Ethanol with a mixed meal increases postprandial triacylglycerol but decreases postprandial non-esterified fatty acid concentrations

Barbara A. Fielding*, Guy Reid, Michelle Grady, Sandy M. Humphreys, Kevin Evans† and Keith N. Frayn

Oxford Lipid Metabolism Group, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, UK

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Plasma triacylglycerol concentrations increase after the acute ingestion of alcohol (specifically ethanol). However, the effect of ethanol when consumed with a mixed meal has not been well studied. The objective of the present study was to determine the perturbations of lipid metabolism that occur after ingestion of ethanol in combination with a mixed meal of specific fatty acid composition. Blood samples were taken from seven healthy male subjects before and after a mixed meal, with and without ethanol. The specific fatty acid composition of the test meal allowed the fatty acids to be traced into the plasma non-esterified fatty acid pool during the postprandial period. Statistical analysis by repeated measures ANOVA showed significant effects of ethanol. For example, postprandial lipaemia was enhanced after the ethanol test meal compared with the control ($P<0{\cdot}05$), mainly due to increases in triacylglycerol-rich lipoproteins in the flotation range S_f 60–400 (VLDL1) ($P<0{\cdot}05$); those in the range S_f 20–60 (VLDL2) and also $S_f>400$ (chylomicrons) were not significantly affected. The later postprandial increase in plasma non-esterified fatty acid concentrations was reduced after the ingestion of ethanol ($P<0{\cdot}001$), but the proportions of palmitoleic acid (a marker of fatty acid content of the test meal) and of linoleic acid (a marker of endogenous lipolysis) were not affected. The results suggest a primary effect of ethanol on the stimulation of secretion of large VLDL particles, which then compete for clearance with chylomicrons by lipoprotein lipase. The results do not support an effect of ethanol on the release of non-esterified fatty acid into the plasma. The suppression of plasma non-esterified fatty acid concentrations during the postprandial period may contribute towards the beneficial effects of moderate ethanol consumption.

Postprandial lipid metabolism: Non-esterified fatty acids: Ethanol

Moderate alcohol (ethanol) consumption has been shown in many studies to have a protective effect on CHD (Rimm *et al.* 1996) which may be mediated through increased HDL-cholesterol concentration (Rimm *et al.* 1996).

However, it is well known that the acute ingestion of ethanol in normal subjects increases lipaemia after a pure fat meal (Nilsson-Ehle, 1978; Franceschini *et al.* 1988; Pownall, 1994) or when given alone (Frayn *et al.* 1990). Ethanol, when consumed before (Talbott & Keating, 1962), or with, a mixed meal (Van Tol *et al.* 1995) potentiates postprandial lipaemia, a state which is normally associated with increased risk of CHD (Zilversmit, 1979). When combined with a mixed meal, the effect of ethanol on postprandial lipaemia has not been extensively studied, despite the fact that ethanol is widely consumed with meals. Moderate alcohol consumption has been advocated as beneficial to health by some (Betteridge & Morrel, 1998), but reservations have been expressed on the use of alcohol as a serious strategy for the prevention of CHD by others (Konstantinov, 1998).

The acute ingestion of ethanol causes large perturbations in lipid metabolism which originate in the liver, its main site of metabolism. Ethanol is oxidized in the liver to acetaldehyde, mainly via alcohol dehydrogenase and most of the ethanol is recovered as free acetate in the hepatic vein (Lundquist *et al.* 1962). The initial effect on the liver is that ethanol is substituted for fatty acids as the major hepatic fuel. Consequently, after the acute ingestion of ethanol (with or without a meal), the concentration of plasma triacylglycerol increases, which is thought to be due to increased esterification of the accumulated fatty acids to triacylglycerol, which exit the liver in VLDL particles (Baraona & Lieber, 1979). Not all studies have reported an increase in VLDL-triacylglycerol (Goldberg *et al.* 1984).

Abbreviations: HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids.

* **Corresponding author:** Dr Barbara Fielding, fax +44 (0) 1865 224872, email barbara.fielding@oxlip.ox.ac.uk

†**Present address:** Department of Clinical Chemistry, Staffordshire General Hospital, Weston Road, Stafford ST16 3SA, UK.

