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Dose-dependent effects of chronic ethanol on mouse adipose tissue lipase activity and cyclic AMP accumulation

Mei-Fen Shih & [1]Peter V. Taberner

Department of Pharmacology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD

1 The effects of two chronic ethanol treatment schedules, which produce different plasma ethanol concentrations, on the specific activities of adipose tissue lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) have been investigated in brown and white fat.

2 Mice provided with 20% ethanol solution as sole drinking fluid for 28 days consumed between 13 and 15 g ethanol kg^{-1} body weight day^{-1} over days 22–28. The mean plasma ethanol concentration was 4.94 ± 1.4 mM ($n=8$) at 09 h 00 min on day 28 when the lipase assays were performed. Mice given ethanol in a liquid diet for 7 days consumed between 15 and 18 g ethanol day^{-1} over days 3–7. The mean plasma ethanol concentration was 15.9 ± 4.7 mM ($n=8$) at 09 h 00 min on day 7. These concentrations of ethanol had no effect on the activity of either LPL or HSL *in vitro*.

3 LPL activity in white and brown fat (expressed as nmol fatty acids released h^{-1} mg^{-1} acetone powder) was unaltered 60 min following an acute injection of ethanol (2.5 g kg^{-1}, i.p.) which produced a mean blood ethanol level of 37.5 ± 6.7 mM. HSL activity in white fat (expressed as nmol fatty acid released h^{-1} mg^{-1} protein) was also unaffected by this acute dose of ethanol, but the activity in brown fat was significantly reduced: 3.07 ± 0.30 ($n=8$) after ethanol compared to 4.36 ± 0.25 ($n=12$) in controls ($P<0.01$).

4 LPL activity in white fat was little altered by either of the chronic ethanol treatment schedules whilst LPL activity in the brown fat from the same animals was significantly increased compared to the respective control values: 0.27 ± 0.03 (ethanol drinking), control: 0.16 ± 0.01; 0.79 ± 0.14 (ethanol liquid diet), control: 0.39 ± 0.05.

5 HSL activity in white fat was significantly increased by the chronic drinking treatment (7.7 ± 0.5; control: 3.78 ± 0.17, $n=8$) at the same time that the activity in brown fat was reduced (3.76 ± 0.2; control: 4.74 ± 0.16). The ethanol liquid diet also reduced HSL activity in brown fat but had negligible effect in white fat.

6 The effects of the two chronic ethanol treatments on adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation in brown and white fat were very similar, both qualitatively and quantitatively, to the effects on HSL.

7 It has been shown that brown and white adipose tissues respond differently to the presence of chronic ethanol and that the response is dependent both upon the concentration of ethanol and the nature of the diet with which the ethanol is administered. The effects of ethanol on adipose tissue HSL activity appear to be mediated via changes in the tissue cyclic AMP level and, in this respect, brown fat is more sensitive to ethanol than white fat.

Keywords: Ethanol; brown adipose tissue; white adipose tissue; lipoprotein lipase; hormone-sensitive lipase; cyclic AMP

Introduction

The clinical effects of chronic ethanol consumption on lipid metabolism have been known for many years (Baraona & Lieber, 1979); alcoholic hyperlipaemia being the second major cause of lipaemia after diabetes, with a consequently increased risk of coronary heart disease (Renaude *et al*., 1993). In contrast, more recent epidemiological studies have confirmed that low to moderate alcohol intake is associated with a reduced risk of heart disease (Suh *et al*., 1992; Gaziano *et al*., 1993; Srivastava *et al*., 1994) and that this may be a consequence of increases in the circulating levels of the high-density lipoprotein (HDL) fractions responsible for the transport and disposal of cholesterol in the blood. However, the mechanism by which chronic ethanol alters plasma lipoprotein levels is still unclear and relatively few studies have investigated the effects of ethanol on the metabolism of lipoprotein-derived lipids at the enzyme level.

The importance of lipoprotein lipase (LPL) activity in regulating serum lipoprotein levels and facilitating the disposal of plasma lipids is well recognized (Taskinen *et al*., 1987; Williams *et al*., 1992); the highest levels of this extracellular enzyme being found on cardio-myocytes and adipocytes. Defects in LPL function are known to be associated with a number of disorders of lipid metabolism. Recent work has demonstrated that low levels of HDL (a major risk factor for coronary artery disease) are associated with a specific mutation in the LPL gene (Reymer *et al*., 1995). In the rat, ethanol has been shown to have no effect on cardiac LPL after chronic treatment (Parkes *et al*., 1990). However, post-heparin LPL in rat heart was found to be decreased after 30 days of 20% ethanol treatment (Sevilla *et al*., 1991). Brown and white adipocyte LPL has not hitherto been measured following chronic ethanol treatment.

The adipose tissues perform a key role in the regulation and storage of lipids, and the hormone-sensitive lipase (HSL) present within adipocytes is the principal step in the regulation of lipolysis. Catecholamines, acting through β-adrenoceptors linked to Gs proteins, activate adenylate cyclase to increase formation of adenosine 3′:5′-cyclic monophosphate (Strålfors *et al*., 1984) which, in turn, activates a cyclic AMP-dependent protein kinase to phosphorylate and activate HSL. The concomitant measurement of HSL and cyclic AMP levels in brown and white fat therefore provides a good indication of the functional response of these tissues to chronic ethanol.

