813.
33. Losowsky, M. S., D. P. Jones, C. S. Davidson, and C. S. Lieber. 1963. Studies of alcoholic hyperlipemia and its mechanism. *Am. J. Med.* **35:** 794–803.
34. Tobias, H., and A. M. Dawson. 1966. Free fatty acid mobilization and plasma triglyceride clearance in alcoholic hyperlipemia. *Gastroenterology.* **50:** 393.
35. Nikkila, E. A., M-R. Taskinen, and J. K. Huttenen. 1978. Effect of acute ethanol load on postheparin plasma lipoprotein lipase and hepatic lipase activities and intravenous fat tolerance. *Horm. Metab. Res.* **10:** 220–223.
36. Mishra, L., N-A. Le, W. V. Brown, and E. Mezey. 1991. Effect of acute intravenous ethanol on plasma lipoproteins in man. *Metabolism.* **40:** 1128–1130.
37. Kessler, J. I., J. C. Kniffen, and H. D. Janowitz. 1963. Lipoprotein lipase inhibition in the hyperlipemia of acute alcoholic pancreatitis. *N. Engl. J. Med.* **269:** 943–948.
38. Austin, M. A. 1989. Plasma triglyceride as a risk factor for coronary heart disease: the epidemiologic evidence and beyond. *Am. J. Epidemiol.* **129:** 249–259.
39. Austin, M. A. 1991. Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb.* **11:** 2–14.
40. Patsch, J. R., G. Miesenbock, T. Hopferwieser, V. Muhlberger, E. Knapp, J. K. Dunn, A. M. Gotto, Jr., and W. Patsch. 1992. Relation of triglyceride metabolism and coronary artery disease: studies in the postprandial state. *Arterioscler. Thromb.* **12:** 1336–1345.