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The change in concentration of plasma non-esterified fatty acids (NEFA) during the postprandial period is also of considerable interest, since it has been suggested that the failure to regulate the plasma NEFA concentration normally, particularly after meals, might underlie a number of risk factors for CHD (Frayn *et al.* 1996). The effect of a moderate dose of ethanol, when combined with a mixed meal, on the plasma NEFA concentration has not, as far as we are aware, been well documented. When given with a pure fat load, ethanol reduced the postprandial elevation of plasma NEFA concentrations (Pownall, 1994); when given alone, ethanol reduced the normal gradual elevation of plasma NEFA concentrations which occurs with prolonged fasting (Frayn *et al.* 1990). In type-2-diabetic subjects, plasma NEFA concentrations were suppressed in response to an oral dose of ethanol followed by continuous intravenous infusion of ethanol (Christiansen *et al.* 1996). In this present study, the acute effect of ethanol on triacylglycerol and NEFA metabolism in healthy subjects was investigated. Ethanol was consumed with a mixed meal that contained palmitoleic acid (16:1 *n*-7) which was used as a natural tracer of dietary fat into plasma lipid fractions (Fielding *et al.* 1996). Some of the results have been published previously in abstract form (Reid *et al.* 1999).

Subjects and methods

Protocol

Studies were conducted on seven healthy male subjects, aged 21–35 years, with BMI 20·7–26·7 kg/m^2, on two occasions following an overnight fast. Subjects were asked to refrain from strenuous exercise, smoking or alcohol for 24 h before each study and were given instructions to consume low-fat meals on the evenings before the studies. Their habitual alcohol intake was from 3–33 units per week, median 22 (1 unit = 8 g ethanol), as assessed from a 7 d diet diary. On the morning of the study, the subject came to the laboratory and a cannula was inserted into a forearm vein and kept patent by a continuous slow infusion of saline (9 g NaCl/l). The forearm was heated to provide arterialized samples. Blood samples were taken before and for 480 min after (see Fig. 1) the consumption of the test meal, which contained 50·9 g fat, 16·2 g protein and 82 g carbohydrate (Table 1). The fat content of the test meal was made up almost entirely of macadamia nuts (Percy Dalton's Famous Peanut Company Ltd, London, UK), which have a high monounsaturated fatty acid content and are relatively enriched in palmitoleic acid (16:1 *n*-7). The fatty acid composition of the test meal was determined by GC, and is shown in the results section. Included with the test meal was 150 ml vodka (Kulov Distillers, Airdrie, UK), containing 47·5 g ethanol. Using a balanced randomized design, control studies were performed in which vodka was omitted from the test meal.

Table 1. Composition of the test meal (g)*

	Quantity	Fat	Protein	Carbohydrate
Cornflakes	50 g	0·4	4·0	43·0
Macadamia nuts	62·5 g	50·0	4·4	5·6
Banana	100 g	0·3	1·2	23·2
Skimmed milk	200 ml	0·2	6·6	10·2
Diet Sprite	330 ml	0	0	0
Total		50·9	16·2	82

* Data was calculated from the food composition tables of Holland *et al.* (1991).

The study was approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

Analytical methods

Blood samples were collected into heparinized syringes and plasma was rapidly separated by centrifugation at 4°. Triacylglycerol-rich lipoprotein fractions were isolated from 1·5 ml portions of plasma by cumulative ultracentrifugation (Karpe *et al.* 1997). Plasma triacylglycerol and glucose concentrations were measured enzymically using test kits (Instrumentation Laboratory, Warrington, Ches., UK) using an IL Monarch centrifugal analyser (Instrumentation Laboratory). Plasma NEFA concentrations were also measured enzymically (WAKO NEFA C kit; Alpha Laboratories Ltd, Eastleigh, Hants., UK, adapted for use in the centrifugal analyser). Plasma 3-hydroxybutyrate concentrations were measured as previously described (Humphreys & Frayn, 1988). Plasma ethanol concentrations were also measured on the centrifugal analyser, using an enzymic test kit from Sigma, Poole, Dorset, UK. Plasma insulin was measured by radioimmunoassay (Kabi Pharmacia Ltd, Milton Keynes, Northants, UK).

