CHRONIC ETHANOL CONSUMPTION AMELIORATES THE MATURITY-ONSET DIABETES–OBESITY SYNDROME IN CBA MICE

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(Received 25 April 1995; in revised form 29 June 1995; accepted 26 July 1995)

Abstract — The effects of a chronic ethanol drinking schedule (20% solution for 6 weeks) on energy balance and carbohydrate and lipid metabolism have been investigated in lean (32–36 g) and obese-diabetic (40–44 g) CBA/Ca mice. The untreated obese-diabetic mice exhibited hyperglycaemia, hypertriglyceridaemia, hyper-insulinaemia and insulin resistance. The chronic ethanol treatment, which yielded plasma ethanol levels of between 1 and 11 mM, lowered the blood glucose, plasma insulin and triacylglycerol levels towards normal in the obese mice, but did not affect these parameters in the lean mice. The body weight of the obese mice tended to return to normal during the 6-week drinking period, although their total energy intake (9.2–10.0 kJ/g/week, food plus ethanol-derived calories) was almost double that of the lean mice (4.8–5.4 kJ/g/week). The blood glucose response to acute insulin injection, which was significantly reduced in the obese mice, became indistinguishable from the response of normal mice after chronic ethanol treatment. Soleus muscle glycogen synthesis in both lean and obese mice was not significantly altered by ethanol drinking, but brown adipose tissue lipogenesis was significantly increased (by 50%) in the obese mice. It is proposed that ethanol is acting chronically to restore insulin sensitivity in the obese diabetic mice at doses which have little or no effect in normal lean animals. This action is exerted, at least in part, at the level of brown adipose tissue lipogenesis.

INTRODUCTION

The hypoglycaemic action of ethanol has been known since the last century and, prior to the discovery of insulin, was even regarded as a potential adjunct in diabetes therapy (Advertisement in The Medical Annual, 1901). However, in spite of the large body of work carried out subsequently on the effects of acute and chronic ethanol administration on carbohydrate metabolism and glucose homoeostasis, the precise cellular basis for the action of ethanol is still open to question. For example, McMongale and Felig (1975) and Nikkila and Taskinen (1975) independently reported that acute ethanol improved glucose tolerance in normal subjects, whereas Dornhorst and Ouyang (1971) and later workers, using the euglycaemic clamp technique (Avogaro et al., 1987), found that acute ethanol impaired insulin sensitivity and glucose disposal in normal subjects. Overall, the metabolic effects of chronic ethanol are to reduce the blood glucose level (BGL) and raise lactate, triglyceride and high density lipoprotein (HDL) levels (Cramp, 1984; Mezey, 1985).

Studies on the effects of ethanol in diabetic subjects have also tended to be contradictory, partly because of the variable nature and severity of the diabetes. Acutely, alcohol consumption can produce a reactive hypoglycaemia which is unrelated to hyperinsulinaemia (Gordon and Southren, 1982), and also cause hypoglycaemic unawareness in both normal and type I diabetic subjects (Kerr et al., 1990). In patients with type II diabetes (non-insulin-dependent diabetes, NIDDM), chronic alcohol has been reported to produce deleterious effects on their metabolic control, tending to raise both the fasting and postprandial BGL (Ben et al., 1991). In contrast, Singh et al. (1988) found no change in glucose tolerance in NIDDM patients. These discrepancies can be ascribed in part to the fact that there were wide variations in the design of the studies, also the doses of alcohol used, and the drinking and dietary history of the subjects or patients involved.