We have previously shown that, in normal CBA mice, chronic ethanol (20% w/v solution as sole drinking fluid for 4–6 weeks) activates brown adipose tissue (BAT) and in-

[1] Author for correspondence.

creases BAT lipogenesis (Al-Qatari *et al*., 1991). In the maturity-onset insulin-resistant obese diabetic CBA mouse line the same drinking schedule had an insulin-like anti-lipolytic effect, reducing plasma triglyceride and blood glucose levels, and eventually ameliorating the obesity (Al Qatari *et al*., 1996). These results suggest that the disposal of lipids from the circulation can be increased by ethanol in the long term. The aim of the present study was therefore to investigate the effects of ethanol on adipose tissue LPL and HSL activity, and cyclic AMP production, initially in normal mice, by use of the same chronic drinking schedule as before. Since the evidence from clinical epidemiological studies suggests that the level of chronic alcohol intake is important in determing whether a beneficial (cardio-protective) or detrimental (dyslipidaemic) effect is observed (Klatsky *et al*., 1981; Friedman & Kimball, 1986; Gaziano *et al*., 1993), we have also employed an alternative liquid diet schedule containing 7% ethanol to produce higher peak plasma ethanol levels in order to compare the effects of different concentrations of ethanol *in vivo* on the lipase activities.

Animals and diet

Normal male CBA/Ca mice were bred within the University of Bristol Medical School. These are derived from the commercially available inbred CBA/Ca strain and, like most other mouse strains, exhibit no positive preference for ethanol. They were aged between 12 and 16 weeks and housed in cages of 8 mice at 19–21°C with a 12 h light-dark cycle (light from 09 h 00 min–21 h 00 min). CRM-pelleted diet (Labsure, Cambs.) and water were provided *ad libitum*. The energy composition of the solid pellet diet was 80% carbohydrate, 18% protein, and 2% fat. Two liquid diets were used: Lieber and DeCarli Liquid Rat diets #710027 and #710260 (Dyets, Pennsylvannia). The first diet was given to control mice, the second was calorifically balanced to the first by the addition of 7% ethanol. The food energy components of the ethanol liquid diet were: 11% carbohydrate, 18% protein, 35% fat, and 36% ethanol; in the control liquid diet: 47% carbohydrate, 18% protein and 35% fat.

Acute and chronic ethanol treatments

Mice received 4% (w/v) ethanol solution for 2 days, then 8% (w/v) ethanol solution for another 2 days, followed by 12% (w/v) ethanol solution for 3 days. After that, 20% (w/v) ethanol solution was given for 4 weeks to complete the chronic ethanol drinking schedule (CED). Daily ethanol consumption was stable for the week before the lipase assay. In the liquid diet schedule mice were initially given control liquid diet (CLD) for 3 days. Subsequently, 3.5% ethanol liquid diet was given for 2 days, followed by 7% ethanol liquid diet (ELD) for 5 days. Pair-fed control mice were given an isocaloric control diet throughout. Ethanol consumption was monitored daily by measuring the overall quantity of ethanol solution or ELD consumed by groups of 8 mice for 5 consecutive days.

Ethanol for i.p. injection was made up in 0.9% (w/v) saline at a concentration corresponding to a dose volume of 0.01 ml g^{-1} body weight.

Ethanol assay

Plasma ethanol concentrations were measured in triplicate samples of 50 μl plasma by a standard alcohol dehydrogenase assay (Bonnichsen & Brink, 1955). Carotid blood samples were collected between 09 h 00 min and 10 h 00 min, immediately after the mice had been killed by cervical dislocation for removal of adipose tissues for lipase assays. The adipose tissue samples were taken 60 min following the acute injection of ethanol since we have previously found that this time coincides with the peak blood ethanol level (Unwin, 1984).

Lipoprotein lipase preparation and assay

Lipoprotein lipase (LPL) activity was assayed in acetone powder extracts prepared from brown (BAT) and white (WAT) adipose tissue. This procedure inactivates the other lipases present. Interscapular BAT was dissected out, cleaned of connective tissues, minced, then homogenized with 15–20 ml of acetone at −10°C, filtered through a pre-weighed Whatman GF/C filter and washed with 3 ml diethyl ether, dried, then stored at −20°C with desiccant for up to one week (Carneheim *et al*., 1984). Epididymal fat pads, used as the source of WAT, were prepared in the same manner.

LPL was extracted from 5 mg of the acetone powder by mixing with 1 ml of 50 mM Tris HCl buffer (pH 8.5) for 5 min before homogenization followed by centrifugation at 10,000 *g* for 10 min at 5°C. The resultant supernatant was used to assay LPL activity.

The triolein emulsion for the assay substrate was prepared freshly by the method described by Nilsson-Ehle & Schotz (1976) to give final concentrations in the 0.25 ml assay volume as follows: 80 mmol l^{-1} Tris HCl, pH 8.5, 3.2 g l^{-1} bovine serum albumin (fatty acid free fraction V), 40% (v/v) inactivated human plasma to provide apolipoprotein CII (the activator of LPL), and 9 mmol l^{-1} of triolein. Assays were carried out for 60 min at 28°C and the reaction begun by adding 150 μl of assay substrate. The reaction was stopped by adding 3.25 ml of methanol: chloroform: heptane (141:125:100 ratio), followed by 50 μl [^{14}C]-oleic acid (2 mM, 0.33 μCi ml^{-1}) as internal standard before separation of the aqueous layer by centrifugation for scintillation counting. The results were calculated as nmol FFA released h^{-1} mg^{-1} acetone powder.