The specific fatty acid compositions of plasma triacylglycerol and NEFA and of lipoprotein-triacylglycerol and phospholipid were measured by GC as previously described (Fielding *et al.* 1996). Total lipid concentrations were calculated by reference to internal standards; these were heptadecanoic acid for NEFA, 1,2,3-triheptadecanoyl glycerol for lipoprotein-triacylglycerol and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine for phospholipid. The triacylglycerol : phospholipid ratio (mole : mole) was calculated in each lipoprotein fraction.

A representative portion of the test meal was homogenized in a blender. The lipid fraction of a small portion of the homogenate was prepared for GC analysis as described earlier in order to determine the fatty acid composition of the total lipid fraction.

Statistical methods

Changes in concentrations with time were assessed by repeated measures ANOVA using time and treatment as within-subject factors. For example, this was used to test if the plasma triacylglycerol concentrations were significantly higher after the test meal with ethanol compared with the test meal alone. Calculations were done with SPSS for Windows Release 7.1 (SPSS Inc., Chicago, IL, USA). Data were also analysed as areas under the curve (AUC). These were calculated for the basal period (−20–0 min) and from the start of eating to 480 min later (postprandial area). Incremental areas (iAUC) were calculated by subtracting, from the postprandial area, the mean baseline value extrapolated over 480 min, reflecting changes occurring after the

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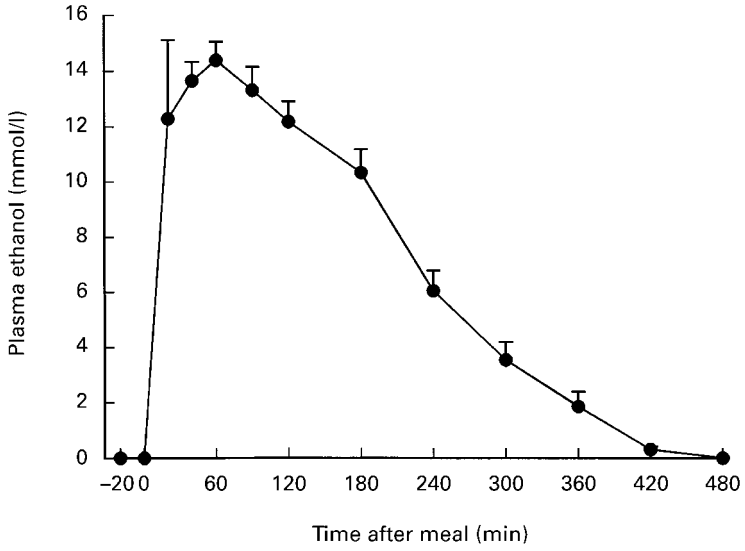

Fig. 1. Plasma ethanol concentrations before and after consumption of a test meal with ethanol. For details of test meal see Table 1 and p. 598. Values are means for seven male subjects with their standard errors represented by vertical bars.

meal. All areas have been divided by the appropriate time-base for presentation (i.e. basal areas by 20, postprandial and incremental areas by 480). The data are therefore presented as time-averaged values. Differences in AUC and iAUC were analysed by paired *t*-test.

Results

Test meal

The major fatty acids in the test meal were (g/100 g): oleic acid (18:1 *n*-9) 59, palmitoleic acid (16:1 *n*-7) 14, palmitic acid (16:0) 11·0, stearic acid (18:0) 5·4 and linoleic acid (18:2 *n*-6) 1·9.

Plasma ethanol

Plasma ethanol concentrations reached a peak after 60 min and ethanol was cleared from the circulation within the duration of the experiment (Fig. 1). Ethanol was not detectable in the plasma of subjects before or after the control meal (data not shown).