Animal studies, on the other hand, have the advantage that the dietary state and alcohol dosing or drinking pattern can be closely controlled. In addition, the cellular changes responsible for the

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overall metabolic effects of ethanol can be studied in detail at the tissue level. Prolonged ethanol treatment has been reported to lower the fasting BGL in normal rats (Imaura et al., 1982), but there have been relatively few animal studies to compare the effect of chronic ethanol under normal and diabetic conditions. Rodents provided with ethanol as part of their diet tend to reduce their carbohydrate intake to adjust for the calorific value of ethanol (30 kJ/g). This has been observed in both mice and rats (Larue-Achagiotis et al., 1990; Richardson et al., 1990). Heat production (thermogenesis) in small rodents, which is normally induced by feeding, can also be induced by ethanol (Stock and Stuart, 1974; Huttunen and Kortelainen, 1990) and this is associated with an increase in adipose tissue lipogenesis (Trayhurn, 1986).

The male CBA/Ca strain of mouse exhibits a mild maturity-onset diabetes–obesity syndrome that is strikingly similar to human type II NIDDM (Connelly and Taberner, 1989) and is associated with insulin resistance and impaired lipogenesis (Mercer et al., 1992). By comparing the effects of chronic ethanol in obese–diabetic CBA mice with their normal (lean) littermates, it should be possible to determine whether the long-term effects of ethanol are different in the normal and the diabetic (insulin resistant) state. The aim of the present work was therefore to investigate the effects of chronic ethanol consumption on energy balance and insulin sensitivity in these mice. A preliminary report of some of these findings has already appeared in abstract form (Al Qatari et al., 1991).

MATERIALS AND METHODS

Animals

Male CBA/Ca mice aged between 20 and 24 weeks were used from an inbred colony maintained in the Bristol University Medical School. By 20 weeks of age, a proportion of these mice develop a mild maturity-onset type II diabetes–obesity syndrome characterized by hyperglycaemia, hypertriglyceridaemia and hyperinsulinaemia (Connelly and Taberner, 1989). They were housed at 20–22°C in groups of four per cage with a 12 h light/12 h dark cycle (light period from 08.00) and fed a commercial low fat/high carbohydrate diet (CRM pelleted diet, Labsure, Cambs., UK) containing 17.9% protein, 57% carbohydrate, 3.6% crude fibre, and 2.4% crude oil, plus vitamins and trace elements. Food and water were freely available.

Monitoring of diabetic state

The mice were divided into two categories at 16 weeks: normal (lean) males were defined as those mice with body weight < 34 g, blood glucose levels between 9–11 mM, and plasma insulin levels < 29 μU/ml. Obese mice were defined as those with a body weight of > 40 g, blood glucose > 15 mM, and plasma insulin > 150 μU/ml. Mice which did not satisfy these criteria were not used.

Food intake was recorded daily in cages of four mice by weighing the food hoppers and allowing for spilt food. Core body temperatures were measured by insertion of a thermistor probe 2.5 cm into the rectum using an electric thermometer (Light Laboratories, Brighton, UK). A preliminary temperature reading was taken 30 min before the initial test reading in order to allow for the rise in temperature associated with the first handling of the mice.

Blood glucose was routinely measured by collecting a 50 μl sample of blood from the tip of the tail onto a BM-test 1-44 diagnostic strip (Boehringer-Mannheim) and reading the colour change on a Glucochek II reflectometer (Medistron, Horsham, UK). Serum immunoreactive insulin was measured by radio-immunoassay (Herbert et al., 1965) using the methods described previously (Connelly and Taberner, 1989). A sample of 200 μl of blood was obtained from a tail vein under light ether anaesthesia and serum prepared using standard procedures. An aliquot of 20 μl of serum was taken for duplicate assay of triglycerides using a standard lipase–glycerol oxidase–peroxidase procedure on a Technicon RA 1000 autoanalyser (Uwajima et al., 1980).

The acute insulin response was measured by monitoring the plasma glucose level over a period of 6 h following the i.p. injection of 67 U/kg insulin between 10.00 and 10.30. The mice were provided with water, but were not fed during this period. Control mice received an equivalent volume of saline. In these experiments, plasma glucose was assayed in 10 μl aliquots in triplicate using a Beckman Glucose Analyzer 2.