Hormone-sensitive lipase (HSL) preparation and assay

Interscapular BAT was dissected out, minced, then homogenized in 10 volumes of medium containing 0.25 M sucrose, 1 mM EDTA, 20 μg ml^{-1} leupeptin, 1 μg ml^{-1} pepstatin A and 1 mM dithiothreitol (pH 7.0). This was centrifuged at 105,000 *g* for 45 min at 4°C. The top 'fat cake' was removed and the clear infranatant fraction decanted and used for the enzyme assay (Huttunen *et al*., 1970). Epididymal fat pads (WAT) were removed and prepared as for BAT, except that 2 volumes of medium were added (Shih & Taberner, 1995). The protein concentrations of the clear infranatant fraction were determined by use of Coomassie Blue (Bradford, 1976).

The assay substrate was the same as for LPL. The final concentrations of reagents in an assay volume of 0.2 ml were as follows: 100 mmol l^{-1} Tris HCl, pH 7.0, 5 g l^{-1} of bovine serum albumin (fatty acid free fraction V), 250 mmol l^{-1} NaCl to inhibit LPL activity, and 4.58 mmol l^{-1} triolein emulsion (Nilsson-Ehle & Schotz, 1976). The reaction was initiated by adding 100 μl of the assay substrate. After 15 min incubation at 37°C, the reaction was stopped by adding 3.25 ml of methanol: chloroform: heptane. The results are expressed as nmol FFA released $min^{-1}mg^{-1}$ protein.

Cyclic AMP preparation

Adipocytes were prepared according to Rodbell (1964) but incubated for 30 min in Krebs-Ringer phosphate buffer (pH 7.4), containing (mM): NaCl 128, $CaCl_2$ 1.4, $MgSO_4$ 1.4, KCl 5.2, and Na_2 HPO_4 10, plus collagenase at a concentration of either 3 mg ml^{-1} (BAT) or 1 mg ml^{-1} (WAT). The cell suspensions were washed three times in collagenase-free Krebs-Ringer phosphate buffer containing 4% BSA, then 20 μl of 2 M HCl was added to a 200 μl volume of cell suspension. This mixture was vortexed and centrifuged at 3000 *g* for 5 min at 4°C.

The supernatant (150 μl) was collected and frozen at −20°C for subsequent cyclic AMP measurement by a competition binding assay based on that described by Gilman (1970). Standard cyclic AMP was diluted over the range 0.125–10 pmol 100 μl^{-1}, and 100 μl of 0.5 mM standard

cyclic AMP used for estimation of the non-specific binding. The reaction was started by adding 100 μl binding protein (prepared from bovine adrenal cortex) into the assay tubes. After 90 min incubation at 4°C, 200 μl of 'Charcoal' (2.5 g charcoal g^{-1} BSA in 500 ml of 50 mM Tris HCl buffer containing 4 mM EDTA) was added and the tubes incubated for a further 20 min at 4°C. The samples were centrifuged at 3000 *g* for 15 min at 4°C and the supernatant decanted into scintillation vials for counting. Sample values were determined from standard curves fitted to a logistic expression. The protein content of the cell suspensions was determined as described above (Bradford, 1976) and the results expressed as pmol cyclic AMP mg^{-1} protein.

Materials

Triolein (C18:1,[*cis*]-9)(1,2,3-tri[cis-9-octadecenoyl] glycerol), oleic acid (*cis*-9-octadecenoic acid), phosphatidylcholine (L-α-lecithin), leupeptin (acetyl-Leu-Leu-Arg-al) hemisulphate, pepstatin A (iso-valeryl-Val-Sta-Ala-Sta), (±)-dithiothreitol, collagenase Type I, and cyclic AMP were obtained from Sigma (Poole, Dorset); sodium heparin (5000 units 5 ml^{-1}) from Leo Laboratories Limited (U.K.); glycerol tri [9,10(n)-^{3}H]-oleate (185 MBq), [1^{-14}C]-oleic acid (1.85MBq) and [8^{-3}H]-adenosine 3′, 5′-cyclic phosphate, ammonium salt (9.25MBq) from Amersham U.K. Binding protein was kindly provided by Dr Eamonn Kelly at the University of Bristol.

Data analysis

The assays, conducted in triplicate unless otherwise stated, have been presented as means ± s.e.mean from $n = 10-12$ mice. Data were combined from 4 or 5 different experimental days with mice randomized between treatment groups. Statistical comparisons were made by ANOVA or by Student's *t* test as appropriate, at a significance level of $P < 0.05$.

Results

The average daily ethanol consumption of the mice over the last 5 days of the CED schedule ranged between 13 and 15 g kg^{-1} body weight, estimated from the overall daily consumption of a cage of 8 mice. During the last 5 days of the liquid diet (ELD) schedule, the average daily consumption varied between 15 and 18 g kg^{-1} body weight. Although the pair feeding design eliminated any differences in the total calorie intake between ethanol-treated mice and their respective control groups, mice provided with pelleted diet consumed fewer calories (2020 ± 60 kJ kg^{-1} day^{-1} (mean ± s.e.mean, $n = 12$)) than those given liquid diet (3262 ± 159 kJ kg^{-1} day^{-1}). Also, the ethanol component of the ELD accounted for 36% of the total energy, whereas the ethanol consumed in conjunction with the pelleted diet represented only 18% of the total energy of the mice.