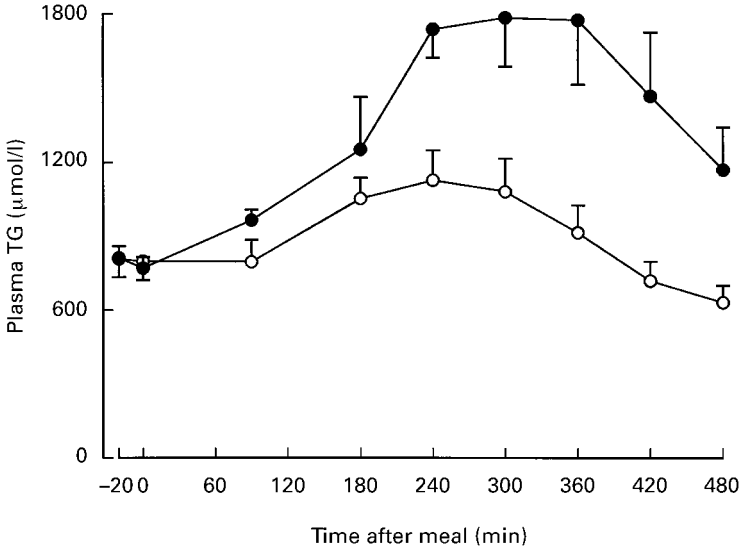

Fig. 2. Effect of a test meal with ethanol (●) and without ethanol (○) on plasma triacylglycerol (TG) concentrations. For details of test meal see Table 1 and p. 598. Repeated measures ANOVA shows main effects of treatment ($P<0·05$) and time × treatment interaction ($P<0·001$). Values are means for seven male subjects with their standard errors represented by vertical bars.

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Plasma triacylglycerol

The expected increases in plasma triacylglycerol concentrations observed after the meal were considerably exaggerated after ethanol consumption (Fig. 2 and Table 2). After ethanol, peak concentrations of plasma triacylglycerol were greater, and the duration of lipaemia was longer. Triacylglycerol concentrations did not return to baseline within 480 min, although the difference in concentrations between 0 and 480 min were not significantly different (P= 0·055). At 480 min after the control test meal, plasma triacylglycerol concentrations were significantly less than at 0 min (P<0·05). At 360 min, the mean concentration of plasma triacylglycerol in the control study was 52 % of that found after ethanol.

Plasma triacylglycerol-rich lipoproteins

After ethanol consumption, triacylglycerol concentrations were significantly higher within the S_f 60–400, but not in the $S_f > 400$, and S_f 20–60 fractions (Fig. 3 and Table 2). The concentrations of triacylglycerol-palmitoleic acid within the lipoprotein fractions largely mirrored those of total triacylglycerol. The triacylglycerol:PL ratio in particles within the $S_f > 400$ fraction increased after both meals (data not shown), indicating a rapid increase in mean particle size. However, there was no significant effect of ethanol on the triacylglycerol:PL ratio during the postprandial period within the lipoprotein fractions.

Plasma non-esterified fatty acids

In contrast to plasma triacylglycerol concentrations, plasma NEFA concentrations (Fig. 4 and Table 2) and also plasma palmitoleic acid concentrations (Fig. 5) were greatly reduced after ethanol, due to a suppression of the normal rise in the later postprandial period. At 180 and 240 min, the mean plasma NEFA concentrations in the ethanol study were less than 50 % of those in the control study. At 480 min, the concentration of plasma NEFA was significantly higher than at 0 min after the control study (P<0·05). After ethanol, there was not such a 'rebound' effect and the plasma NEFA concentrations were not