Tissue glycogen synthesis was estimated essentially by the [14C]glucose incorporation method of...
Espinal et al. (1983). Individual soleus muscles were rapidly dissected out and incubated in Krebs Ringer bicarbonate medium containing \([U-^{14}C]D-\)glucose (0.25 µCi/ml) for 60 min at 37°C. The muscle was then removed, rinsed, and rapidly frozen in solid CO\(_2\) prior to extraction of glycogen (Cuendet et al., 1976). Glycogen synthesis was calculated as µmol of glucose incorporated/mg wet weight of tissue/h.

Adipose tissue lipogenesis

Fatty acid synthesis was measured in vivo by following the incorporation of \(^3\)H\(_2\)O into the tissue fatty acids as described by Mercer and Trayhurn (1983). The mice, which had been allowed 4 weeks to recover from the blood sampling procedure necessary to monitor their diabetic state, were injected i.p. with 0.5 mCi (0.1 ml) \(^3\)H\(_2\)O between 08.30 and 10.00. One hour later, the mice were killed by cervical dislocation and exsanguination. A sample of blood was collected into a heparinized microcentrifuge tube and the interscapular brown adipose tissue (BAT) and epididymal white adipose tissue (WAT) were rapidly removed and weighed. The entire BAT and 500 mg of WAT were saponified separately in 1.5 ml of 30% (w/v) KOH at 70°C for 15 min followed by the addition of 1.5 ml of ethanol for a further 2 h. The samples were then cooled on ice, acidified with 1.5 ml of 9 M sulphuric acid and the fatty acids extracted twice into 5 ml light petroleum ether. The ether fraction was washed twice with water. This procedure reduces contamination with non-lipid \(^3\)H to negligible levels (Stansbie et al., 1976). The samples were then air-dried overnight in pre-weighed glass vials preparatory to scintillation counting in Emulsifier-safe LSC cocktail (Packard, Groningen, The Netherlands). An aliquot of 20 µl of plasma from the blood sample was also counted in order to estimate the specific activity of the body water (Mercer and Trayhurn, 1983). Fat-free BAT weight was calculated by subtracting the weight of the saponified fatty acids extracted from the tissue from the total tissue wet weight.

Chronic ethanol treatment

Mice were weaned onto a solution of 20% (w/v) ethanol as their sole drinking fluid over a period of 7 days as described previously (Unwin and Taberner, 1980). Mice were maintained on this regime for a period of 4–6 weeks prior to experimentation. Plasma ethanol was assayed routinely using standard gas chromatographic methods on a Pye Unicam PU 4500 GLC (Hammond, 1975).

Drugs and reagents

Reagents were obtained from either BDH or Sigma Chemical Co., Poole, Dorset, UK as indicated and were of Analar quality wherever possible. Radio-labelled compounds were from Amersham International, Amersham, UK.

Statistical analyses

Mice were randomly allocated to the different treatment groups on each day in order to eliminate any time-dependent variation. The data are presented as means ± SEM from samples of at least \(n = 6\) (number shown in parentheses). The statistical significance of differences between the means of independent groups were determined using either an unpaired Student's \(t\)-test or a one-way analysis of variance (ANOVA) to analyse the effects of treatment group on repeated measures.

RESULTS

Blood ethanol during chronic drinking

Mice which received 20% (w/v) ethanol as their sole drinking fluid consumed on average 11.4 ± 0.8 g of ethanol/kg body weight over 24 h (range 9.2–13.0 g, \(n = 12\)). There was no significant difference in overall alcohol consumption between the lean and obese mice. The blood ethanol concentrations showed a wide, but consistent, diurnal variation which reflected the daily drinking pattern of the mice. In a preliminary experiment the blood ethanol concentration was monitored in groups of eight mice at intervals over 24 h. In lean mice, the peak blood level (11.0 ± 1.6 mM) was observed at midnight and the lowest (1.6 ± 0.3 mM) at 14.00. The blood alcohol levels in the obese mice were not significantly different from these values (10.4 ± 1.2 at 24.00; 1.2 ± 0.4 at 14.00).