The plasma ethanol concentrations following a single acute dose of 2.5 g kg^{-1} ethanol and at different times of day during the chronic treatment schedules are shown in Table 1. The blood ethanol concentration at 60 min after this acute dose was similar to the peak level measured in the CED group at 24 h 00 min. This dose of ethanol produced mild ataxia but no loss of righting reflex in the mice. The CED and ELD schedules yielded similar variations in blood ethanol levels over a 24 h period, the lowest levels being observed at 14 h 00 min and the highest at midnight or 05 h 00 min.

Effect of ethanol on lipase activity in vitro

Although, by the nature of the tissue preparation methods used, minimal ethanol should have been carried over into the assay medium from the tissues obtained from ethanol-treated mice, the possibility of an effect of ethanol *in vitro* on LPL and HSL activity could not be eliminated. When ethanol was added to replicate aliquots of LPL assay medium prepared from

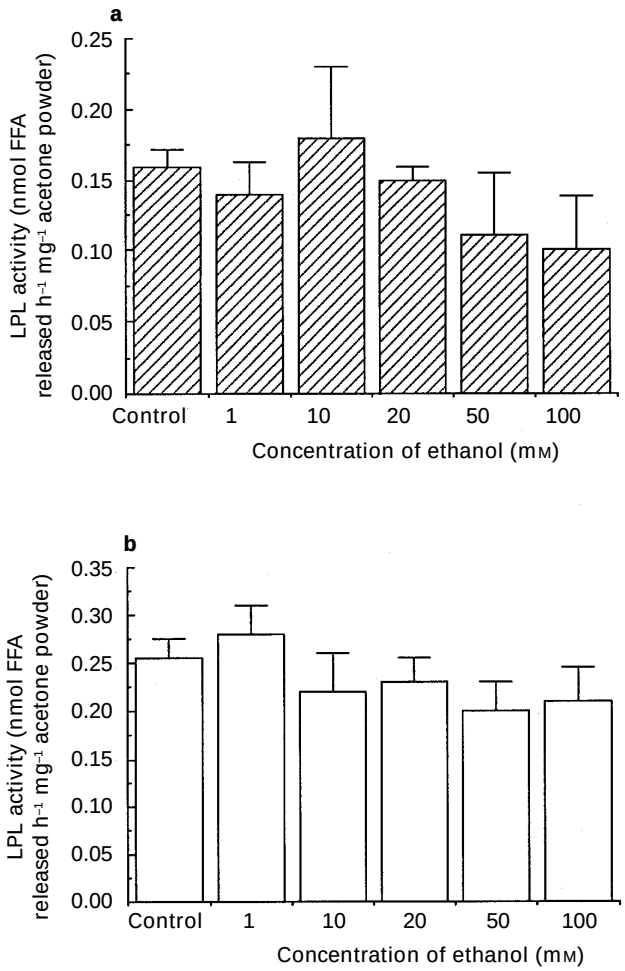

Figure 1 Effect of ethanol *in vitro* on lipoprotein lipase (LPL) activity in (a) brown fat, and (b) white fat. Ethanol was added to the LPL assay medium 30 min before the addition of substrate to produce the final concentrations shown. Results (combined from different experimental days) are shown as means ± s.e.mean from 6 independent samples assayed in triplicate. None of the differences between control and ethanol groups was statistically significant.

Table 1 Plasma ethanol concentrations

	Time of day			
Treatment	*09 h 00 min*	*14 h 00 min*	*24 h 00 min*	*05 h 00 min*
Acute 2.5 g kg^{-1}, i.p.	37.5 ± 6.7			
Chronic				
CED schedule	4.94 ± 1.4	0.8 ± 0.5	34.3 ± 8.2	11.2 ± 3.9
ELD schedule	15.9 ± 4.7	1.2 ± 0.3	68.2 ± 6.7	78.4 ± 8.8

Data are expressed in mmol l^{-1} and as means ± s.e.mean ($n = 8$). In the acute experiments, the blood for ethanol assay was collected 60 min following an i.p. injection at 09 h 00 min. Plasma levels during the chronic ethanol drinking (CED) and ethanol liquid diet (ELD) schedules were measured on treatment days 26–28 and day 7 respectively. For further details see Methods.

brown and white fat derived from control mice, there was no significant effect on the specific activity of the LPL at ethanol concentrations up to 100 mM (Figure 1). The same range of concentrations of ethanol was added to adipose tissue samples immediately before homogenisation for assay of HSL (Figure 2). In the case of brown fat HSL activity (Figure 2a) there was significant inhibition at 50 mM and 100 mM ethanol, whereas white fat HSL activity was only inhibited at 100 mM ethanol (Figure 2b).

Effects of acute ethanol in vivo

The acute dose of ethanol produced no change in lipoprotein lipase (LPL) activity in either brown or white fat, assayed 60 min following i.p. injection (Figure 3). However, the same dose of ethanol produced a significant ($P<0.01$) reduction in both hormone-sensitive lipase (HSL) and cyclic AMP production in brown fat (Figure 4a) whilst HSL and cyclic AMP in white fat remained unaffected (Figure 4b).

Effects of chronic ethanol on lipoprotein lipase (LPL) activity

LPL activity in brown fat was significantly increased by the chronic drinking treatment (Figure 5a), whilst LPL activity in the white fat from the same animals was unaltered (Figure 5b).

The control liquid diet produced a highly significant ($P<0.01$) increase in LPL activity in both brown and white fat compared to the levels in control mice fed on the pelleted diet. The addition of ethanol to the liquid diet produced a further significant ($P<0.01$) stimulation of LPL activity in brown fat (Figure 5a) but no effect in white fat (Figure 5b). Thus, the effects of the two ethanol treatment schedules were both qualitatively and quantitatively similar.