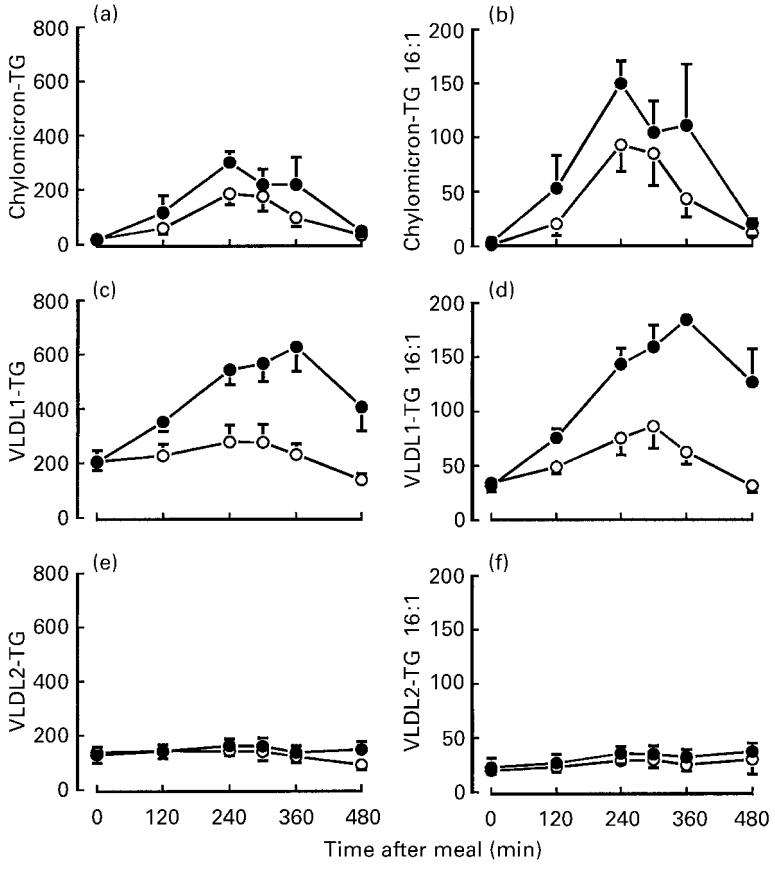

Fig. 3. Effect of a test meal with ethanol (●) and without ethanol (○) on plasma triacylglycerol (TG)-rich lipoproteins. For details of test meal see Table 1 and p. 598. TG (a, c, e) and TG-palmitoleic acid (16:1 *n*-7) (b, d, f) concentrations (μmol/l) are shown for each of the following fractions: (a), (b) S_f>400 (labelled 'chylomicron'); (c), (d) S_f 60–400 (labelled 'VLDL1'); (e), (f) S_f 20–60 (labelled 'VLDL2'). Repeated measures ANOVA shows main effects of treatment (P<0·05) and time × treatment interactions (P<0·001) for both VLDL1 TG and VLDL1 TG 16:1 *n*-7. No significant differences were found for the chylomicron and VLDL 2 fractions. Values are means for seven subjects with their standard errors represented by vertical bars.

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Table 2. Plasma metabolites and lipoprotein triacylglycerol measurements in seven male subjects after consumption of a test meal with and without ethanol. Values are expressed as time averaged area under the curve (AUC) and time averaged incremental area under the curve (iAUC)*
(Mean values with standard errors)

	AUC					iAUC				
	Ethanol		Control			Ethanol		Control		
Plasma variable	Mean	SE	Mean	SE	Statistical significance of difference between means†	Mean	SE	Mean	SE	Statistical significance of difference between means†
Glucose (mmol/l)	5·1	0·1	4·9	0·1	NS	−0·05	0·12	−0·1	0·06	NS
Insulin (mU/l)	13·5	4·5	11·2	2·1	NS	6·2	1·0	4·9	0·9	NS
NEFA (μmol/l)	245	27	400	29·7	$P < 0·001$	−236	70·6	−0·24	33·8	$P < 0·05$
Triacylglycerol (μmol/l)	1343	112	905	88·4	$P < 0·05$	575	118	106	38·5	$P < 0·05$
Lipoprotein triacylglycerol										
$S_f > 400$ (μmol/l)	161	30·3	95·6	15·2	NS	145	33·1	76·7	19·8	NS
S_f 60–400 (μmol/l)	454	39·7	230	42·4	$P < 0·05$	251	49·4	24·1	32·5	$P < 0·05$
S_f 20–60 (μmol/l)	146	24·3	131	18·8	NS	17·8	15·2	−7·4	8·6	NS

NEFA, non-esterified fatty acid.
*For details of test meal see Table 1, and for procedures p. 598.
†Paired *t*-test.

Table 3. Plasma 3-hydroxybutyrate (μmol/l) in seven male subjects after consumption of a test meal with and without ethanol*

Time (min)	Control		Ethanol†	
	Mean	SE	Mean	SE
0	75	27	101	53
60	24	4	99	8
120	19	2	104	8
240	98	24	128	13
360	285	94	107	21
480	360	81	107	23

*For details of test meal see Table 1, and for procedures see p. 598.
†Mean values were significantly different from those of the control group (repeated measures ANOVA, effect of treatment $P < 0·001$).

significantly different at 0 and 480 min. There was no significant effect of ethanol on the proportion of palmitoleic acid in total plasma NEFA (Fig. 5). The concentration of linoleic acid can be used as a marker of endogenous lipid metabolism, since it was a very minor component of the test meal. The proportion of linoleic acid in total plasma NEFA was also similar following the two test meals (Fig. 5).