Chronic ethanol and the diabetic syndrome

The relevant blood biochemistry of the obese–diabetic mice compared with the lean control mice is summarized in Table 1. Obese mice exhibited significantly higher circulating levels of plasma glucose and serum immunoreactive insulin and
### Table 1. Effects of chronic ethanol treatment on blood biochemistry of lean and obese mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Chronic ethanol-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose (mM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>8.8 ± 0.3 (12)</td>
<td>8.3 ± 0.5 (12)</td>
</tr>
<tr>
<td>Obese</td>
<td>13.4 ± 0.8** (12)</td>
<td>11.0 ± 0.4§ (12)</td>
</tr>
<tr>
<td><strong>Serum immunoreactive insulin (μU/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>18.1 ± 3.2 (12)</td>
<td>24.2 ± 5.4 (8)</td>
</tr>
<tr>
<td>Obese</td>
<td>194.0 ± 22** (12)</td>
<td>21.8 ± 4.2†† (8)</td>
</tr>
<tr>
<td><strong>Serum triglycerides (mM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>1.65 ± 0.25 (8)</td>
<td>1.50 ± 0.35 (8)</td>
</tr>
<tr>
<td>Obese</td>
<td>3.50 ± 0.40** (8)</td>
<td>2.35 ± 0.25† (8)</td>
</tr>
</tbody>
</table>

Results are the means ± SEM of the number of observations shown in parentheses. Chronic ethanol-treated mice received 20% (w/v) ethanol solution as their sole drinking fluid for a period of 5–6 weeks. Obese versus lean, **P < 0.01; obese chronic ethanol versus obese control, †P < 0.05; ††P < 0.01; by Student’s unpaired t-test.

The chronic ethanol treatment had no significant effect on any of these parameters in the lean mice, but there was a highly significant reduction (P < 0.01) in all three parameters in the obese mice. After 6 weeks ethanol drinking, the glucose and triglyceride levels were still higher than in control mice, but the immunoreactive insulin level had returned to the normal level found in lean mice. If the ethanol treatment is acting chronically to ameliorate the diabetic syndrome in these mice, then this should be manifested by an improvement in their energy balance.

**Chronic ethanol and energy balance**

The mean body weight of the groups of obese–diabetic mice used in these studies was, by definition, >40 g, compared with an average of between 34 and 36 g for lean mice of the same age (see Fig. 1). In lean mice the chronic ethanol treatment produced a statistically significant (P < 0.001) fall in the mean body weight after the first 2 weeks of treatment, but from week 3 the body weights did not differ from those of untreated lean mice. In contrast, the obese mice showed a highly significant fall in body weight (P < 0.001) which was maintained throughout the 6-week period of treatment and approximated to 10% of the body weight. Two weeks following withdrawal from ethanol, the average body weight of these mice had risen slightly, but was still less than that of the untreated obese mice (Fig. 1).

Since ethanol itself has a calorific value of 30 kJ/g, the presence of ethanol in the drinking water may have reduced the appetite of the mice. For this reason, the daily energy intake (derived from either food alone or food plus ethanol) of the mice was monitored at the same time. The results are shown in Fig. 2. It is clear that the mean weekly energy intake, over the 5 weeks it was recorded, was highly consistent within each of the four different treatment groups. The lean mice during chronic ethanol treatment showed the same total energy intake as lean controls, by virtue of a...
Fig. 2. Effect of chronic ethanol treatment on total energy intake of normal (lean) and obese mice.