Effects of chronic ethanol on hormone-sensitive lipase (HSL) activity

In contrast to the effect on LPL activity, brown fat HSL activity was significantly reduced ($P<0.05$) by the chronic drinking treatment (Figure 6a), although at the same time HSL activity in white fat was significantly increased ($P<0.01$; Figure 6b). It is evident from these data that the control liquid diet had far less effect on HSL activity than on LPL activity. Brown fat HSL activity during control liquid diet feeding was very similar to that observed in control mice fed with the pelleted diet, and the activity in white fat was only slightly, albeit significantly, greater than in the control group. The addition of 7% ethanol to the liquid diet had no effect on HSL activity in white fat (Figure 6b), although a highly significant inhibition of HSL in brown fat was observed (Figure 6a). In this case, the effects of the two ethanol treatments appear to be qualitatively different between the different types of adipose tissue.

Effects of chronic ethanol on cyclic AMP accumulation

The effects of the two ethanol treatment schedules on cyclic AMP accumulation (Figure 7) very closely paralleled the observed changes in HSL activity (Figure 6). In white fat there was little difference between the cyclic AMP accumulation rates after ethanol liquid diet compared to the corresponding control levels (Figure 7), although there was a highly significant increase after the chronic drinking schedule. Interestingly, in contrast to the results from the LPL assays, the liquid diet itself had no effect on the levels of cyclic AMP or HSL activity in either brown or white fat.

Discussion

The two chronic ethanol treatment schedules used in these studies have demonstrated that circulating levels of ethanol at

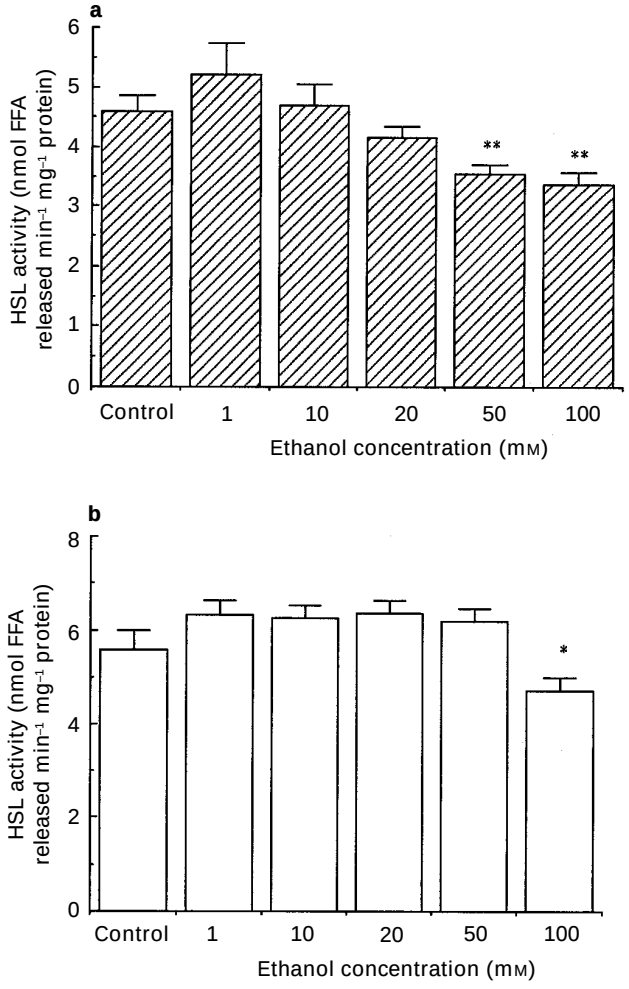

Figure 2 Effect of ethanol *in vitro* on hormone-sensitive lipase (HSL) activity in (a) brown fat, and (b) white fat. Ethanol was added to the tissue homogenization medium to produce the final concentrations shown. Results (combined from different experimental days) are shown as means ± s.e.mean from 6 independent samples assayed in triplicate. HSL activity in presence of ethanol less than in control: $*P<0.05$, $**P<0.01$.

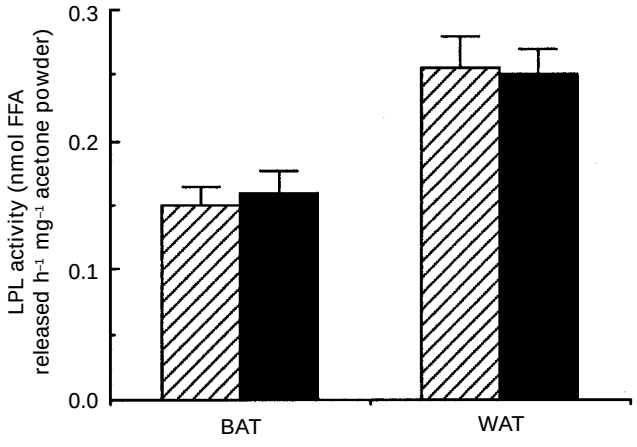

Figure 3 Effect of acute ethanol *in vivo* on LPL activity in brown (BAT) and white (WAT) fat. Mice were injected with (hatched columns) saline ($n=10$) or (solid columns) ethanol 2.5 g kg^{-1} i.p. ($n=8$) 60 min before the removal of tissue for assay in triplicate. Results (combined from different experimental days) are shown as means ± s.e.mean. The differences between the saline control and ethanol-treated groups were not statistically significant.