Plasma insulin and glucose

There were no significant effects of ethanol on plasma insulin and glucose concentrations (Table 2).

Plasma 3-hydroxybutyrate

The mean concentration of plasma 3-hydroxybutyrate followed the same pattern as plasma NEFA concentrations in the control studies but remained constant, with no suppression, during the whole period of the study after ethanol consumption (Table 3).

Discussion

As expected, the plasma triacylglycerol concentrations were significantly elevated, compared with the control meal, after the meal containing ethanol. The appearance and clearance of ethanol in the plasma was rapid compared with the metabolism of the dietary fat. Mean plasma ethanol concentrations reached a peak at 60 min, at a time when the plasma triacylglycerol concentration was not increased above baseline in the control studies. By 240–360 min, corresponding to peak lipaemia in the ethanol studies, the plasma ethanol concentration was almost back to baseline.

The increase in VLDL1-triacylglycerol concentration, together with our previous observation of elevated triacylglycerol concentrations following ethanol alone (Frayn *et al.* 1990), might suggest a primary stimulation of the secretion of large VLDL particles which then compete with chylomicron particles for clearance by lipoprotein lipase (LPL). In a recent study in which the same amount of ethanol was given with a pure fat load, no effect of ethanol on VLDL-triacylglycerol production rate, measured isotopically, was found (Siler *et al.* 1998). The authors attributed the elevation of plasma triacylglycerol concentrations to reduced

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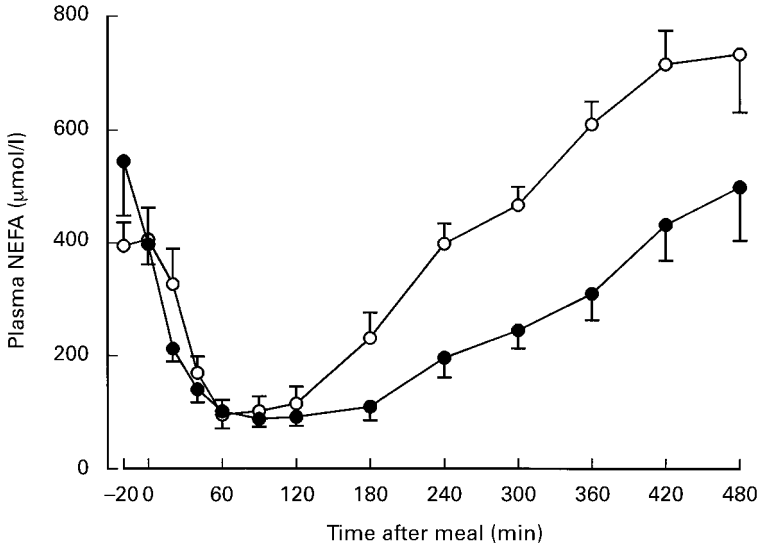

Fig. 4. Effect of a test meal with ethanol (●) and without ethanol (○) on plasma non-esterified fatty acid (NEFA) concentrations. For details of test meal see Table 1 and p. 598. Repeated measures ANOVA shows main effects of treatment ($P<0.001$) and time × treatment interactions ($P<0.001$). Values are means for seven male subjects with their standard errors represented by vertical bars.

VLDL-triacylglycerol clearance (Siler *et al.* 1998). The situation may be different when ethanol is given with a mixed meal, however, as the insulin response to the meal would normally tend to suppress VLDL-triacylglycerol secretion, especially in the large VLDL1 fraction (Malmström *et al.* 1998). In that situation it could be envisaged that ethanol would maintain VLDL1-triacylglycerol secretion rates against the suppressive effect of insulin. Inhibition of