Energy intake was calculated by daily monitoring of the quantity of food (CRM diet: 13.3 kJ/g) and, where appropriate, the volume of 20% ethanol solution (4.75 kJ/ml) consumed. Data points are the means (± SEM) from the average daily reading from four groups of four mice each (n = 4). Lean mice (top): controls, O; chronic ethanol-treated total energy, Δ; chronic ethanol-treated food energy, ■. Obesity (bottom): controls, •; chronic ethanol-treated total energy, ▲; chronic ethanol-treated food energy, ▼. Lean chronic ethanol-treated consumed significantly less food energy than lean controls (P < 0.01). Obese mice (bottom): controls, ●; chronic ethanol-treated total energy, ▲; chronic ethanol-treated food energy, ▼. Obese control mice consumed significantly more energy than lean controls (P < 0.01). Obese ethanol-treated mice consumed significantly more energy than obese controls (P < 0.001). *Obese ethanol-treated mice consumed significantly less food energy than obese controls (P < 0.01); **unpaired ANOVA and Duncan’s multiple range test. There was no significant effect of time on the energy consumption in any group (2-way ANOVA).

Chronic ethanol and lipogenesis

Since body weight is determined by the balance between energy intake and energy expenditure, it is possible that ethanol is acting to raise the basal metabolic rate of the mice by increasing non-shivering thermogenesis. This can be estimated indirectly by measuring adipose tissue fatty acid synthesis (lipogenesis) in vivo. Brown and white fat lipogenesis was measured in control (untreated) and chronic ethanol-treated lean and obese mice. The results are summarized in Table 2. Untreated obese mice showed significantly lower rates of interscapular brown fat lipogenesis compared with their lean littermates. The chronic ethanol treatment slightly increased brown fat lipogenesis in lean mice, but produced a highly significant increase in lipogenesis (P < 0.005) in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Chronic ethanol treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown adipose tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean controls</td>
<td>118.0 ± 6.8</td>
<td>128.0 ± 8.5</td>
</tr>
<tr>
<td>Obese</td>
<td>70.5 ± 8.2**</td>
<td>106.0 ± 6.2††</td>
</tr>
<tr>
<td>White adipose tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean controls</td>
<td>22.5 ± 4.5</td>
<td>22.0 ± 5.2</td>
</tr>
<tr>
<td>Obese</td>
<td>34.5 ± 6.8</td>
<td>25.5 ± 4.6</td>
</tr>
</tbody>
</table>

Results are shown as means ± SEM of n = 12–16 observations.

**Obese versus lean controls, P < 0.001; ††chronic ethanol-treated versus untreated, P < 0.005; by Student’s unpaired t-test.
Table 3. Effects of chronic ethanol treatment on brown adipose tissue weight and protein content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Chronic ethanol treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue wet weight (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>158.00 ± 12 (12)</td>
<td>162.00 ± 15 (10)</td>
</tr>
<tr>
<td>Obese</td>
<td>261.00 ± 15** (12)</td>
<td>163.00 ± 15† (12)</td>
</tr>
<tr>
<td>Total protein (mg/100 mg wet weight)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>8.45 ± 0.12 (12)</td>
<td>7.88 ± 0.28 (12)</td>
</tr>
<tr>
<td>Obese</td>
<td>4.40 ± 0.24†† (8)</td>
<td>7.75 ± 0.36§ (8)</td>
</tr>
</tbody>
</table>

Results are the means ± SEM of the number of observations shown in parentheses. Obese versus lean: **P < 0.01; obese versus lean: ††P < 0.01; obese chronic ethanol versus obese control: ‡P < 0.01; obese chronic ethanol versus obese control: §P < 0.01; by Student's unpaired t-test.

Obese mice compared with untreated controls. The rates of lipogenesis recorded in epididymal white fat were generally much lower than those in brown fat from the same animals. The chronic ethanol treatment produced no significant changes in white fat lipogenic rates in either lean or obese mice.

The tissue weight and protein content of the BAT was also measured (Table 3). The total wet weight of the interscapular BAT was significantly greater in obese compared to lean animals. In contrast, the protein concentration of the tissue was significantly reduced in obese mice. This suggests that the size of the adipocytes had increased rather than the cell number. Chronic ethanol treatment had no effect on these parameters in tissue from lean mice, but reduced the tissue weight, thus increasing the protein concentration, in tissue from obese mice.