a specific time point cannot be reliably predicted from the average daily dose consumed. That is to say, although the overall daily ethanol intake was similar during chronic ethanol drinking (CED) and ethanol liquid diet (ELD) schedules, the plasma ethanol concentration measured between 09 h 00 min and 10 h 00 min was three-fold higher in the ELD than in the CED group. This could be due either to behavioural or pharmacokinetic factors (Shih *et al*., 1996). It is known that the pattern of feeding and drinking in rodents exhibits a diurnal variation, with the greatest consumption occurring during the dark (behaviourally active) phase (Deimling & Schnell, 1980). The temporal pattern of ethanol consumption in the two schedules may be slightly different since, in the ELD schedule the alcohol is consumed with the food, whereas in the CED schedule it is consumed with the water and is independent of the food. Alternatively, the rate of ethanol metabolism tends to become zero order at higher levels of ethanol as the concentration of NAD^+, the co-factor for alcohol dehydrogenase, becomes rate-limiting. Consequently, the rate of plasma clearance is independent of the blood level. The blood levels of ethanol observed here are of the same order of magnitude as those associated with moderate drinking in human subjects. The U.K. legal limit for driving (80 mg% in blood) is 17.4 mM, which can be achieved by an acute oral dose of about 0.4 g kg^{-1} (Holford, 1987). Levels in excess of 50 mM are associated with severe intoxication. The peak levels observed in the mice on the ELD schedule are double those observed after an acute dose of 2.5 g kg^{-1} and would be sufficient to produce loss of righting reflex in non-tolerant animals.

The lack of effect of ethanol LPL activity *in vitro* indicates that the effects observed after the chronic *in vivo* treatments are not due to the presence of residual ethanol in the assay medium. Similarly, the lack of effect of an acute dose of ethanol on LPL activity in WAT suggests that the significant activation observed during the CED schedule represents an adaptive change to the chronic presence of ethanol.

In the case of HSL, inhibition was only observed *in vitro* at concentrations of ethanol well in excess of those occurring *in vivo* at the time the adipose tissues were dissected (Table 1). However, the acute inhibition of brown fat HSL and cyclic AMP production by ethanol (Figure 4a) in the same animals in which these parameters were unaltered in WAT (Figure 4b), suggests that BAT is intrinsically more sensitive to ethanol than WAT.

Both the CED and ELD schedules increased LPL activity in BAT. This may be a consequence of an increase in serum lipoprotein levels. It is well known that ethanol raises high density lipoprotein fractions (Fraser *et al*., 1983) and that long term ethanol intake increases serum LPL activity in man (Belfrage *et al*., 1977). The higher LPL activity observed in the control liquid diet group compared to controls fed pelleted diet is not surprising in view of the higher fat content of the diet and the consequently higher daily calorie intake. This particular liquid diet schedule was selected because it has been widely used

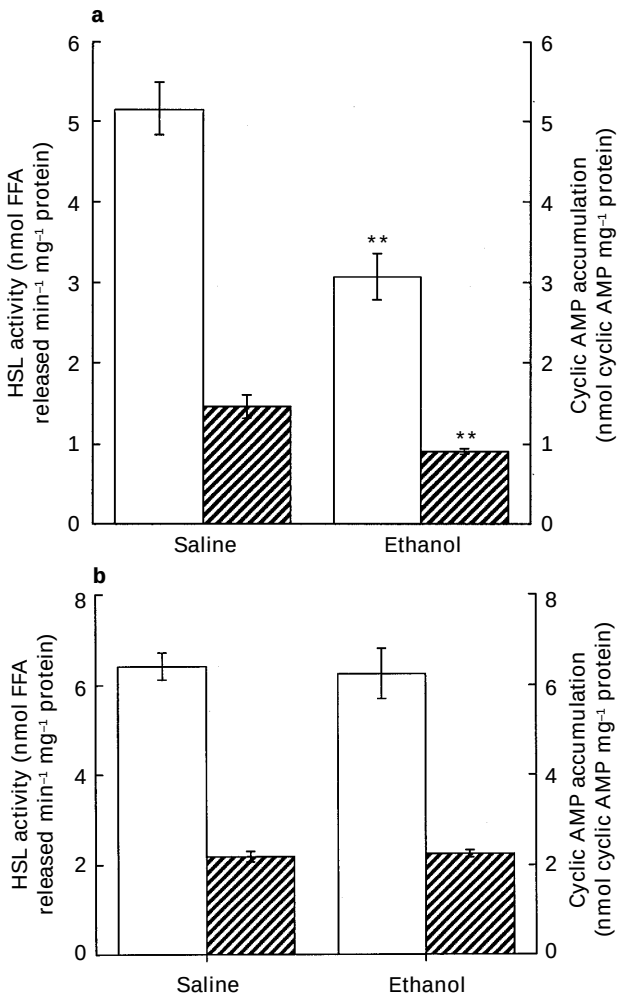

Figure 4 Effects of acute ethanol *in vivo* on HSL activity (open columns) and cyclic AMP accumulation (hatched columns) in (a) brown fat and (b) white fat. Mice were injected with saline ($n=12$) or ethanol 2.5 g kg^{-1}, i.p. ($n=8$) 60 min before the removal of tissue for assay in triplicate. $^*P<0.01$, ethanol treated group < control.