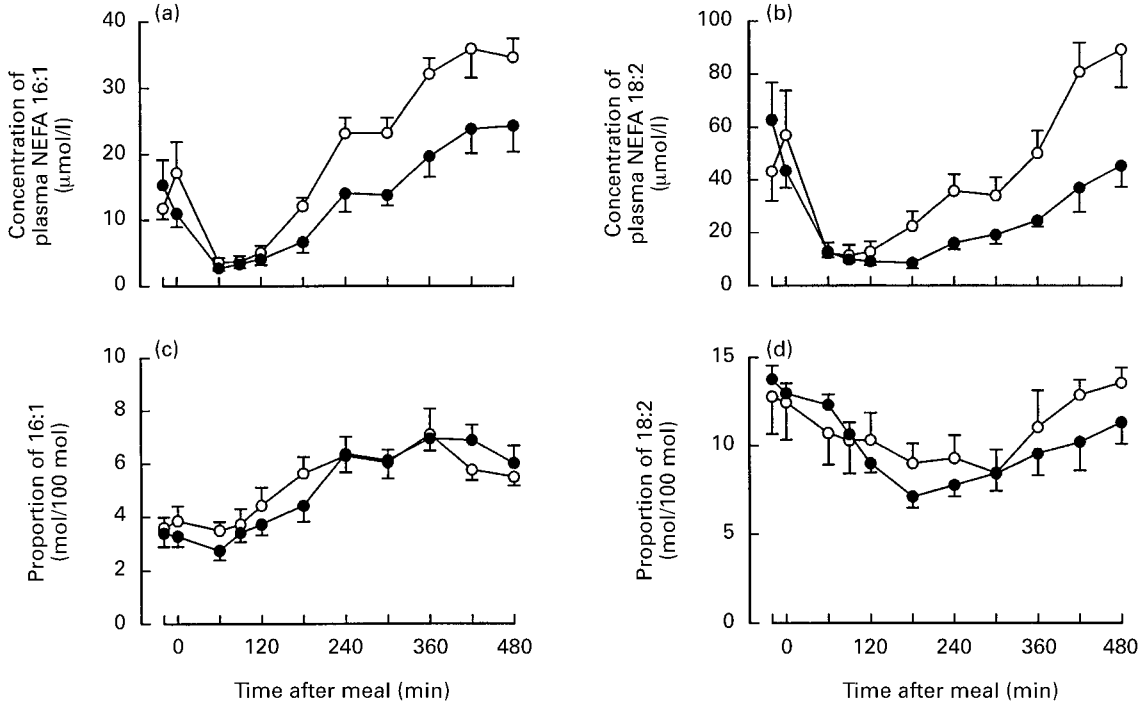

Fig. 5. Effect of a test meal with ethanol (●) and without ethanol (○) on plasma non-esterified fatty acids (NEFA) (a) palmitoleic acid (16:1 *n*-7) and (b) linoleic acid (18:2 *n*-6) concentrations and the proportion of (c) 16:1 and (d) 18:2 in the plasma NEFA fraction. For details of diet see Table 1 and p. 598. Repeated measures ANOVA shows main effects of treatment ($P<0.05$) and time × treatment interactions ($P<0.001$) for concentrations. No significant differences were found for the proportions of 16:1 and 18:2 in the plasma NEFA fraction. Values are means for seven male subjects with their standard errors represented by vertical bars.

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hepatic lipase following ethanol ingestion (Goldberg *et al.* 1984) may also have contributed to the increased concentration of VLDL-triacylglycerol that we observed. The (non-significant) increase in concentration of chylomicron-triacylglycerol would therefore be a secondary effect. The lack of difference in the triacylglycerol: phospholipid ratio in the VLDL fractions suggests that the increase in concentration of VLDL-triacylglycerol could be accounted for by a greater number of particles.

The potentiation of postprandial lipaemia by ethanol is superficially similar to that seen with fructose (Cohen & Schall, 1988; Jeppesen *et al.* 1995; Abraha *et al.* 1998), and it has also been suggested that the effect of fructose is mediated via increased hepatic triacylglycerol synthesis (Truswell, 1994). However, the present results highlight some important differences between the effect of fructose and that of ethanol. In the case of fructose ingested with a fat load, the increase in plasma triacylglycerol reflects mainly an increase in the fraction $S_f > 400$ (Cohen & Schall, 1988), whereas with ethanol we found the effect mostly in the S_f 60–400 fraction. In addition, there are differences in the relationship between plasma NEFA and triacylglycerol concentrations: whilst plasma NEFA concentrations were suppressed by ethanol during the later part of the postprandial response, the effect of fructose is biphasic, with increased NEFA concentrations at this stage of the response (Abraha *et al.* 1998). These differences imply that the mechanism of potentiation of postprandial lipaemia by ethanol may be different from that of fructose.