Although mice rapidly become tolerant to the hypothermic actions of high doses of ethanol, a rebound hyperthermia can often be observed in ethanol withdrawal in severely dependent animals. To determine whether the chronic ethanol drinking schedule used in these experiments was affecting thermo-regulation in mice, the rectal temperature of obese mice was monitored over 24 h from the time of withdrawal. The results are shown in Fig. 3. It is apparent that the pattern of variation in body temperature with time is the same in both ethanol-treated and control (untreated) mice. There is no evidence of a rebound hyperthermia in the mice withdrawn from the chronic ethanol treatment.

Chronic ethanol and insulin sensitivity

Since insulin resistance is a feature of the diabetes-obesity syndrome in the CBA mice, and insulin is an important regulator of adipose tissue lipogenesis and thermogenesis, the acute blood glucose response to insulin was measured in order to determine whether the effect of chronic ethanol was exerted at the level of insulin-dependent glucose disposal as well as adipose tissue lipogenesis. Blood glucose levels were monitored following an acute dose of insulin (67 U/kg i.p.) in groups of control and ethanol-treated lean and obese mice. The results are shown in Fig. 4. The initial (zero-time) blood glucose level in the obese untreated mice was significantly higher than that of the other three groups. In addition the effect of the insulin, in terms of both the magnitude and duration of the fall in blood glucose, was less than that in the other groups. In contrast, the initial blood glucose level of the ethanol-treated obese mice was no different from that of the lean groups. The acute response to insulin was virtually identical in the control and ethanol-treated lean mice, and the ethanol-treated obese mice.

The ability of insulin to lower blood glucose level is a consequence of the increased glucose uptake into liver and muscle (increasing glycogen synthesis) and into adipose tissue (increasing lipogenesis). The effect of chronic ethanol treatment on muscle glycogen synthesis was measured in
CHRONIC ETHANOL AND DIABETES

Blood glucose (mM)

Time (hours)

Fig. 4. Effect of chronic ethanol treatment on the acute response to insulin.

Control lean (○) and obese (●) mice and chronic ethanol-treated lean (□) and obese (■) mice were given insulin (67 IU/kg i.p.) immediately following the initial blood glucose assay at zero-time. Further blood samples were collected hourly up to 6 h post-insulin. Food and water or 20% ethanol solution were freely available throughout this time. Each data point represents the mean (± SEM) from groups of six mice. Chronic ethanol treatment did not significantly affect the blood glucose level in lean mice. The blood glucose level was significantly reduced in chronic ethanol treated obese mice compared to untreated obese mice (P < 0.0001, unpaired ANOVA).

DISCUSSION

Previous studies which have employed the same ethanol drinking schedule in other strains of mice have demonstrated no fall in body weight, and a normal weight gain in younger animals, over a 6-week treatment period (Unwin and Taberner, 1980). Higher doses of ethanol, however, have been found to reduce the normal weight gain of rats (Larue-Achagiotis et al., 1990; Singh and Patel, 1978). At these higher chronic doses (drinking 36% v/v), or following acute doses sufficient to produce behavioural symptoms of sedation and ataxia, the effects of ethanol on energy balance will be complicated by the reduced locomotor activity of the animals and also the direct hypothermic effect of ethanol. At doses of ethanol which produce hypothermia, mice have been shown to become briefly hyperglycaemic following acute treatment (Risinger and Cunningham, 1991). However, we found no evidence of ethanol-induced hypothermia or rebound hyperthermia in the ethanol-treated obese mice (Fig. 3) and no visible signs of sedation or ataxia in any of the ethanol-treated groups.