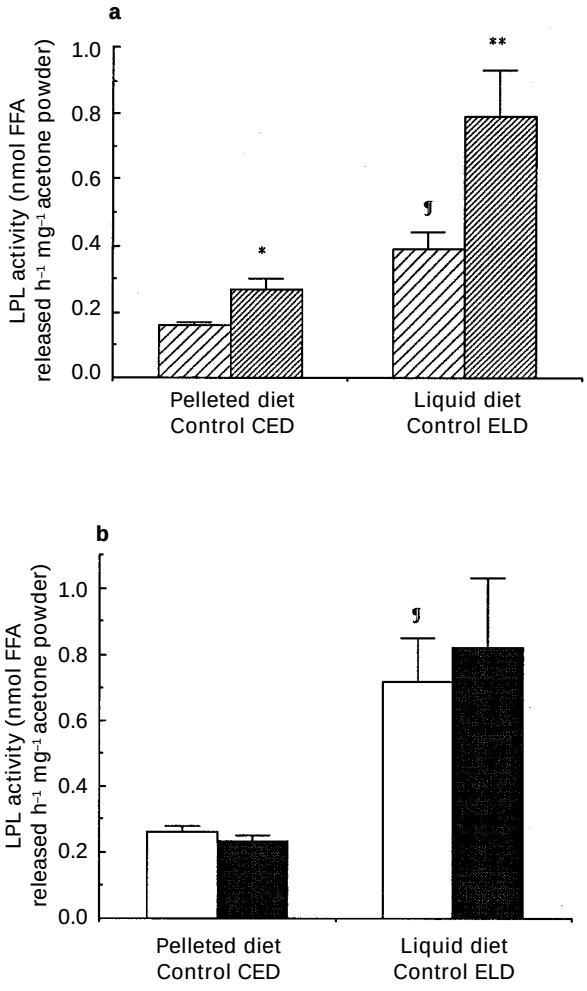

Figure 5 Effects of chronic ethanol treatments on lipoprotein lipase (LPL) activity in (a) brown fat and (b) white fat. LPL activity is expressed as nmol free fatty acid released h^{-1} mg^{-1} acetone powder. The data are shown as means ± s.e.mean from $n=10-12$ observations. Significant differences are indicated as follows: $^*P<0.05$, chronic ethanol drinking > control; ¶$P<0.01$, control liquid diet > control pelleted diet; $^{**}P<0.01$, ELD > control.

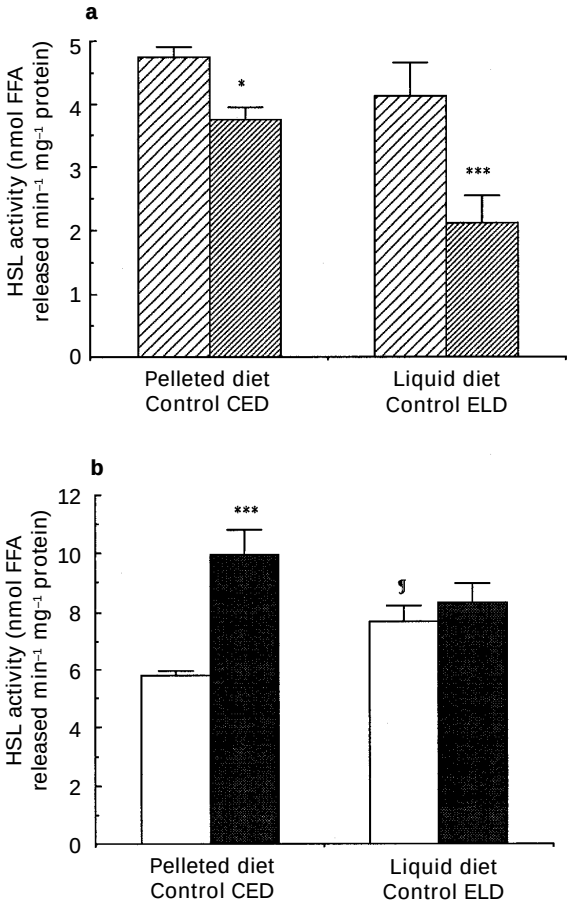

Figure 6 Effects of chronic ethanol treatments on hormone-sensitive lipase (HSL) activity in (a) brown fat and (b) white fat. HSL activity is expressed as nmol free fatty acid released h^{-1} mg^{-1} protein. The data are shown as means ± s.e.mean from $n = 10-12$ observations. Significant differences are indicated as follows: $*P < 0.05$, $***P < 0.005$, chronic ethanol drinking different from control; $¶P < 0.01$, control liquid diet > controlled pelleted diet.