After a typical mixed meal, plasma NEFA concentrations normally decrease rapidly to reach a nadir at about 60–90 min. This reflects suppression of the enzyme hormone-sensitive lipase (HSL) and a rapid reduction in the rate of release of NEFA from adipose tissue stores of triacylglycerol (Coppack *et al.* 1992). As circulating plasma NEFA are utilized by the body, the plasma concentration of NEFA typically falls to approximately one quarter of the fasting value (Coppack *et al.* 1992). In the later postprandial period, there is rapid entry of chylomicron-triacylglycerol into the circulation, and LPL, situated at the capillary endothelium, hydrolyses the component fatty acids for storage in the adipose tissue. A major proportion of the plasma NEFA during this period is likely to be derived from 'spillover' of LPL-derived fatty acids into the plasma. This value has been shown to be as much as 90 % in human subcutaneous adipose tissue (Frayn *et al.* 1997) and was reflected in an increase in the proportion of palmitoleic acid in the plasma NEFA fraction in the present study. The entry of fatty acids into the plasma is therefore largely controlled by the coordinated regulation of adipose tissue HSL activity and LPL activity (Coppack *et al.* 1992). The fatty acids released by the action of these two lipases will have different compositions reflecting different sources; the products of the action of HSL are endogenous fatty acids, whereas products of LPL action in the postprandial period are largely dietary, or exogenous fatty acids. In order to try to explain the present observation of suppressed plasma NEFA concentrations in the later postprandial period, we examined the specific fatty acid composition of the plasma NEFA pool.

Inhibition of adipose tissue lipolysis has been thought to be the cause of reduced plasma NEFA concentrations when ethanol is given without a meal in normal subjects (Frayn *et al.* 1990) and type 2 diabetics (Christiansen *et al.* 1996). This may be mediated through an increase in plasma acetate concentration (Crouse *et al.* 1968). That mechanism would also explain the divergence of the time-courses for ethanol concentration and suppression of NEFA concentration, since acetate levels in plasma remain elevated while ethanol concentrations are falling to baseline (Frayn *et al.* 1990). However, in this present study, the proportion of linoleic acid in the plasma NEFA fraction after both test meals was similar, suggesting that the proportion of endogenous fatty acids, and therefore the action of HSL, was not affected. Reduced action of LPL has also been implicated as the cause for reduced plasma NEFA concentrations when alcohol was given with a pure fat load (Pownall, 1994). Likewise, as we did not find relative depletion of palmitoleic acid, a component of the test meal, reduced action of LPL is not likely to be the cause. In other studies, LPL activity was found not to change during 10 h following acute alcohol ingestion (Goldberg *et al.* 1984). When ethanol was given in conjunction with glucose, there was significant suppression of LPL activity compared with glucose alone. However, this effect was not seen using the combination of ethanol and a pure lipid load (Nilsson-Ehle, 1978).

The unchanged composition of the plasma NEFA fraction after ethanol consumption indicated that effects on LPL or HSL action could not account for the observation of reduced plasma NEFA concentration. Therefore, the results might be explained by enhanced uptake of plasma NEFA to tissues or organs. The suppression of plasma NEFA concentrations in response to alcohol could, in part, be accounted for by increased incorporation into hepatic triacylglycerol. The lack of rise in 3-hydroxybutyrate concentrations implies that the fatty acids were directed towards esterification rather than oxidation. Studies in rats have found that the increased rate of hepatic triacylglycerol synthesis could be accounted for by increased uptake of plasma NEFA (Abrams & Cooper, 1976*b*). The increased delivery of plasma NEFA was shown to be due to increased hepatic blood flow (Abrams & Cooper, 1976*a*). An earlier paper reported increased hepatic blood flow in man after intravenous infusion of ethanol (Mendeloff, 1954).

Reduction in postprandial plasma NEFA concentrations may partly explain the reduced risk of CHD in moderate alcohol consumers. Further studies are required to determine the origin of reduced plasma NEFA concentrations when ethanol is consumed with a mixed meal.

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