For these reasons, and in order to compare our results more readily with those from other studies, the concentration of ethanol in the blood was monitored during the chronic treatment. The diurnal pattern of drinking and consequent blood ethanol levels that we observed are in accord with those reported in C57BL mice drinking 10% ethanol (Gentry et al., 1983). These workers also found that the peak plasma ethanol concentration corresponded to the daily periods of maximal behavioural activity and feeding. It should be remembered that the plasma half-life of ethanol in mice is very much shorter (<30 min) than in human subjects where ethanol exhibits zero-order kinetics at blood levels above 5 mM. Ethanol does show similar saturation kinetics in mice following acute high doses (Greaven and Roach, 1969; Gessner, 1973). The range of plasma ethanol levels recorded in the present experiments (0.7–11 mM) is very much lower than those which occur acutely following the i.p. injection of behaviourally active doses of ethanol (the ED50 for loss of righting reflex being ~48 mmol/kg (2.2 g/kg) (Unwin and Taberner, 1982), and these levels are also lower than those likely to occur in human subjects during social drinking.

In this context, it is worth noting that the chronic ethanol treatment specifically lowered the body weight of the obese mice, although they were consuming the same daily dose of ethanol as their lean littermates, whose weight remained constant. This could reflect a reduction in overall energy intake, but that this is not the case is clear from the fact that the obese mice consumed almost twice the total daily calories of the lean controls. [Fig. 2 (bottom)], and yet maintained the same body weight as the lean mice (Fig. 1). Any effect of ethanol to reduce their appetite can therefore be discounted, leaving the alternative possibility of increased expenditure of metabolic energy.

The level of energy intake and the nutrient
composition of the diet can exert marked effects on energy balance in both experimental animals and humans. In normal rats, it has already been shown that food intake is decreased to accommodate the energy derived from ethanol, possibly through the release of cholecystokinin (Larue-Achagiotis et al., 1990). It has long been known from human studies that replacing food-derived calories with ethanol-derived calories can lead to a reduction in body weight, and also that adding ethanol-derived calories to the diet does not increase body weight to the same extent as when other food calories are added (Pirola and Lieber, 1972). These latter authors also demonstrated that the energetic cost of oxidizing ethanol can contribute to the overall metabolic rate. However, the microsomal ethanol-oxidizing system, which does not yield ATP when alcohol is metabolized, has a higher $K_m$ for ethanol (10 mM) than alcohol dehydrogenase (Mezey, 1985), so that the contribution of the microsomal system is likely to be minimal at the ethanol levels encountered in the present treatment schedule.

The mature male obese diabetic CBA mice used in these studies were characterized by mild obesity, moderate hyperglycaemia and hypertriglyceridaemia, and severe hyperinsulinaemia. When untreated, obese mice maintain their higher body weight on the same energy intake as lean animals (Connelly and Taberner, 1989). They are thus not hyperphagic, and ethanol does not reduce their satiety threshold. From the present data, it can be calculated that ~50% of the calorific intake of the obese mice is being derived from ethanol, suggesting that ethanol is acting in some way to increase energy expenditure.

Since the obese mice are relatively insulin-resistant, part of the effect of ethanol could be due to an improvement in insulin sensitivity. This would result in an increase in the rate of glucose uptake and metabolism, glycogen synthesis, and a reduction in lipolysis. There was a striking improvement in the acute insulin response of obese mice after chronic ethanol in terms of the BGL (Fig. 4), but there was no potentiation of the insulin response in lean mice. The lack of effect of ethanol treatment on muscle glycogen synthesis may reflect the limitations of an in vitro assay in which the normal neuroendocrinological control is lacking, and we are therefore currently undertaking the measurement of hepatic and muscle glycogen synthesis in vivo by following the incorporation of [U-14C]glucose. Other groups have shown that high levels of ethanol are associated with a deterioration in the acute insulin response as a result of the desensitization of tissues to insulin (Nikkila and Taskinen, 1975; Lomeo et al., 1982). In some circumstances, ethanol can also inhibit the release of insulin (Tiengo et al., 1981).