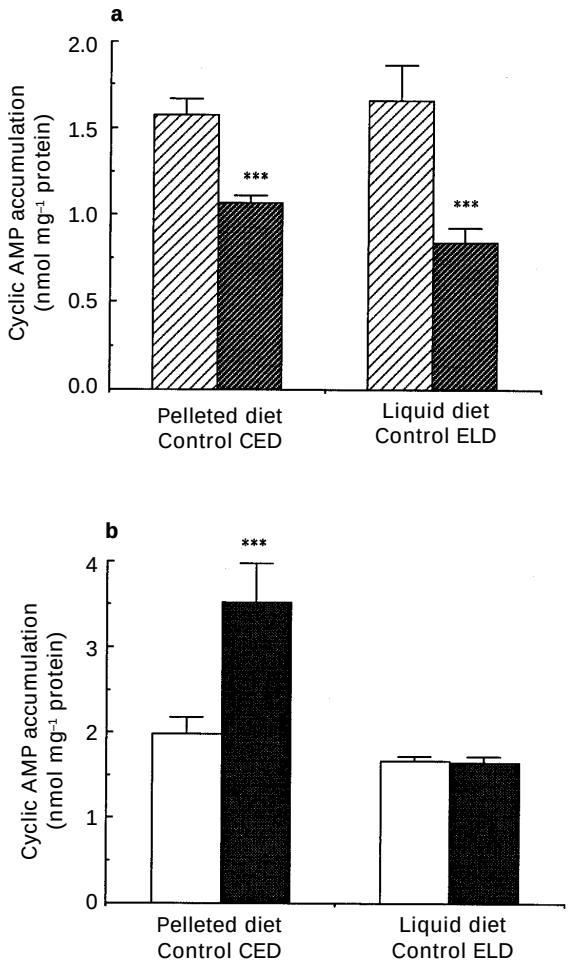

Figure 7 Effects of chronic ethanol treatments on cyclic AMP accumulation in (a) brown fat and (b) white fat. Cyclic AMP accumulation is expressed as nmol cyclic AMP mg^{-1} protein. The data are shown as means ± s.e.mean from $n = 10-12$ observations. Significant differences are indicated as follows: $***P < 0.005$, ethanol drinking different from control.

in alcohol research and achieves high ethanol levels within a short period of time (Lieber & DeCarli, 1989). It is not feasible to match the energy content of the solid diet by using a low fat liquid diet because the lower energy content of carbohydrate compared to fat becomes volume limiting. However, the pair-feeding regime that we have employed in all these studies ensured that the effects of the ethanol treatments were always compared against control animals which had experienced the same calorie intake in the 7 day period before the lipase assays.

Similar ethanol liquid diets have been used to study white fat LPL in miniature pigs (Woollett *et al.*, 1987) and Wistar rats (Parkes *et al.*, 1990). In both cases LPL activity in WAT was unaffected by the chronic ethanol. Our results confirm that LPL activity in WAT is unaffected by ELD, but show that, at the same time, brown fat LPL is stimulated. This suggests that brown fat LPL is either more sensitive to the continued presence of ethanol or that the response to ethanol is specific to this tissue.

The effects of ethanol on HSL activity and cyclic AMP accumulation were also qualitatively different between brown and white fat during the same treatment schedules, although the changes in HSL activity were closely paralleled by the alterations in cyclic AMP accumulation. In this case HSL in BAT was inhibited whilst the activity in WAT was unchanged or increased. These findings support earlier studies in which the effects of CED on adipose tissue lipogenesis *in vivo* were also found to be qualitatively different (Al-Qatari *et al.*, 1991). Again, it appeared that BAT was more sensitive to ethanol than WAT. The inhibition of HSL in BAT will tend to reduce the rate of lipolysis in the tissue, so that the chronic ethanol treatment is, in effect, exerting an insulin-like antilipolytic action. The differential responses of the brown and white adipose tissues may be due to the extensive sympathetic innervation and vascularization of BAT, which can rapidly respond to changes in diet, stress or temperature by alterations in local blood flow and sympathetic tone (Girardier & Seydoux, 1986). WAT, on the other hand, requires long-term stimulation to induce lipase activities. Since WAT is essentially an energy store, and HSL is the controlling enzyme for lipolysis, the selective increase in HSL activity in WAT after chronic ethanol treatment may play an important role in regulating energy balance and body weight. This may explain the ameliorating effect of the CED schedule on the hypertrigyceridaemia and obesity exhibited by the inbred diabetic CBA mouse line (Al Qatari *et al.*, 1996). Al Qatari *et al.* (1991) earlier demonstrated a rebound decrease in lipogenesis which peaked after 9 h withdrawal from CED. This finding will be the subject of further investigation of LPL and HSL activity in both the normal and obese-diabetic CBA mouse lines.

In human studies, the substitution of more than 50% of calories by ethanol results in a decline in body weight; an observation which has led to the proposal that ethanol-derived calories somehow do not contribute to energy intake (Lieber, 1991). On the other hand, it has recently been demonstrated that a combination of alcohol and a high-fat diet leads to overfeeding (Tremblay *et al.*, 1995). In an earlier study, Lieber

et al., (1966) showed that ELD containing high fat (43% total calories), increased lipid and triglyceride accumulation in the liver, and that this was mainly due to dietary fat. The effect of chronic ethanol on HSL activity in WAT could therefore be dependent upon the presence or absence of high fat levels in the diet. The pair-fed dietary regime is therefore crucial in assessing the specific pharmacological rather than caloric effect of ethanol.

In adipose tissues, cyclic AMP is an important intracellular mediator involved in the regulation of lipolysis; catecholamine-mediated activation of β-adrenoceptors leading to activation of a cyclic AMP-dependent kinase and phosphorylation of HSL (Stralfors *et al*., 1984). In contrast, the activity of the extracellular LPL is independent of cyclic AMP. The changes in cyclic AMP formation we have observed after chronic ethanol treatments could indicate that the effects of ethanol on lipid mobilization within adipose tissues might be exerted via this second messenger system. It has recently been shown that, in the mouse cerebral cortex, chronic ethanol can downregulate adenylyl cyclase activity (Tabakoff *et al*., 1995). Future studies are therefore aimed at comparing the effect of chronic ethanol on the activities of both adenylyl cyclase and phosphodiesterase in brown and white adipose tissues.

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