The possibilities remain that in the obese mice chronic ethanol is acting either to inhibit hepatic gluconeogenesis or facilitate glucose uptake. The relatively high acute dose of insulin (67 IU/kg) used in the blood glucose response experiments described here might be expected to suppress hepatic glucose output to the extent that any modulatory effect of ethanol on gluconeogenesis would be obscured. However, our previous studies in vitro have indicated that chronic ethanol had little effect on 2-deoxyglucose uptake into adipose tissue or muscle, although insulin-independent brain glucose uptake was slightly increased (Connelly et al., 1987). On the other hand, it has recently been shown that ethanol can inhibit the type 1 facilitative glucose transporter (GLUT 1) in vitro in a dose-dependent manner, but only at concentrations above 25 mM (Krauss et al., 1994). This concentration does occur in vivo but is double the peak blood levels recorded in the present studies.

A similar dose-dependent effect has also been observed on lipid metabolism. Moderate drinking in human subjects is associated with raised plasma levels of HDL which transport cholesterol, believed to be one possible basis for the cardioprotective action of alcohol (Fraser and Upsdell, 1981). Heavy drinking, on the other hand, is associated with a variety of dyslipoproteinaemias and fatty liver (Taskinen et al., 1982; Schneider et al., 1983; Lieber and Pignon, 1989). Similarly, animal studies have shown that relatively low concentrations of ethanol (0.65–5 mM) can stimulate adipocyte lipogenesis (Scheig, 1971), whereas high levels, well in excess of those in the present studies, stimulate lipolysis (Baraona and Lieber, 1979). Thus, it is only at low doses that ethanol appears to have an insulin-like (i.e. anti-lipolytic) effect in adipose tissue.

Since brown adipose tissue is the main site for non-shivering thermogenesis, particularly in rodents (Foster and Frydman, 1978, 1979), and chronic ethanol intake has been shown to activate
brown fat and increase its oxidative capacity by induction of mitochondrial enzymes (Huttunen and Kortelainen, 1990), it might be expected that chronic ethanol consumption should lead to an increase in energy expenditure as heat. The present results support this view and are in agreement with the earlier report by Rothwell and Stock (1986), who showed that chronic ethanol in vivo, at doses slightly lower than those used here, increased energy expenditure in rats by activating brown adipose tissue thermogenesis. Diet-induced brown fat thermogenesis is known to be defective in a number of obese rodent models (Trayhurn, 1986), and we have already demonstrated that this is also the case in the obese CBA mouse (Mercer et al., 1992). It is interesting therefore that the chronic ethanol treatment can restore the lipogenic rate of the obese mice towards normal, at dose levels which have little or no effect in normal animals.

The present findings emphasize the essentially opposite effects of low and high levels of ethanol on carbohydrate metabolism; the blood glucose, triglyceride, and insulin lowering effects of chronic ethanol consumption in the obese diabetic mice occur at relatively low circulating concentrations of ethanol. The precise nature of the metabolic defects which lead to the development of the maturity onset NIDDM syndrome in the CBA mouse is still unclear, but the reduced level of Gβ protein expression reported to occur in their adipose tissue is one possibility (Palmer et al., 1992). Various animal models of the insulin resistance, which is often present in NIDDM and obesity, exhibit alterations in the inhibitory regulation of adipocyte adenyl cyclase. This could affect the cAMP-dependent activation of hormone-sensitive lipase activity and thus reduce lipolysis. The identification of the precise site at which ethanol, within a specific concentration range, is capable of restoring normal insulin responses in the obese CBA mouse has important implications, not only for the eventual elucidation of the cellular basis for the lipid-lowering and potential cardioprotective action of ethanol, but also the aetiology of maturity-onset insulin resistance.

Acknowledgements — We are grateful to Ian Leighton and Isla Ogilvie who provided valuable assistance with some of these experiments. We thank the British Diabetic Association and the Taberner Trust for financial support, and the Al Tajir Trust for a studentship to M.A.Q.

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